Current Applied Science and Technology Vol. 21 No. 2 (April-June 2021)

Evaluation of Nutrient Contents, Antioxidant and Antimicrobial Activities of Two Edible Mushrooms Fermented with *Lactobacillus fermentum*

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Received: 15 May 2020, Revised: 22 September 2020, Accepted: 7 October 2020

Abstract

In this study, the edible mushrooms; *Termitomyces robustus* and *Pleurotus ostreatus* were fermented with lactic acid bacterium. The proximate composition, minerals, amino acids and fatty acids of unfermented and lacto-fermented mushrooms (mushrooms fermented with *Lactobacillus fermentum*) were revealed. The free radical scavenging and antimicrobial activities of ethanolic extracts from the mushrooms were carried out. The protein content of *P. ostreatus* and *T. robustus* fermented with *L. fermentum* increased ($p<0.05$) up to 17.7±1.9% and 10.4±0.4%, respectively. The crude fiber (7.8±0.0%) and total carbohydrates (76.6±7.9%) in lacto-fermented *T. robustus* as well as crude fiber (9.0±0.6%) and total carbohydrates (67.3±8.4%) in lacto-fermented *P. ostreatus* were reduced ($p<0.05$) when compared to unfermented mushroom samples. Lacto-fermented *P. ostreatus* had the highest valine content of 11.1±0.2 mg/100 g mushroom, while palmitic acid was found to be the most abundant saturated fatty acids (SFA) with 23.0±2.1 % in lacto-fermented *T. robustus*. The phenolic content of the studied mushrooms ranged from 5.6±0.0-7.8±0.0 mg GAE/g extract, while flavonoid was within 3.1±0.0 - 4.9±0.1 mg QE/g extract. The scavenging activity of the unfermented and lacto-fermented mushrooms against DPPH ranged from 62.8±6.8% to 91.3±10.2%. The extracts from lacto-fermented mushrooms showed better zones of inhibition ranging from 5.0±0.0 mm to 12.5±0.7 mm against tested isolates. The research suggests that the probiotic fermentation of mushrooms is a food processing method that can be adopted to enhance nutritional and functional properties of edible mushrooms.

Keywords: fermentation; preservatıon; lactic acid; fatty acid; amino acid
DOI 10.14456/cast.2021.23

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1. Introduction

Edible mushrooms are often regarded as vegetables or meat substitutes due to their culinary usage [1]. Mushrooms are commonly consumed as popular dishes and are frequently used as partial substitutes for red meat due to their attractive sensory attributes like flavour, texture, aroma and taste [2]. Edible mushrooms are highly appreciated and in demand for human consumption due to the significant amounts of nutrients [3]. Nutritional excellence and biological activities of edible mushrooms contribute to their efficacious uses as fresh ingredients in soups, salads, sauces, stuffing and meat dishes, and to their inclusion as value-adding ingredients into novel products like pickles, chips, paste, soup-powder formulation, ketchup, pâté, noodles and pasta, biscuits, and nuggets [4, 5].

Mushrooms are widely consumed and can be eaten in various processed forms following different innovative and unconventional food processing methods like drying, frying, canning, salting, blanching, and freezing [5]. In recent times, some traditional methods have been adopted to preserve wild-grown and cultivated edible fungi [6, 7]. Lactic fermentation is a simple food biotechnological approach to preserve, to enhance the nutritional quality and improve functional properties of mushrooms. The studies of Jabłonska-Rys *et al*. [8] and Liu *et al*. [9] reported the effect of lactic acid bacteria (LAB) fermentation on the nutritional and functional activities of edible mushrooms. Mushrooms such as *Lenzites quercina, Lactarius deliciosus*, *Boletus edulis*, *Suillus luteus*, *Armillaria* spp., *Leccinum* spp., *Paxillus* spp., *Cantharellus cibarius, Amanita muscaria, Russula* spp., *Ganoderma* spp., *T. robustus*, *Flammulina velutipes*, *Agaricus bisporus, Pleurotus* spp. and certain members of the genus of *Tricholoma* were reportedly fermented to improve their nutritional profiles and their free radical scavenging and antimicrobial properties [10, 11]. Hence, edible macrofungi are very good sources of bioactive constituents and are increasingly used as functional foods, dietary supplements, and traditional medicines [12].

Lactic fermentation expedites food preservation and renders food products resistant to microbial spoilage due to the availability of naturally occurring secondary metabolites. Thus, it provides foods with attractive organoleptic qualities and longer shelf life [13]. The presence of viable LAB and their secreted metabolites in fermented foods enhances the health-promoting properties of the final product [14]. On this note, lactic fermentation is widely available and acceptable as a cheap technique that can be adopted for various types of food processing [15]. Hence, there is a need for more research about nutritional composition and functional potentials of certain mushrooms fermented via lactic fermentation. In this study, the nutrient contents, free radical scavenging and antimicrobial potentials of *T. robustus* or and *P. ostreatus* fermented with *L. fermentum* were investigated.

2. Materials and Methods

2.1 Chemical reagents

Folin-Ciocalteu's-reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), gallic acid, quercetin, and dimethyl sulfoxide (DMSO) were products of Sigma-Aldrich (Steinheim, Germany); trichloro acetic acid, hydrogen peroxide (H_2O_2) were from Acros Organics (Geel, Belgium); deoxyribose, aluminium trichloride, and ethylenediaminetetraacetic acid (EDTA) were from Amresco (Ohio, USA); sodium carbonate (anhydrous), chloroform, and methanol were from Univar (Downers Grove, IL, USA), boron trifluoride, Fatty Acid Methyl Ester (FAME) Standards (C_4-C_{22}) were from Sigma-Aldrich (Saint Louis, MO, USA). All the reagents used during this study were of analytical grade.

