PROXIMATE, MINERALS, PHYTOCHEMICALS, AMINO ACIDS, LIPIDS COMPOSITION AND SOME FOOD PROPERTIES OF THE SCLEROTIUM OF *PLEUROTUS TUBER-REGIUM* (RUMPH.EX FR.) SINGER1951)

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ABSTRACT

Proximate, minerals, phytochemicals, amino acids, lipidscomposition and some food properties were determined in the edible part of *Pleurotus tuber- regium*. In the proximate composition: protein,ash and carbohydrate were high. Energy due to proteinand utilizable energy due to protein were high.High levels wereobserved in Ca, Mg, K, Na and P; the Ca/P ratio was excellent. In the mineral safety index, P, Na and Ca were above the MSI by values of 79.0-94.0%.Total amino acid value was 93.6 g/100 g. On amino acid scores, *P. tuber-regium* was better than Gly, Ala, Pro, Val, Thr, Glu, Tyr and His under whole hen's egg comparison; under provisional amino acid scoring pattern, the sample was better than Val, Thr, Phe + Tyr and total amino acid (AA) concentration; under pre-school child comparison, sample was better concentrated in Val, Thr, Met +Cys, Phe +Tyr, His and total AA.Essential amino acid index was high at 90.3 and biological value was 86.7. The trend in the fatty acid composition was (% total FA): ΣMUFA > ΣPUFA > ΣSFA and< ΣUFA. MUFA/SFA (=1.93), PUFA/SFA (=1.83) and n-6/n-3 (=1.37). Phospholipids level was low;in sterols, only cholesterol was significant at 44.8 mg/100 g (about 100 %). These functional property values were high: water absorption capacity, oil absorption capacity, emulsion capacityand the lowest gelation concentration was 6.00 ±0.00 %. Protein solubility was high at both acid and base sides of the pH with pI value of pH 5.

Keywords: Chemical composition, functional properties, *Pleurotus tuber-regium*

No of Tables: 12 **No of Figures: 1** **No of Plate: 1** **References: 92**

INTRODUCTION

Pleurotus tuber-regium (Fr.) Sing. is a basidiomycete found in the tropical and subtropical regions of the world (Zoberi, 1993). It forms a large, spherical to ovoid, subterranean sclerotium, composed of fungus tissue, sometimes up to 30 cm (11.8 inches) or more diam. The sclerotium is dark brown on the outside and white inside (Plate 1).

P.tuber-regium initially infects dry wood where it produces the sclerotium which may become buried in the soil. The fungus is common in Nigeria where farmers usually lift the sclerotium out of soil or wood while cultivating their farm. The fungus is consumed popularly in Nigeria (Oso, 1977). *P. tuber-regium* as a fungus is found to grow on the drill dust of the wood of *Treculia africana* (bread fruit), *Elaeis guineensis* and *Daniella oliveri*tree (Olukoya and Okogbo, 1990).

Olukoya and Okogbo (1990) reported that when the sclerotium was grown on drill dust of *Eleais guineensis* and *Daniella oliveri* tree, the fungus produced sclerotia but on other substrates it produces fruit bodies with sclerotis. In nature, sclerotia typically form in response to adverse growing conditions as a methodof carrying the life of the fungus through difficult conditions. When the growth medium dries out or available nutrients are used up, the fungus responds by forming a scloretium (Olukoya and Okogbo, 1990).

The scientific classification of *P. tuber-regium* is as follows: Kingdom (Fungi), Phylum (Basidiomycota), Class (Agaricomycetes), Order (Agaricales), Family (Pleutotaceae), Genus (*Pleurotus*), Species (*P.tuber-regium*), Binomial name (*Pleurotus tuber-regium*) (Rumph.ex Fr.)Singer 1951. Synonyms are *Pachyma tuber-regium* Fr. 1822 and *Lentinus tuber-regium* (Fr.) Fr. 1836 (Wikipedia).

