*Original Research Article*

# EFFECT OF PROCESSING ON THE CHEMICAL COMPOSITION AND PHYTOCHEMICAL PROPERTIES OF *Lentinus squarrosulus* MUSHROOM FLOUR




## ABSTRACT

**Aims:** To assessing the effect of boiling and fermentation on Proximate, mineral, dietary fibre and phytochemical compositions of the raw and processed *Lentinus squarrosulus* mushroom.
**Study design**: The mushroom was cultivated, processed into boiled and fermented flour and then analysed.
**Place and Duration of the study**: Department of Food Science and Technology, the Federal University of Technology, Akure, Ondo State and Department of Food Technology, The Federal Polytechnic, Ado Ekiti, between February 2020 and March 2022.
**Methodology**: *Lentinus squarrosulus* spawn was obtained and grown into edible mushroom. The mushroom was divided into three, two portions were processed into boiled and fermented mushroom and the third one was used as control. The three samples were analysed for proximate, mineral, dietary fibre and phytochemical compositions.
**Results**: The proximate composition revealed that moisture content ranged between 10.78-11.66%, ash 5.58-6.11%, fat 3.93-5.10%, crude fibre 6.79-7.29%, protein 16.88-20.53%, carbohydrate 51.24-53.88% and the energy value 321.41-330.15 kcal/100g. Fermentation helped to improve the protein content of the sample. Mineral composition revealed that the samples were significantly different ($p<0.05$). Fermented sample had higher value in sodium, phosphorus, magnesium and iron, boiled sample was high in calcium and selenium while raw mushroom was high in manganese and zinc. Boiled sample has the least soluble fibre but high in insoluble fibre, this may be as a result of the soluble fibre being dissolved in water during boiling, but fermentation improved the soluble fibre content. The phytochemical composition revealed that fermentation improved the saponin and cardiac glycoside, boiling improved the terpenoid and cardiac glycoside while phlobatannin was only present in a small quantity in raw sample.
**Conclusion:** Fermentation and boiling process helped to improve some nutrients in the processed mushroom flour and they can be used as food supplements.


*Keywords:* *Lentinus squarrosulus*, Proximate, Mineral and Phytochemicals

## 1. INTRODUCTION

*Lentinus squarrosulus* Mont. is a white rot saprophytic fungus, which lives on dead or decayed wood in forest and found in many countries in African and Asia. Its fruit bodies are consumed as food in Sub-Saharan Africa and Southeast Asia [1].The fruit body, if harvested within 3 days of fruiting, is used as a meat substitute. It was not until recently that research started to emerge on its application in food [2] and medicine [3]. The mycelium of *L. squarrosulus* contains high protein (57.6%) and low total fat (0.5%) and is also rich in magnesium (0.4%), potassium (3.8%), and vitamin B3 (0.2%). The extract has good antioxidant properties in vitro and it has no toxic effects, even at high doses [4]. Mushroom mycelia have been reported to be a good source of antioxidants. The water soluble glucans from *L. squarrosulus* have immune-enhancing properties [5], while water soluble extract from mycelia eliminated ulcer in rats within 72 hours [4].

Medicinal mushrooms are mushrooms or extracts from mushrooms that are used or studied as possible treatments for disease. Some mushroom materials include polysaccharides, glycoprotein and proteoglycans modulated immune system. Some medicinal mushrooms isolates that have been identified also show cardiovascular, antiviral, antibacterial, antiparantic, antiinflammatury antiulcer and antidiabetic properties [6]. Mushroom is a macro fungus with a distinctive fruiting body that is large enough to be seen by the naked eyes. It includes both edible and non-edible species. Some mushrooms serve as food because of their nutrient contents while some have been used extensively in traditional medicine. Out of the hundreds of known mushroom varieties, several have been studied for their ability to enhance the human immune system and fight infections. Edible mushrooms have been widely utilized as human foods for centuries and have been appreciated for texture, flavor as well as some nutritional and medicinal attributes [7]. However, the awareness of mushrooms as a healthy food and as an important source of biological active substances with medicinal value has recently emerged [8]. Nowadays, mushroom is being relished throughout the world as food and medicine. Researcher reports revealed that mushrooms, being a complete food source are rich in proteins, vitamins, fats, carbohydrates, dietary fibers, and minerals. The rich source of proteins (19 – 35%), vitamins and minerals, low fat content (2 - 8%) and unique chemicals constitution of mushrooms make them low calorie food and choice diet for those suffering from hypertension, atherosclerosis, diabetes, obesity etc. The objective of this study is to determine the proximate, mineral and phytochemical properties of processed *Lentinus squarrosulus* mushroom flour.