2.2 Source of microorganisms

Termitomyces robustus and *Pleurotus ostreatus* were harvested from a farmland in Akure, Nigeria. *Lactobacillus fermentum* was isolated from a locally fermented cereal food (Ogi) using standard microbiological techniques described by Cheesbrough [16] and identified according to Cowan *et al*. [17]. The indicator microorganisms were Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Candida troplicalis* and *Candida albicans*. The microorganisms were obtained from Nigeria Institute of Medical Research (NIMR), Lagos. The microorganisms were maintained at -4^{o}C until they were needed for the experimental study.

2.3 Fermentation of mushrooms and extract preparation

The fermentation procedure reported by Liu *et al*. [9] with a little modification was adopted. Briefly, freshly harvested mushrooms were cleaned with 75% v/v alcohol and rinsed with sterile distilled water. The mushrooms were blanched in boiling water at 96^{o}C for 4 min. The pre-treated mushrooms (500 g each) were placed in 2000 ml glass jars followed by the addition of 2% w/w NaCl and 3% sucrose at a solid-liquid ratio of 2:1 (w: v). The starter culture (*L. fermentum*) was adjusted to 10^5 cfu/ml and mixed with the prepared samples. The mixtures were incubated at 20±2^{o}C for 14 days in 3.5 l anaerobic culture jar (Sigma-255, Sigma Scientific Glass Company, India). Thereafter, the mushroom samples were freeze dried at -65±3^{o}C using a vacuum freeze dryer (FD-10-MR, Xiangtan Xiangyi Instrument Ltd, China) until constant weight was achieved. Then, the unfermented and lacto-fermented mushroom powder (100 g) samples were separately soaked in ethanol (1000 ml of 95% v/v) for 48 h. Thereafter, the samples were filtered with Whatman No. 1 filter paper. The filtrates were concentrated in a rotary evaporator (RE-52A, UNION Laboratories, England) and lyophilized. The extracts obtained were re-dissolved in 0.1 % v/v DMSO prior to further analysis.

2.4 Proximate and mineral analysis of mushrooms

The proximate composition like moisture, ash, crude fiber, protein, fat, total carbohydrates the mineral content of unfermented and lacto-fermented mushrooms were carried out according to the methods of Association of Official Analytical Chemists [18]. Minerals were analyzed by using mushroom ash obtained at 600^{o}C. The ash (1.0 g) was digested in 5 ml of HCl and 2 ml of 5 % v/v lanthanum chloride, boiled, filtered and made up to standard volume with deionized water. Atomic Absorption Spectrometer (Buck Scientific, Model 200, Inc. East Norwalk, Connecticut, U.S.A) was used at the appropriate wavelength to determine minerals; Zn, Fe, Ca, Mg, Na, and K. In order to determine Na and K contents in mushroom samples, a flame photometer (Jenway PFP 7, Staffordshire, UK) was used.

2.5 Determination of amino acids and fatty acids in mushrooms

The unfermented and lacto-fermented mushrooms (2.0 g) were defatted with chloroform: methanol (1:1v/v) in a Soxhlet apparatus equipped with a thimble. Defatted mushrooms (40 mg) were weighed into glass ampoules followed by the addition of 7.0 ml of 6 M HCl. Nitrogen gas was passed into each ampoule to expel oxygen, which can cause oxidation of amino acid during hydrolysis. After sealing the ampoules, they were put in an oven at 105±3^{o}C for 22 h and cooled to remove humins through filtration. Filtrate obtained was concentrated at 40^{o}C in a rotary evaporator, and the residue was dissolved with acetate buffer (5 ml), stored in a specimen bottle

and kept at 4ºC. The amount of hydrolysate loaded was between 5-10 μl and each aliquot was dispensed into the cartridge of the analyzer. Technicon Sequential Multisample (TSM-1 Technicon Instruments Company Ltd, Basingstoke, UK) was used to analyze free acidic, neutral and basic amino acids of the hydrolysate for 76 min. The net height of chromatogram peak representing an amino acid was measured. The approximate area of each peak was obtained by multiplying height with width at half-height [19]. The fatty acids of mushroom samples were determined by the trans-esterification method described by Stojković *et al*. [20]. Briefly, fat extracted from the mushrooms was esterified with 0.5 M KOH in methanol (3.4 ml) for 5 min at 95°C. After neutralizing the mixture with 0.7 M HCl, 3 ml of boron trifluoride (14%) in methanol was added and heated for 5 min at 90 °C. To obtained Fatty Acid Methyl Esters (FAMEs), each mixture was extracted with *n*-hexane three times and concentrated to 1 ml. From concentrated sample, 1 µl was injected into gas chromatography (GC-2010, Shimadzu, Japan) with auto injector (AOI) and capillary column (BPX-70). Quantification of FAMEs was achieved using a mix standard (C_4-C_{22}). The concentration and area of each peak of FAMEs was computed using GC post-run analysis software (Shimadzu, Japan).