Agomuo (2011) had reported the proximate, phytochemical and mineral element analysis of the sclerotium of *Pleurotus tuber-regium*. This mushroom is used as a food source by many Nigerians but chemical composition information on it is scanty. In trying to bridge this gap of paucity of information, this research reports on the analytical determination of the proximate, minerals, phytochemicals, amino acids, lipids (fatty acids, phospholipids, sterols) composition and food properties of *Pleurotus tuber-regium* Sing.

MATERIALS AND METHODS

Collection and treatment of sample

The balls of the sclerotium of *Pleurotus tuber-regium* were purchased from Oba market in Akure and identified at the Taxonomy Unit of the Department of Plant Science, Ekiti State University,

Ado-Ekiti. The mushroom was washed, dried and milled into powder.

Proximate analysis

The proximate analysis of the powdered sample for crude fat, total ash, crude fibre and moisture was determined using the methods described by AOAC [2006]. Crude protein was determined by the method described by Pearson [1976] while carbohydrate was determined by difference. The calorific value in kilojoule (kJ) was calculated by multiplying the crude fat, crude protein and carbohydrate by Atwater factor of 37, 17 and 17 respectively or in Calories by multiplication with 9, 4 and 4 respectively. Determinations were in duplicate.

Mineral analysis

The mineralswere analysed from the solution obtained by first dry ashing the sample at 550^0 C. The mineral analysis was performed using atomic absorption spectrophotometer (Buck Scientific Model-200 A/210, Norwalk, Connecticut 06855) and phosphorus was determined colorimetrically by Spectronic 20 (Gallenkam, UK) using phosphovanado molybdate method (AOAC, 2006). Ca/P, Na/K, Ca/Mg and the milliequivalent ratio [K/(Ca +Mg)] (Hathcock, 1985); the mineral safety index (MSI) (Hathcock, 1985) of Ca, P, Na, Fe, Cu, Zn, Mg and Se were also calculated.

Phytochemical analysis

The phytochemical analysis (tannins,total phenol, phytic acid, oxalate, alkaloids, flavonoid and saponins) was carried out using the methods described by (AOAC, 2006). All determinations were in duplicate. All chemicals used were of analytical grade obtained from British Drug House (BDH, London, UK).

Amino acid analysis

Amino acid analysis was done by ion-exchange chromatography (FAO/WHO, 1991) using the Technicon Sequential Multisample Amino Acid Analyzer (TSM) (Technicon Instruments Corporation, New York). The dried sample was defatted, hydrolysed, filtered to remove humins and evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5 mL of acetate buffer (pH 2.0) and stored in a plastic specimen bottle kept inside the deep freezer pending subsequent analysis. The TSM is designed to separate free acidic, neutral and basic acids of the hydrolysate. The amount loaded for the sample was 5-10 µL and about 76 min elapsed for the analysis. The column flow rate was 0.50 mL/min at 60 °C with reproducibility consistent within ± 3 %. The net height of each peak produced by the chart of the TSM was measured and calculated for the amino acid it represented. All chemicals were of analytical grade.

Norleucine was used as internal standard.

From the amino acid data, other calculations were made.

The isoelectric point (pI) was calculated (Olaofe and Akintayo, 2000); calculation of predicted protein efficiency ratio (C-PER or P-PER) (Alsmeyer et al., 1974); leucine/isoleucine ratio, their differences and their percentage differences were calculated; estimation of essential amino acid index (EAAI) (Oser, 1959) using the egg protein amino acids as the standard; estimation of biological value (BV) following the equation of Oser (1959):

Biological value = 1.09 (EAAI) - 11.73

computation of Lys/Trp and Met/Trp ratios; computation of amino acid scores using three different procedures: scores based on amino acid values compared with whole hen's egg amino acid profile (Paul and Southgate, 1978), scores based on essential amino acid scoring pattern (FAO/WHO, 1973) and scores based on essential amino acid suggested pattern of requirements for pre-school child (FAO/WHO/UNU, 1985); distribution of amino acids into groups (Nieman et al., 1992).