## 2. MATERIAL AND METHODS

### 2.1 Sources of Materials

An edible mushroom was grown by obtaining spawn from Mushroom research section of Federal Institute for Industrial Research , Oshodi (FIIRO), Lagos and the substrate (sawdust) was obtained from ‘Egbewa’ sawmill in Ado Ekiti. Rice bran was bought from a rice miller in Ado Ekiti, while other materials were obtained from ‘Oja Oba’ in Ado Ekiti.

### 2.2 Preparation of *Lentinus squarrosulus* (mont.) Powder

The fruiting body of the mushroom was divided into three portions each, the first portion was boiled at 100 $^{o}$C for 5 minutes, the second portion was soaked in water and the water was drained, wrapped in polyethylene bag and allowed to ferment for three days while the third portion was used as control. All the samples were allowed to dry at 60 $^{o}$C using hot air oven, milled into powder and packaged in the high density polyethylene bag.

## 2.3 Chemical Analysis

### 2.3.1 Determination of proximate composition

Moisture content of the raw, boiled and fermented mushroom was determined by using the oven drying method which was based on weight loss and expressed as percentage (%) moisture content [9]. Crude protein was determined from the total nitrogen (TN) determined by the micro-Kjeldahl method by multiplying the total nitrogen by a factor of 6.25. Crude fat was determined by using the Soxhlet extraction method using petroleum ether as the solvent [9]. Ash was measured by burning the sample at 550 °C to constant weight. Carbohydrate was determined by difference. The Atwater factor of 4:9:4 was used to obtain the energy value of the sample.

### 2.3.2 Determination of mineral composition

Mineral compositions of dried mushroom samples were determined by wet-ashing method. The solutions of ash obtained from the samples were dissolved in a drop of trioxonitrate (V) acid made up to 50 ml with deionized water and analyzed. Sodium (Na) and Potassium (K) were estimated using flame photometer while Phosphorus (P) was determined using UV-visible spectrometer after making Ammonium vanado-molybdate at 436 nm according to the established procedures [9]. Calcium (Ca), magnesium (Mg) and other mineral elements of the dried mushroom samples were determined using an Automated Atomic Absorption Spectrophotometer (Perkin-Elmer, Model 2380), [9].

### 2.3.3 Determination of soluble and insoluble dietary fibers

Insoluble dietary fibers (IDF) and soluble dietary fibers (SDF) were extracted according to [9] Official Methods of Analysis using dietary fiber assay kit.

### 2.3.4 Determination of phytochemicals

#### *2.3.4.1 Determination of tannin*

About 0.2 g of finely ground sample was weighed into a 50 ml sample bottle. 10 ml of 70 % aqueous acetone was added and properly covered. The bottle were put in an ice bath shaker and shaken for 2 hrs at 30 $^{o}$C .Each solution was then centrifuge and the supernatant store in ice. 0.2 ml of each solution was pipette into the test tube and 0.8 ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folinciocateau reagent was added to both sample and standard followed by 2.5 ml

of 20% $Na_2CO_3$ the solution were then vortexed and allow to incubate for 40 mins at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared [10].