2.6 Total phenolic content in mushroom extracts

Total phenolic content in extracts from the unfermented and lacto-fermented mushrooms was determined using the method of Singleton *et al*. [21]. Briefly, 2.5 ml of 10% Folin-Ciocalteu's reagent was mixed with the extract (0.5 ml). After 2 min, 2 ml of 7.5% sodium carbonate was added. The blank contained all the reaction reagents except the mushroom extract. The solution was incubated for 1 h at 25ºC. Thereafter, absorbance was measured at 765 nm and compared to a gallic acid calibration curve. Total phenols were determined as gallic acid equivalents from a standard curve, which was prepared with a calibration range of 0-100 µg/ml (coefficient of determination R^2=0.9941) and the results were expressed as mg of gallic acid equivalents (GAE)/g of extract).

2.7 Flavonoid content in mushroom extracts

The total flavonoid content of the extracts from the unfermented and lacto-fermented mushroom was determined using aluminum chloride method described by Meda *et al*. [22]. Mushroom extract (0.5 ml) and 0.5 ml of methanol were mixed with 0.1 ml of 10% w/v aluminum trichloride ($AlCl_3$), followed by 0.5 ml of potassium acetate (1.0 M) and 1.4 ml of distilled water. Each solution was incubated at 28ºC for 30 min. Absorbance was taken at 415 nm in a spectrophotometer. Quercetin was used to prepare a standard curve. The concentration of total flavonoids in mushroom samples was calculated as equivalent of quercetin (mg Quercetin (QE)/g extract).

2.8 Ferric reducing property of mushroom extracts

The reducing power of the unfermented and lacto-fermented mushroom was determined by assessing the ability of extracts to reduce $FeCl_3$ solution as described in the studies of Oyaizu [23]. Extract (2.5 ml) of each sample was mixed separately with 2.5 ml of 200 mM sodium phosphate buffer and potassium ferricyanide (2.5 ml of 1%). After incubation of the mixture at 50ºC for 20 min, 2.5 ml of 10% trichloroacetic acid was added and centrifuged. Each resulting supernatant, after centrifugation at 650 rpm for 10 min, was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. Absorbance was measured at 700 nm. Ascorbic acid at various

concentrations was used to prepare calibration curve and the reducing property of each extract was expressed in mg of Ascorbic Acid Equivalent (AAE) per g of extract.

2.9 DPPH scavenging activity of mushroom extracts

The free radical scavenging ability of the mushroom extracts against DPPH (1, 1-diphenyl-2-picryhydrazyl) was determined using the method described previously by Gyamfi *et al*. [24]. Each extract (0.1 ml) was mixed with 1.0 ml of 0.4 mM DPPH in methanol. The mixture was then incubated at 29°C for 30 min in the dark. Butylated hydroxytoluene (BHT) was used as a positive control and absorbance was measured at 517 nm using a UV-visible spectrophotometer. The capacity of free radical scavenging was calculated using the following equation:

Scavenging activity (%) = {(A control –A sample) / A control} x 100%

2.10 Scavenging ability of mushroom extracts on hydroxyl radical

The scavenging potential of the mushroom extracts against hydroxyl radical was assessed using the method of Halliwell *et al*. [25]. The reaction mixture containing an aliquot of each extracts (0.1 ml) or standard, 20 mM deoxyribose (120 µl), 0.1 M phosphate buffer (400 µl), 20mM hydrogen peroxide (40 µl), 500 µM $FeSO_4$ (40 µl), and the volume was made up to 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30 min. Thereafter, 0.5 ml of trichloro acetic acid (2.8%) was added, followed by 0.4 ml thiobarbituric acid (0.6%). The mixture was heated for 20 min and cooled. The absorbance of solution was measured at 532 nm in spectrophotometer.

Scavenging activity (%) = {(Ab – As)/ Ab} × 100

Where Ab is absorbance of blank and As is absorbance of the sample at 532 nm.

2.11 Nitric oxide radical scavenging assay

The scavenging activity of the mushroom extracts against nitric oxide was performed according to the method described by Jagetia and Baliga [26]. Sodium nitroprusside (1.0 ml) in 0.5 mM phosphate buffer saline was mixed with extract (100 µl). The mixture was incubated at 25°C for 48 h. Thereafter, 1.0 ml of aliquot solution was mixed with 1.0 ml of Griess reagent containing 1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride. After incubating the solution at 34°C for 30 min, absorbance was recorded at 546 nm and scavenging activity was calculated using:

NO Scavenging activity (%) = {(A control –A sample) / A control}x 100%

2.12 Antimicrobial activity of mushroom extracts

The antimicrobial activity of the extracts was tested against microorganisms using the agar well diffusion method [16]. Briefly, the microorganisms were cultivated on nutrient broth or potatoes dextrose broth for bacterial or fungi at 37°C for 24 h and 25°C for 48 h, respectively. The inoculum size was adjusted to 0.5 McFarland turbidity standard at 600 nm using a visible spectrophotometer. A sterile swab stick was moistened with inoculum and spread on Mueller Hinton agar. Subsequently, wells of 4 mm diameter were bored into the agar medium and filled with 50 µl of 50 mg/ml of crude extract. Amoxicillin and ketoconazole were used as positive control against bacteria and fungi, respectively, while sterile distilled water served as a negative control. The plates were incubated at 37°C for 24 h and 25°C at 48-72 h for fungi and bacteria,

respectively. After incubation, zones of inhibition were measured and recorded in millimeters (mm).