Preparation of methyl ester and analysis

The extracted fat (50 mg) was saponified for 5 min at 95 °C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralised by 0.7 M HCl. A volume of 3 mL of 14 % BF_3 in methanol (Supelco Inc., Bellefonte, PA, USA) was added (AOAC, 2006). The mixture was heated for 5 min at 90 °C to achieve complete methylation. All the fatty acid methyl esters (FAME) were extracted into redistilled n-hexane (2x3 mL). The content was concentrated to 1mL for analysis and 1 µL was injected into the injection pot of the GC. The FAME was analysed using these GC conditions: (GC; HP 5890 Series II, autosampler 7673, powered with HP 3365 ChemStationrev. A09.01[1206] software; Hewlett-Packard Co., Avondale, PA, USA) fitted with a flame ionization detector. Split injection type used, split ratio was 20:1 and carrier gas was nitrogen. Inlet temperature was 250 °C, column type was HP INNOWAX capillary column (30 m, 0.25 mmid, 0.25 µm film thickness) (Supelco, Inc. Bellefonte, PA, USA). The oven programme was: initial temperature at 60 °C, first ramping at 10 °C/min for 20 min (260 °C), maintained for 4 min; second ramping at 15 °C/min for 4 min (320 °C), maintained for 10 min. Flame ionization detector temperature was 320 °C. Hydrogen pressure was 22 psi and compressed air was 35 psi. The peaks were identified by comparison

of their retention times with authentic standards of FAME.

Sterol analysis

For the analysis of sterols, the gas chromatographic conditions of analysis were similar to the GC conditions for the methyl esters analysis.

Phospholipids analyses

Modified method of Raheja et al. (1973) was employed in the analysis of phospholipids. A weight of 0.01 g of the extracted fat was added to the test tube. To ensure complete dryness of the oil for phospholipids analyses, the solvent was completely removed by passing a stream of nitrogen gas on the oil. A volume of 0.40 mL of chloroform was added to the tube followed by the addition of 0.10 mL of chromogenic solution. The tube was heated at 100 °C in water bath for about 1 min and 20 sec. The content was allowed to cool to the laboratory temperature and 5 mL of hexane was added and the tube shaken gently several times. The solvent and the aqueous layer were allowed to separate. The hexane layer was recovered and concentrated to 1.0 mL for analysis. The phospholipids were analysed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. A09.01 [1206] software [GMI, Inc., Minnesota, USA] fitted with a pulse flame photometric detector (PFPD). Nitrogen was used as the carrier gas with a flow rate of 20-60 mL/min. The oven programme was: initial temperature at 50 °C, first ramping at 10 °C/min for 20 min (250 °C), maintianed for 4 min, second ramping at 15 °C/min for 4 min (310 °C) and maintained for 5 min. The injection temperature was 250 °C whilst the detector temperature was 320 °C. A polar (HP5) capillary column (30 m, 0.25 mm id, 0.25 µm film thickness) was used to separate the phospholipids. The split injection type used had a split ratio of 20:1. Hydrogen pressure was 20 psi and compressed air was 30 psi. The peaks were identified by comparison with standard phospholipids.

Quality assurance

For the purpose of ensuring the accuracy of the results obtained for the various lipid parameters, the followings were carried out: standard chromotographs were prepared for sterols, phospholipids and fatty acids methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient determination for each fatty acid parameter (32), same for sterols (7) and phospholipids (5). Correlation coefficient should be ≥ 0.95 for the result to be acceptable. It is a statistical index that shows the quality assurance of the calibration curve

performed. It was performed with the Hewlett-Packard Chemistry (HPCHEM) software. Fatty acids were listed with the chain length and double bond members.

Functional properties

Protein solubility

The solubility of the sample as a function of pH was determined using the method described by Sathe et al. (1982). The pH was checked and adjusted then centrifuged at 4000 rpm for 20 min at room temperature and the nitrogen in the supernatant or in aliquot (2.0 mL) of the clear supernatant was estimated by the micro Kjeldahl method (AACC, 1983).