#### *2.3.4.2 Determination of saponin*

The spectrophotometric method was used for saponin determination [11]. Precisely, 2 g of the finely grinded sample was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol or (But-2-ol) was added. Shaker was used to shake the mixture for 5 hrs to ensure uniform mixing. The mixture was filter with No 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40 % saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained was filtered though No 1 Whatman filter paper to obtain a clean colourless solution. 1 ml of the solution was taken into 50 ml volumetric flask using pipette, 2 ml of 5 % Iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm.

#### *2.3.4.3 Determination of terpenoid*

The procedure described by Sofowora [12] was used. Precisely, 0.5 g of finely grounded sample was weighed into a 50 ml conical flask, 20 ml of chloroform: methanol 2:1 was added and the mixture shaken thoroughly and allowed to stand for 15 min at room temp. The suspension was centrifuge at 3000 rpm the supernatant was discarded and the precipitate was re-washed with 20 ml chloroform: methanol 2:1 and then re-centrifuge again the precipitate was dissolve in 40 ml of 10% SDS solution. 1ml of 0.01M ferric chloride was added and allowed to stand for 30 min before taken the abs at 510 nm. The STD Terpenoid (alpaterpineol) concentration ranging from 0-5 mg/ml from the stock solution.

#### *2.3.4.4 Determination of cardiac glycosides*

The procedure described by Sofowora [12] was used, 10 ml the extract pipetted into a 250 ml conical flask. 50 ml chloroform was added and shaken on vortex mixer for 1 hr. The mixture was filtered into 100 ml conical flask, 10 ml of pyridine and 2 ml of 29 % of sodium nitroprusside were added and shaken thoroughly for 10 min. 3 ml of 20 % NaOH was added to develop a brownish yellow colour. Glycosides standard (Digitoxin). A concentration which range from 0 – 50 mg/ml were prepared from stock solution the absorbance was read at 510 nm.

### 2.3.5 Statistical Analysis

The data obtained were analysed using the analysis of variance (ANOVA) and means were separated using the Duncan multiple range test at the level of $p$=.05 [13].

## 3. RESULTS AND DISCUSSION

### 3.1 Proximate composition of *Lentinus squarrosulus* mushroom

Table 1 shows the proximate composition of *Lentinus squarrosulus* flour. The results of the research show that boiling and fermentation has great effect on the proximate composition of *Lentinus squarrosulus* flour. The result of the proximate analysis showed that the moisture content of the raw sample (11.30%) and fermented sample (10.78%) was lower than the boiled sample (11.66%). The percentage moisture content of the samples was in line with the earlier work, [14] and [15] on *P.squarrosulus* but lower than that of *P.tuber-regiumas* [16] and higher than that of *Pleurotus ostreatus* [17] .The relatively low moisture content of the samples was an indication that the fermented *Lentinus squarrosulus* flour will have high shelf life especially when properly packaged against external conditions. The higher moisture content reported in boiled sample (11.66%) indicates that boiling increases the moisture content of food material thereby having a disadvantage of been stored for a longer periods. The ash content of the boiled sample (6.11%) was higher than the raw (5.88%) and fermented sample (5.58%). The decrease in the ash content of the fermented sample could be as a result of partial consumption of minerals by fermenting microorganisms in the process of metabolism. The ash content of the samples were lower compared to *P.squarrosulus* (7.84%) [15] and higher than that *P.tuber-regium* [16]; *P. ostreatus*, *Pleurotus eryngii* and *Pleurotus sajor-caju* [18]. Mushrooms are rich in protein, [17]. The fat content of the samples ranged from 3.93 to 5.10%. The importance of having fats in edible foods cannot be overemphasized [19]. Dietary fats increase palatability of food by absorbing and retaining flavors. The fat content of the raw sample is higher than the fermented and boiled samples which indicated that fermentation and boiling process affected the fat content of the mushroom. The observed decrease of fat content in the fermented sample could be as a result of the breakdown of fatty acid and glycerol by lipolitic microorganisms present in the sample during fermentation, and the breakdown of the fatty acid and glycerol resulted in the increase of aroma, taste, odour and texture of fermented sample [20]. The fats content of the studied sample agreed with *P. squarrosulus* (4.90%) [15], higher than those of *P. giganteus* and *P.erynii* [18], lower than those of *P. ostreatus* and *P. pulmonarius* [17]. The reduction in the fat content of the fermented and boiled mushroom will increase the shelf life of the sample. The crude fibre content ranged from 6.79 to 7.29%. The crude fibre content of the boiled and fermented sample was low compared to the raw sample, which indicated that boiling and fermentation process reduced the fibre content. The crude fiber content of the study was lower than those of *P. tuber-regium* [16] and *P. pulmonarius* [17] but higher than those of *P. ostreatus* [17] and *P. squarrosulus* [15]. The results of the study showed appreciable levels of fibre which is known as anti-tumorigenic and hypochlestrolaemic agent. Evidence from epidemiological studies suggest that increased fiber consumption may contribute to a reduction in the incidence of certain diseases such as diabetes, coronary heart disease, colon cancer, high blood pressure, obesity, and various digestive disorders. The protein content of the samples ranged from 16.88 to 20.53%.The protein content of the fermented sample was significantly higher than the raw and boiled sample. The observed protein content could be an indication that it can contribute significantly to