2.13 Statistical analysis

Data from the experimental study were reported as mean ± standard deviation (SD) of replicates (n=3). Data were subjected to One-way Analysis of Variance (ANOVA) using SPSS version 20 software (USA). For all tests, mean values were compared by Duncan's new multiple range test and significance was determined at $p \leq 0.05$.

3. Results and Discussion

Lactic fermentation contributes to nutritional enhancement or dietary significance of various foods, and also improve the shelf life of different foods with possible production of new functional foods with nutraceutical potentials [27, 28]. In this study, the nutritional composition and functional properties of unfermented mushrooms and mushrooms fermented with *L. fermentum* was studied. Table 1 shows the proximate composition and mineral contents of unfermented and lacto-fermented mushrooms. Unfermented and fermented mushrooms have very low fat content, with values ranging from 0.5±0.0 to 1.0±0.0%. This agreed with the findings of Adebiyi *et al.* [29], who reported that fresh *T. robustus* was virtually low in fat, and had no cholesterol. The nutritional benefits of low fat content in commonly consumed mushrooms implies that *P. ostreatus***,** *T. robustus* and their fermented products can be recommended for people with cholesterol related ailments or as a suitable component of body weight management. Reduced fat content was recorded after fermentation of *P. ostreatus* or *T. robustus* with *L. fermentum* with the values of 0.5±0.0% and 0.6±0.0%, respectively. The findings of Khetarpaul and Chauhan (30) revealed that natural fermentation increased the fat content whereas pure culture fermentation involving *Lactobacillus brevis* and *L. fermentum* decreased the fat content of fermented food. Hence, the choice of starter cultures and type of substrates may affect the nutritional composition of fermented foods. In this study, the contents of fat, protein, carbohydrates, crude fiber, fatty acids and amino acids in the studied unfermented and lacto-fermented mushrooms were within the recommended Dietary reference intakes (DRI) by Food and Nutrition Board of the Institute of Medicine [31].

The protein contents of fermented *P. ostreatus* and *T. robustus* with *L. fermentum* increased to 17.7±1.9% and 10.4±0.4% and were significantly different ($p<0.05$) when compared to unfermented *P. ostreatus* and *T. robustus* which have values of 4.7±0.0 % and 6.8±1.0%. The increase in protein content of the lacto-fermented mushrooms could have occurred due to the separation of proteins bound with polysaccharides within the cell wall of mushrooms and could also have occurred in connection with the proteolytic activity of LAB metabolites. Ogidi *et al*. [10] reported that an increase in protein content of fermented *Lenzites quercina* occurred as a result of dissociation of protein bound with polysaccharides at the fungal cell wall. The increased protein content in the lacto-fermented mushrooms could also have been due to the protein-like metabolites (bacteriocins and enzymes) produced by LAB biomass during fermentation. This study found appreciable quantities of crude fiber in unfermented mushrooms, at the level of 11.5±1.6% for *T. robustus* and 13.8±0.0% for *P. ostreatus*, respectively, which were reduced to 7.8±0.0% and 9.0±0.6% in *T. robustus* and *P. ostreatus* fermented with *L. fermentum.* This is an indication that LAB is capable of utilizing mushroom crude fiber to produce other metabolites. The studies of Liang *et al*. [32] revealed that some fermenting microorganisms have the ability to degrade crude fiber of food depending on the food composition. The total carbohydrates of

unfermented and lacto-fermented mushrooms varied from 67.3±8.4 to 78.4±11.0%. The studies of Guillamón *et al.* [33] revealed that carbohydrates are the largest portion (35% to 70%) in fruiting bodies, which are the main ingredient in mushrooms and could serve as a raw material for lactic fermentation. Fungal carbohydrates like -saccharides, sugar alcohols, polysaccharides (glucans), glycogen, and chitin serve as a short-term energy source for the growth of microorganisms [34].

The mineral contents (Na, K, Mg, Ca, Zn and Fe) of unfermented and fermented mushrooms with *L. fermentum* are shown in Table 1. The reduction of Ca and Na contents in fermented mushrooms may have been due to ability of lactic acid organisms to utilize the minerals for their metabolism. Potassium was the most abundant element in *P ostreatus* fermented with *L. fermentum*, having the highest value of 1408.8±40.5 mg/100g, while the potassium concentration in *T. robustus* fermented with *L. fermentum* was 1126.0±30.7 mg/100g. The levels of Fe, Mg and Zn increased in fermented mushrooms. Fermentation has reportedly increased the bioavailability of some minerals like potassium, calcium, phosphorous, magnesium, zinc, and iron. Utilization of carbohydrates, protein and anti-nutrients by microorganisms during fermentation improves mineral contents of fermented products [35, 36]. Calcium, potassium, iron, zinc, and magnesium have been reported with high concentrations in edible mushrooms [37, 38]. These macro- and micro-elements are important in various metabolic and physiological functions in humans, which include regulation or maintaining of cellular functions, acid-base balance, osmotic fluid and oxygen transport in the body, catalysis of metabolic growth and enzyme activities, lowering of blood pressure, and the building of immune systems [39].