daily human protein requirements, usually about 23 to 56 g [21]. According to [22], the high protein content in the fermented sample could be attributed to the ability of microorganisms that was present in the course of the fermentation to secrete some extracellular enzymes (protein) into the fermented sample during their metabolic activities. The high protein content of fermented mushroom has a good implication in a society with high protein deficiency and will no doubt complement protein from cereals and other plant foods. The protein content of the present study is lower than that of *P. squarrosulus* (21.31%) [15], *Pleurotus pulmonarius* [17], P. sajor-caju [18] and P. tuber-regium [16] but higher than those of *P.erynii and P.giganteus* [18]. The carbohydrate content ranged from 51.24 to 53.55%. The raw sample has higher value followed by boiled and the least was in fermented sample. The carbohydrate content of the studied sample agreed with value obtained for *P. squarrosulus* (51.58%) [15], lower than those of *P. tuber-regium* [16], *Lentinus edodes, P.eryngii and P.sajor-caju* [18], but higher than those of *P. pulmonarius* and *P.ostreatus* [17], also some other fungi species such as *Tetanocerarobusta, Volvariella volvacea, Termitomyces microcarpus,* [17]. Wild-grown mushrooms have low energy levels [18]. The energy value ranged from 321.41 to 330.15 Kcal/100 g. The energy value of the present study is lower than those of *P. tuber-regium* [16], *P. ostreatus*, *P. eryngii*, and *P. giganteus* [18] and *P. squarrosulus* [15].

**Table 1: Proximate composition of *Lentinus squarrosulus* mushroom**

| Sample | Raw | Boiled | Fermented |
|---|---|---|---|
| Moisture (%) | 11.30±0.01$^{b}$ | 11.66±0.02$^{a}$ | 10.78±0.02$^{c}$ |
| Ash (%) | 5.88±0.03$^{b}$ | 6.11±0.04$^{a}$ | 5.58±0.06$^{c}$ |
| Fat (%) | 5.10±0.03$^{a}$ | 3.93±0.04$^{c}$ | 4.79±0.04$^{b}$ |
| Crude fibre (%) | 7.29±0.01$^{a}$ | 6.79±0.04$^{c}$ | 7.09±0.04$^{b}$ |
| Protein (%) | 16.88±0.01$^{c}$ | 18.60±0.04$^{b}$ | 20.53±0.03$^{a}$ |
| Carbohydrate (%) | 53.55±0.06$^{a}$ | 52.91±0.07$^{b}$ | 51.24±0.12$^{c}$ |
| Energy value (Kcal/100g) | 327.64±2.04$^{b}$ | 321.41±1.12$^{c}$ | 330.15±2.06$^{a}$ |

Mean values ± standard deviation along the same row with different superscript are significantly different from each other ($p$ = .05).

### 3.2 Mineral composition of *Lentinus squarrosulus* mushroom

Table 2 showed the minerals found in raw, boiled and fermented *Lentinus squarrosulus*. Important mineral elements such as sodium, calcium, potassium, manganese, zinc, phosphorus, magnesium, selenium and iron and were observed in the mushroom samples. The mineral elements were significantly different ($p$ = .05). Raw mushroom sample have highest value for manganese and zinc, boiled mushroom sample was high in calcium, potassium and fermented mushroom sample was high in sodium, phosphorus, magnesium and iron. Boiling and fermentation of mushroom helped to improve the mineral content of the mushroom. Edible mushrooms contain high amounts of ash, which were mainly potassium, phosphorus, magnesium, calcium and zinc [18]. The elements found in mushroom samples were lower than those of *P. tuber-regium* [16] but the iron content was higher than those of other *Pleurotus* species [16, 17].The presence of

potassium, sodium, calcium, magnesium, and phosphorus found in this study was in line with the earlier study on *P. squarrosulus* [23]. High zinc content in the mushroom suggests that increased consumption of edible mushrooms could help reduce the growing incidence of micronutrient deficiency. Minerals in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others. The Potassium levels of the mushroom samples were found to be higher than Na. A high intake of Na is attributed to high blood pressure and hypertension; therefore, fruits and vegetables that are low in sodium but high in potassium are highly preferred for their health benefit [24]. Potassium can help regulate blood pressure and this may decrease the risk of hypertension and cardiovascular disease. Na/K ratio was found to be less than 1 in all the mushroom samples, which indicate that the mushroom have potentials to be suitable for hypertensive patients [25]. Likewise, Ca/P ratio of the mushroom samples was higher than one. The higher the value of Ca/P ratio above one (1), the better it is as a food supplement [26]. Studies have shown that low Ca/P diets cause low bone densities [27], and higher ratios are reported to contribute to a lower prevalence of obesity [28]. The importance of these elements cannot be overemphasized because they are required by many enzymes as cofactors to function effectively, which is an indication that mushroom could be essential for bone and teeth formation.

**Table 2:** Mineral composition of *Lentinus squarrosulus* mushroom (mg/g)

| Sample | Raw | Boiled | Fermented |
|---|---|---|---|
| Sodium | $67.43\pm0.12^{c}$ | $72.83\pm0.25^{b}$ | $80.60\pm0.25^{a}$ |
| Calcium | $120.40\pm0.20^{c}$ | $188.50\pm0.01^{a}$ | $153.76\pm0.21^{b}$ |
| Potassium | $190.60\pm0.30^{c}$ | $220.76\pm0.015^{a}$ | $207.46\pm5.00^{b}$ |
| Manganese | $0.25\pm0.01^{a}$ | $0.26\pm0.01^{b}$ | $0.18\pm0.01^{c}$ |
| Zinc | $0.51\pm0.01^{a}$ | $0.42\pm0.01^{b}$ | $0.39\pm0.01^{c}$ |
| Phosphorus | $60.89\pm0.02^{b}$ | $60.30\pm0.02^{c}$ | $72.18\pm0.01^{a}$ |
| Magnesium | $10.80\pm0.04^{c}$ | $12.79\pm0.02^{b}$ | $13.28\pm0.03^{a}$ |
| Selenium | $0.19\pm0.01^{a}$ | $0.14\pm0.01^{b}$ | $0.12\pm0.01^{c}$ |
| Iron | $0.57\pm0.02^{b}$ | $0.51\pm0.02^{c}$ | $0.63\pm0.01^{a}$ |
| Na/K | $0.35^{b}$ | $0.33^{c}$ | $0.39^{a}$ |
| Ca/P | $1.98^{c}$ | $3.13^{a}$ | $2.13^{b}$ |

Mean values ± standard deviation along the same row with different superscript are significantly different from each other ($p$ = .05).