Table 1. Proximate (DW%) and mineral (mg/100 g) contents of unfermented and fermented mushrooms with *L. fermentum*

Proximate	***T. robustus***	***P. ostreatus***	**Lacto-fermented *T. robustus***	**Lacto-fermented *P. ostreatus***
Fat	$1.0^{ab} \pm 0.0$	$0.8^{b} \pm 0.0$	$0.6^{c} \pm 0.0$	$0.5^{c} \pm 0.0$
Protein	$6.8^{c} \pm 1.0$	$4.7^{d} \pm 0.0$	$10.4^{b} \pm 0.4$	$17.7^{a} \pm 1.9$
Crude fiber	$11.5^{a} \pm 1.6$	$13.8^{a} \pm 0.0$	$7.8^{b} \pm 0.0$	$9.0^{b} \pm 0.6$
Ash	$2.3^{c} \pm 0.0$	$3.3^{b} \pm 0.0$	$4.6^{a} \pm 0.0$	$5.5^{a} \pm 0.0$
Carbohydrates	$78.4^{a} \pm 11.0$	$77.4^{a} \pm 9.6$	$76.6^{a} \pm 7.9$	$67.3^{b} \pm 8.4$
Na	$3.0^{a} \pm 0.0$	$4.0^{a} \pm 0.0$	$0.6^{b} \pm 0.0$	$0.4^{b} \pm 0.0$
K	$681.0^{d} \pm 20.3$	$884.7^{c} \pm 27.0$	$1126.0^{b} \pm 30.7$	$1408.8^{a} \pm 40.5$
Mg	$442.0^{a} \pm 17.0$	$340.1^{b} \pm 8.1$	$223.3^{c} \pm 11.6$	$458.6^{a} \pm 20.4$
Ca	$103.1^{c} \pm 8.0$	$215.2^{a} \pm 18.2$	$84.0^{d} \pm 9.7$	$164.4^{b} \pm 10.6$
Fe	$346.7^{c} \pm 27.0$	$282.0^{d} \pm 11.5$	$710.7^{a} \pm 21.0$	$583.1^{b} \pm 9.2$
Zn	$208.4^{b} \pm 13.7$	$115.2^{c} \pm 9.3$	$261.2^{a} \pm 8.4$	$210.5^{b} \pm 3.8$

Values are mean±SD of replicates (n=3), values with different alphabets along row are significantly different from each other when $p \leq 0.05$. SD of 0.0 is <0.1.

The amino acids profile of unfermented and lacto-fermented mushrooms is shown in Table 2. Unfermented and lacto-fermented mushrooms contain valuable amino acids. Edible mushrooms contain proteins, having quality more satiating than meat [40]. Fermented mushrooms with *L. fermentum* showed increased levels of both essential and non-essential amino acids. The highest amino acid content found in lacto-fermented *P. ostreatus* was valine (11.1±0.2 mg/100 g mushroom) followed by alanine (10.2 mg/100g mushroom), while lacto-fermented *T. robustus* had the highest value of methionine (10.4 mg/100g mushroom). High concentrations of essential amino acids were found in *Agaricus bisporus*, *A. brasiliensis*, *Flammulina velutipes*, *Lentinula edodes*, *Pleurotus djamor*, *P. eryngii* and *P. ostreatus*, thus indicating that edible mushrooms are a potential and alternative source of amino acids [41]. The use of *L. fermentum* as a starter culture in mushroom fermentation increased amino acids contents. Akabanda *et al*. [42] revealed that different amino acids were secreted when milk was fermented with various single and combined starter cultures (LAB) during production of 'Nunu'. The proteolytic system of LAB with different enzymes such as cell-wall bound proteinase PrtP, aminopeptidases: PepC, PepN and PepM, and proline peptidases: PepX and PepQ, converts proteins to oligopeptides, peptides and then to amino acids [43]. With the presence of these enzymes in LAB, this study evidently showed that the lacto-fermented mushrooms are rich sources of essential and non-essential amino acids.

The fatty acids (%) of unfermented and fermented mushrooms with *L. fermentum* is shown in Table 3. Palmitic acid (C16:0) was the most abundant saturated fatty acid (SFA) in lacto-fermented *T. robustus* with value of 23.0±2.1% and 20.4±0.8% in lacto-fermented *P. ostreatus*, 10.3±1.5% in *P. ostreatus* and 6.0±0.0% in *T. robustus*. Palmitoleic acid (C16:1) is 22.2±2.8% in lacto-fermented *P. ostreatus* and 17.0±1.1% in lacto-fermented *T. robustus*, 21.0±4.2% in *P. ostreatus* and 10.0±1.1% in *T. robustus*. Oleic acid was the most abundant monounsaturated fatty acid (MUFA) with 21.8±4.8% in lacto-fermented *P. ostreatus*, 13.3±0.9% in lacto-fermented *T. robustus*, while in unfermented mushrooms, it was 9.4±0.2% in *P. ostreatus* and 10.4±0.6% in *T. robustus*. The findings of Barros *et al*. [44] and Ribeiro *et al*. [45] revealed fatty acids in some edible mushrooms ranging from 0.03 to 76.50% and 30 to 3175 mg/kg, respectively. The presence of linoleic, oleic and palmitic acids as important fatty acids in human diet is evidence for edible mushrooms being a healthy food. Several edible mushrooms that are nutritionally well-balanced with monounsaturated and polyunsaturated fatty acids are receiving increased attention due to their functionality and nutraceutical potential [46]. Polyunsaturated fatty acids (PUFAs) such as linoleic acid is an essential nutrient required by the body; plays distinctive roles in the structure and function of biological membranes in the retina, central nervous system and in the prevention of cardiovascular diseases [47].