### 3.3 Dietary fibre of raw, boiled and fermented *Lentinus squarrosulus* mushroom flour

The result of the dietary fibre is shown in Figure 1. The values obtained for the soluble fibre were 9.30, 7.70 and 11.30% for raw, boiled and fermented samples respectively, while the insoluble fibre were 90.70, 92.30 and 88.70% for raw, boiled and fermented samples respectively. There was significant difference ($p$=.05) in the soluble and insoluble dietary fibres. Fermented sample (11.30) as the highest value for the soluble dietary fibre followed by the raw sample (9.30%) while the least was in boiled sample (7.70%). The insoluble fibre revealed that boiled sample (92.30) has the highest value followed by the raw sample (90.70%) while the least was fermented sample (88.70%). Dietary fibre (soluble and insoluble fibre) or

roughage is the indigestible portion of food derived from plants conferring lots of health benefits and is essential in reducing risk of chronic diseases, obesity and cardiovascular disease [29]. The health benefits that has been attributed to dietary fibre could be due to the presence of phytoc hemicals from plant foods and antioxidant dietary fibres has describe as a potential food ingredient [30]. The decrease in the value obtained for the boiled sample for the soluble fibre may be as a result of the soluble fibre being dissolved in water during boiling. The soluble fibre dissolves in water and is readily fermented in the colon into gases and physiologically active by-products and can also be prebiotic and viscous [31]. The insoluble fibre does not dissolve in water and it is metabolically inert and provides bulking when it absorbs water as it moves through the digestive system, easing defecation [32].