Table 4 shows the total phenolic and flavonoid contents of unfermented and lacto-fermented edible mushrooms. The phenolic contents of 5.6±0.0, 6.4±0.0, 7.1±0.1 and 7.8±0.0 mg GAE/g extract were obtained for *T. robustus*, *P. ostreatus*, *T. robustus* fermented with *L. fermentum*, and *P. ostreatus* fermented with *L. fermentum*, respectively. Lacto-fermented *P. ostreatus* had the highest flavonoid of 4.9±0.1 mg QE/g extract, followed by lacto-fermented *T. robustus* with value of 4.4±0.0 mg QE/g. In the findings of Skąpska *et al*. [48], the total polyphenol content of 12.3 g/kg d.m. and 5.8 g/kg d.m. (expressed as gallic acid) were reported for *Agaricus bisporus* and *P. ostreatus*, respectively. Phenolic content of 67.6 mg GAE/g extract was obtained for fermented *Lenzites quercina*, a wild medicinal mushroom [49] when compared to an unfermented sample. Microbial fermentation is a method that enhances release of bound phenolic compounds through some biological processes like glycosylation, deglycosylation, ring cleavage, methylation, glucuronidation, and sulfate conjugation [50]. The ferric reducing property of the mushroom extracts ranged from 72.8±6.3 to 121.8±10.1 mg AAE/g extract. The scavenging activities of unfermented and lacto-fermented mushrooms against DPPH, HO^- and NO ranged from 62.8±6.8% to 91.3±10.2%, 50.5±5.4 to 80.5±6.1% and 40.3±2.7% to 70.3.6±7.2%,

Table 2. Amino acids (mg/100 g mushroom) of unfermented and lacto-fermented mushrooms

Amino acids	*T. robustus*	*P. ostreatus*	Lacto-fermented *T. robustus*	Lacto-fermented *P. ostreatus*
Alanine	3.0 ^{d} ±0.0	5.5 ^{c} ±0.0	8.6 ^{b} ±0.7	10.2 ^{a} ±0.0
Arginine	2.2 ^{c} ±0.2	3.2 ^{b} ±0.0	6.1 ^{a} ±0.3	6.5 ^{a} ±0.0
Aspartic	2.1 ^{d} ±0.0	5.5 ^{b} ±0.0	4.8 ^{c} ±0.0	7.2 ^{a} ±0.0
Cysteine*	3.0 ^{c} ±0.0	1.8 ^{d} ±0.1	4.9 ^{a} ±0.0	4.1 ^{b} ±0.0
Glutamine	8.1 ^{a} ±0.3	5.9 ^{c} ±0.2	6.8 ^{b} ±0.2	5.0 ^{d} ±0.0
Glycine	4.5 ^{a} ±0.0	3.5 ^{b} ±0.0	1.0 ^{d} ±0.0	2.0 ^{c} ±0.0
Histidine^	0.7 ^{a} ±0.0	0.5 ^{b} ±0.0	0.6 ^{a} ±0.0	0.4 ^{b} ±0.0
Isoleucine*	2.3 ^{b} ±0.2	2.9 ^{b} ±0.4	5.3 ^{a} ±0.2	5.1 ^{a} ±0.0
Leucine*	5.3 ^{b} ±0.0	2.7 ^{d} ±0.0	6.3 ^{a} ±0.0	4.2 ^{c} ±0.0
Lysine*	2.6 ^{c} ±0.0	2.5 ^{c} ±0.0	6.8 ^{b} ±0.2	8.3 ^{a} ±0.8
Methionine*	6.8 ^{bc} ±0.0	6.1 ^{c} ±0.0	10.4 ^{a} ±0.2	8.2 ^{ab} ±1.1
Phenylalanine*	3.6 ^{c} ±0.2	2.6±0.0	6.0 ^{a} ±0.1	6.2 ^{a} ±0.6
Proline	2.5 ^{c} ±0.0	1.2 ^{d} ±0.0	3.8 ^{b} ±0.0	4.5 ^{a} ±0.1
Serine	2.6 ^{b} ±0.0	2.0 ^{b} ±0.0	5.2 ^{a} ±0.0	4.6 ^{a} ±0.0
Threonine*	0.3 ^{d} ±0.0	4.3 ^{bc} ±0.1	5.5 ^{b} ±0.0	7.1 ^{a} ±0.0
Tyrosine*	4.0 ^{b} ±0.0	3.8 ^{b} ±0.0	4.0 ^{b} ±0.0	5.7 ^{a} ±0.0
Tryptophan*	5.1 ^{c} ±0.4	6.2 ^{b} ±0.0	6.3 ^{b} ±0.0	8.6 ^{a} ±0.0
Valine*	5.6 ^{d} ±0.0	7.0 ^{c} ±0.8	9.8 ^{b} ±0.3	11.1 ^{a} ±0.2

^ An indispensable amino acid in human adults according to FAO/WHO/UNU [39]
* Essential amino acids
Values are mean±SD of three replicates (n=3)
Values with different alphabets along row are significantly different from each other when $p \leq 0.05$.
SD of 0.0 is <0.1.