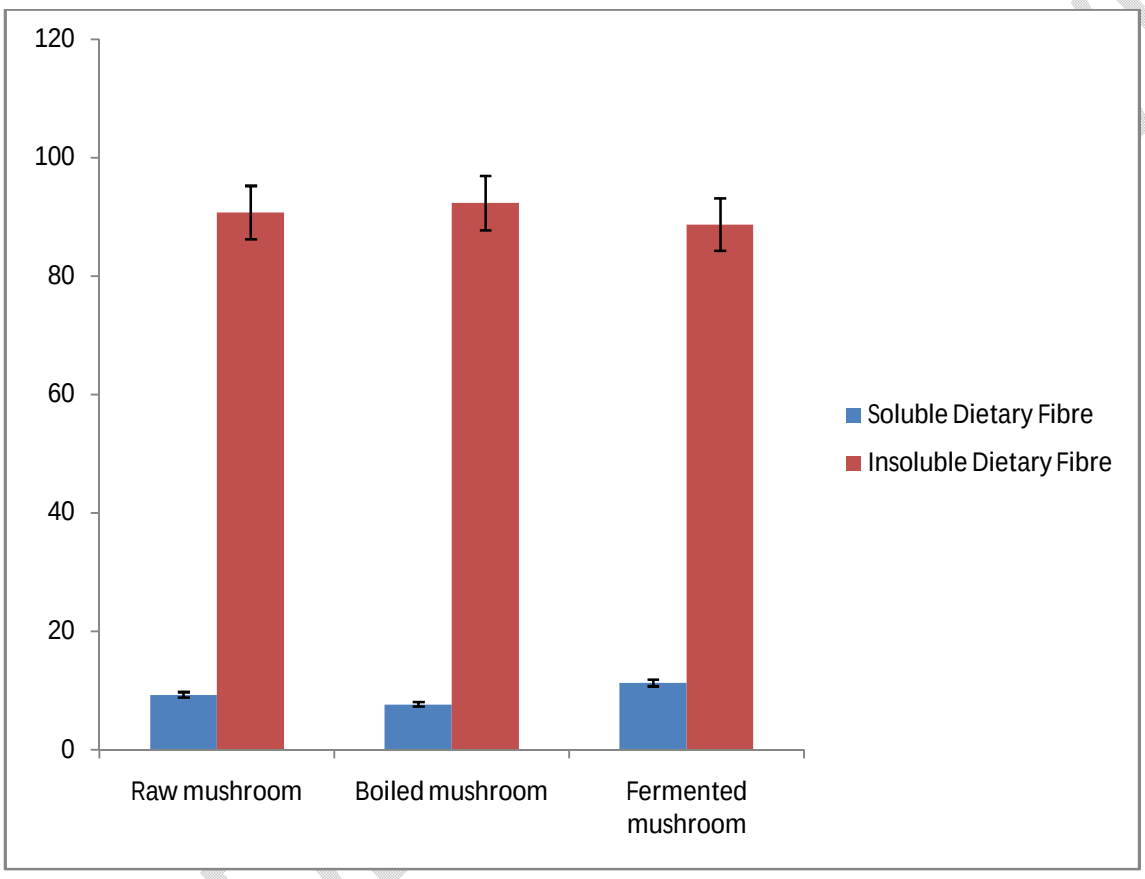


**Figure 1:** Dietary fibre (%) of raw, boiled and fermented *Lentinus squarrosulus* mushroom flour

### 3.4 Phytochemical composition of the *Lentinus squarrosulus* flour

The results of phytochemical composition of the raw, boiled and fermented mushroom are shown in Table 3. The phytochemical composition indicates that the mushroom sample is rich in saponins, tannin, terpenoid and cardiac glycoside. The phytochemical content of the mushroom samples were significantly different ($p$ = .05). Raw sample has the highest value for tannin (1.23 mg/g), boiled sample was high in terpenoid (25.93 mg/g) and fermented sample was high in saponin (69.64 mg/g), and cardiac glycoside (24.79 mg/g). Phlobatannin was only present in small quantity in the raw sample and was absent in boiled and fermented samples. This indicated that boiled and fermentation process reduced the

amount of phlobatannin in the mushroom sample. The saponins and tannins which could be toxic at high amount were found to occur in small amount. The tannin content (1.23 mg/g) obtained in this study was low compared to the results obtained for wild edible mushrooms (Pleurotus ostreatus) [33]. Tannin is a bioactive substance quickens the healing of wounds, useful for the prevention of cancer and treatment of inflamed mucous membranes and ulcerated tissues [33]. Saponin (69.65mg/g) and Terpenoid (25.93mg/g) contents were high compared to the result obtained for wild edible mushrooms (Pleurotus ostreatus) [33]. Presence of saponin and terpenoid suggests the usefulness of *Lentinus squarrosulus* mushroom as a potential fertility agent and has demonstrated antimicrobial, anticarcinogenic, antimalarial, anti-ulcer and hepaticidal effects [34].

**Table 3: Phytochemical quantitative analysis of the *Lentinus squarrosulus* flour (mg/g)**

| Samples | Raw | Boiled | Fermented |
|---|---|---|---|
| Saponin | $39.27\pm0.18^{b}$ | $19.45\pm0.16^{c}$ | $69.64\pm0.19^{a}$ |
| Tannin | $1.23\pm0.01^{a}$ | $0.26\pm0.01^{c}$ | $0.94\pm0.01^{b}$ |
| Phlobatannin | 0.01±0.00 | 0.000 | 0.000 |
| Terpenoid | $14.29\pm0.03^{b}$ | $25.93\pm0.03^{a}$ | $8.43\pm0.03^{c}$ |
| Cardiac glycoside | $9.91\pm0.04^{c}$ | $24.79\pm0.03^{a}$ | $15.79\pm0.03^{b}$ |

Mean values ± standard deviation along the same row with different superscript are significantly different from each other (*p* = .05).

## 4.0 Conclusion

Fermentation and boiling helped in improving the protein, ash and crude fibre content of the *Lentinus squarrosulus* mushroom and also the mineral composition. This will help in complementing the protein and minerals supply deficiencies prevalent in developing countries since mushrooms are highly nutritional and can compare favourably with some proteinous food such as meat, milk and egg. The results of proximate, mineral and phytochemical shows that the mushroom is a potential food supplement.

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