Table 3. Fatty acids (%) of unfermented and lacto-fermented mushrooms

Fatty acids	No. of carbon	*T. robustus*	*P. ostreatus*	Lacto-fermented *T. robustus*	Lacto-fermented *P. ostreatus*
Butyric acid	C4:0	5.9 ^{c} ±0.7	6.8 ^{b} ±0.2	7.0 ^{a} ±0.0	4.0 ^{d} ±0.0
Caproic acid	C6:0	11.0 ^{c} ±2.6	4.8 ^{d} ±0.0	16.0 ^{a} ±2.8	13.0 ^{b} ±1.4
Caprylic acid	C8:0	4.0 ^{d} ±0.0	4.8 ^{c} ±0.0	8.6 ^{b} ±0.9	10.7 ^{a} ±1.2
Capric acid	C10:0	13.4 ^{a} ±0.8	10.1 ^{b} ±0.0	13.0 ^{a} ±1.7	9.4 ^{b} ±0.0
Lauric acid	C12:0	3.0 ^{c} ±0.1	13.0 ^{a} ±2.1	10.7 ^{b} ±2.5	10.4 ^{b} ±0.0
Myristic acid	C14:0	8.0 ^{a} ±0.4	5.0 ^{b} ±0.0	4.5 ^{b} ±0.2	7.0 ^{a} ±0.2
Myristoleic	C14:1	17.0 ^{a} ±3.0	9.4 ^{b} ±0.6	6.0 ^{c} ±0.0	17.3 ^{a} ±1.9
Palmitic acid	C16:0	6.0 ^{d} ±0.0	10.3 ^{c} ±1.5	23.0 ^{a} ±2.1	20.4 ^{b} ±0.8
Palmitoleic acid	C16:1	10.0 ^{d} ±1.1	21.0 ^{b} ±4.2	17.0 ^{c} ±1.1	22.2 ^{a} ±2.8
Margaric acid	C17:0	12.1 ^{a} ±2.6	8.5 ^{b} ±0.1	11.0 ^{a} ±0.9	3.2 ^{c} ±0.0
Stearic acid	C18:0	7.8 ^{b} ±0.3	6.8 ^{c} ±0.0	8.0 ^{b} ±0.0	14.2 ^{a} ±1.1
Oleic acid	C18:1	10.4 ^{c} ±0.6	9.4 ^{c} ±0.2	13.3 ^{b} ±0.9	21.8 ^{a} ±4.8
Linoleic	C18:2	8.3 ^{c} ±0.0	10.6 ^{c} ±1.0	12.6 ^{b} ±0.0	14.4 ^{a} ±1.8
Linolenic	C18:3	9.0 ^{c} ±0.0	15.9 ^{a} ±3.1	9.1 ^{c} ±0.3	11.1 ^{b} ±1.5
Behenic acid	C22:0	4.0 ^{a} ±0.1	2.8 ^{c} ±0.2	4.4 ^{a} ±0.0	3.2 ^{b} ±0.0
Erucic acid	C22:1	1.8 ^{bc} ±0.0	2.1 ^{a} ±0.0	1.6 ^{c} ±0.0	1.9 ^{a} ±0.0
Arachidonic acid	C22:4	3.7 ^{b} ±0.0	1.3 ^{c} ±0.0	6.0 ^{a} ±0.0	3.5 ^{b} ±0.3

Values are mean±SD of replicates (n=3)
Values with different alphabets along row are significantly different from each other when $p \leq 0.05$.
SD of 0.0 is <0.1.

Table 4. Total phenolic, flavonoid contents and FRAP of unfermented and lacto-fermented mushrooms

Extract	Phenol mg GAE/ g of extract	Flavonoid mg QE/g extract	FRAP mg AAE /g of extract
T. robustus	5.6 ^{b}±0.0	3.8 ^{b} ±0.0	72.8 ^{d} ±6.3
P. ostreatus	6.4 ^{b} ±0.0	3.1 ^{c} ±0.0	90.0 ^{c} ±8.1
T. robustus fermented with *L. fermentum*	7.1 ^{a} ±0.1	4.4 ^{ab} ±0.0	102.6 ^{b} ±4.7
P. ostreatus fermented with *L. fermentum*	7.8 ^{a} ±0.0	4.9 ^{a} ±0.1	121.8 ^{a} ±10.1

Values are mean±SD of replicates (n=3), Values with different alphabets along column are significantly different from each other when $p \leq 0.05$. SD of 0.0 is <0.1.

respectively (Figure 1). The scavenging activity of ethanolic extract from lacto-fermented *P. ostreatus* against DPPH were not significantly different ($p<0.05$) when compared to BHT. Jabłonska-Rys *et al.* [8, 51] reported that blanching reduced the phenolic content of edible mushrooms but fermented mushrooms with probiotic strains possessed higher level of phenolic compounds and often displayed better antioxidant activities. The scavenging activity of unfermented and lacto-fermented mushrooms could be attributed to the presence of phenolic compounds in the extracts. Medicinal mushrooms contain many polyphenolic compounds that are able to transfer electrons to free radicals and thereby scavenge free radicals [6]. The synergistic action of bioactive molecules in mushrooms have led to their emerging as pleasing sources of nutraceuticals, antioxidants, anticancer, prebiotic, immune-modulating, anti-inflammatory, anti-viral, antimicrobial, and antidiabetic [52].

The zones of inhibition displayed by ethanolic extracts of unfermented and lacto-fermented mushrooms are presented in Table 5. Extracts from lacto-fermented mushrooms exhibited better inhibition of 5.0±0.0 to 12.5±0.7 mm against tested microorganisms than extracts from unfermented mushrooms, which ranged from 5.5±0.0 to 8.4±0.1 mm. The inhibitory potential of studied extracts against the growth of Gram-negative, Gram-positive and fungi was of great interest. The ability of extracts to inhibit all tested organisms suggests that unfermented and lacto-fermented mushroom contain potential antimicrobial agents. This is an indication that mushrooms contain biologically active compounds that possess antimicrobial properties [53]. In addition, the pronounced inhibition displayed by lacto-fermented mushrooms could be linked to antagonistic metabolites, which are mainly organic acids, hydrogen peroxide, bacteriocins, and reuterin produced by *L. fermentum* [54]. Bacteriocins are small peptides (bioactive proteins) that are ribosomally synthesized by Gram-positive and Gram-negative bacteria with antimicrobial activity [55], and may exert bio-preservative effects on mushrooms. This is in accordance with the findings of Bintsis [56] who reported that bacteriocins produced by LAB during fermentation prevented food spoilage by inhibiting food pathogens. The health benefits of LAB-induced fermentation are attributed to the inhibition of enteropathogenic bacteria, the promotion of safe metabolic activity, improvements in palatability and acceptability of food products as a result of change in texture, flavour, and colour [57].

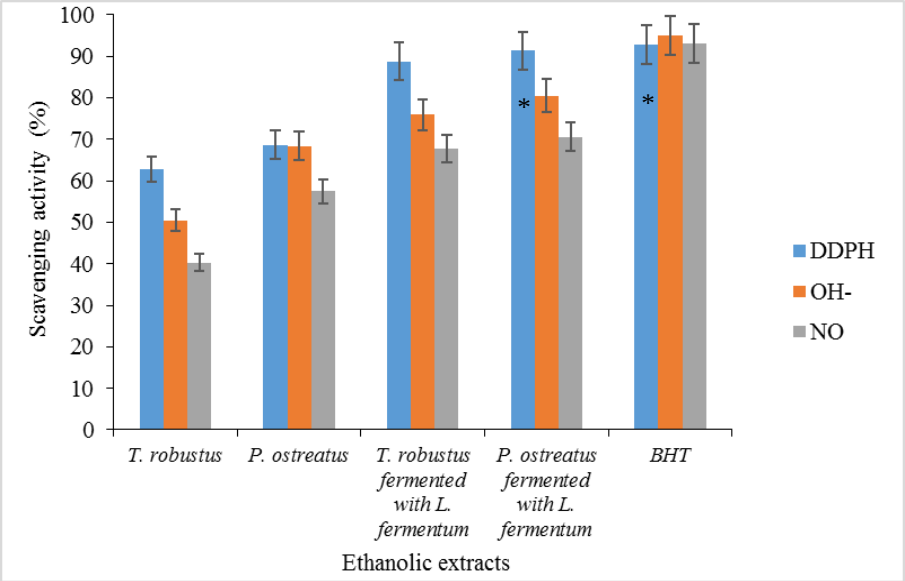

Figure 1. Scavenging activity of unfermented and lacto-fermented mushrooms at 1.0 mg/ml
* Indicates scavenging activity against DPPH were not significantly different from each other at p≤0.05. Error bar is SD.

Table 5. Zones of inhibition (mm) displayed by ethanolic extracts from unfermented and lacto-fermented mushrooms at 50 mg/ml

Extracts	**MRSA**	***E. coli***	***S. dysenteriae***	***P. aeruginosa***	***C. troplicalis***	***C. albicans***
T. robustus	7.4 [b] ±0.0	6.0 [c] ±0.0	8.0 [a] ±0.1	0.0	5.5 [c] ±0.0	0.0
P. ostreatus	8.0[a]±0.0	0.0	8.4[a]±0.1	0.0	0.0	0.0
T. robustus fermented with *L. fermentum*	12.5 [a] ±0.7	7.5 [b] ±0.1	10.0 [b] ±0.4	0.0	7.0 [b] ±0.0	6.0 [c] ±0.0
P. ostreatus fermented with *L. fermentum*	11.7 [a] ±0.9	5.0 [c] ±0.0	11.0 [a] ±1.8	0.0	9.5 [b] ±0.2	5.0 [c] ±0.0
amoxicillin / ketoconazole*	15.0 [a] ±1.2	10.6 [c] ±0.8	14.0 [a] ±2.1	8.0 [d] ±0.0	11.0 [b] ±0.0	12.2 [b] ±0.0

0.0: no zones of inhibition at 50 mg/ml, MRSA: Methicillin resistant *Staphylococcus aureus* and *tested against fungi. Values are mean±SD of replicates (n=3). Values with different alphabets along row are significantly different from each other when p≤0.05. SD of 0.0 is <0.1

4. Conclusions

Mushrooms fermented with *Lactobacillus fermentum* have the highest protein and ash contents. Presence of some essential amino acids and fatty acids as well as minerals in lacto-fermented mushrooms was higher than unfermented mushrooms. Hence, fermented mushrooms, as rich sources of proteins, amino acids, fatty acids, minerals, and antioxidants, can have positive effects on human health. *Lactobacillus fermentum* can be used as a starter culture to ferment edible mushrooms. The results in this study for free radical scavenging and antimicrobial activities indicate that lactic acid fermentation cannot reduce the bioactivity of edible mushroom.

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