Water and oil absorption capacity

Water and oil absorption capacity of the sample was determined according to the method described by Beuchat (1977).

Emulsifying capacity

Emulsifying capacity was determined according to the procedure of Beuchat et al. (1975). Emulsifying capacity was calculated as follows:

$$\text{Emulsifying capacity} = \frac{\text{Weight of oil emulsified}}{\text{Weight of sample taken}}$$

where weight of oil emulsified = Total volume of oil emulsified x Specific gravity of oil used.

Gelation capacity

Least gelation concentration was determined using the method of Coffmann and Garcia (1977).

RESULTS AND DISCUSSION

Proximate composition

Table 1 presents the proximate composition of the sample. These parameters were on the high side in the sample: crude protein, carbohydrate, total ash and metabolizable energy. Not shown in Table 1 was the organic matter which was high at 70.8 g/100 g. The crude protein of 19.2 g/100 g was close to the value of 19.6 g/100 g (dry weight) in the cotyledon of *Irvingia gabonensis*(Adeyeye, 2013); same could be said for *I. gabonensis* in the moisture content since this sample had a value of4.39 g/100 g. The value of moisture content of 41.6 % for sclerotium of *P. tuber-regium* obtained by Agomuo (2011) might be because his sample was not fully ripe by the time of collection. *P. tuber-regium* normally contains low level of moisture when fully ripe. The high total ash would ensure high mineral content in the sample. The energy content of 1148 kJ/100 g (270 kcal/100 g) showed the sample to be a good source of concentrated energy due to its high protein and carbohydrate contents. Energy from cereals range from 1.3-1.6 MJ/100 g (Paul and Southgate, 1978).

Table 1 also shows the various energy values as contributed by protein, fat and carbohydrate. Whilst the fat had

the least contribution (2.68 %), carbohydrate contributed the highest level of 68.9 %. The fat contribution of 2.68 % fell far short of the 30 % recommended energy from fat particularly for adults (Davies and Dickerson, 1991); this makes the sample good for heart problem patients. The utilizable energy due to protein (UEDP %) for the sample (assuming 60 % utilization)was 17.0. This value is higher than the recommended safe level of 8 % for an adult man who requires about 55 g protein per day with 60 % utilization. However, 19.2 g/100 g would just be 34.9 % of 55 g adult requirement per day.

It is well known that water is indispensable for the efficient utilization and conservations of food within the body (Snively and Wessner, 1954). The most important connection of water with other food substances is the fact that the water content of the body changes with the type of diet (White House Conferences, 1932). The biochemical basis for this relationship arises from the fact that the water deficit created by protein metabolism is about seven times that for equivalent calories of carbohydrate or fat. Hence, it is not surprising to find in studies with experimental animals and young children that an increase in calories from carbohydrate causes hydration; whereas an increase in calories from proteins causes dehydration (Pratt andSnyderman, 1953). Albanese (1959) had given values of grams of water needed for complete metabolism of 100 calories of some food substances: food material: protein, starch and fat (preformed water = 0.00); gained by oxidation: protein (10.3), starch (13.9)and fat (11.9); lost in dissipating heat: 60 for all (protein, fat and starch); lost in excreting end products: protein (300), both starch and fat (0.00); deficit, protein (350), starch (46) and fat (48). One calorie of protein requires 3.0 mL of water for the excretion of the urea and sulphate formed from it; 1 g of ash requires 65 mL of water for its excretion(Albanese, 1959). Assuming complete metabolism of 76.8 Calories (kcalories) of protein, 230 mLof water would be required for the samples. This water loss can be offset by the high carbohydrate content of the samples or from water intake.

Mineral composition and mineral safety index (MSI)

The mineral levels of the sample are all shown in Table 2. The sample contained sufficient quantities of these minerals to meet nutritional requirement of man and animals(mg/100 g): Ca (12445), Mg (685), K (211), Na(987), P(2146). The values of Mn, Cu, Ni and Fe were lower than in the report of Agomuo (2011)in sclerotium of *P. tuber-regium*. The lower level of K compared to Na was not in agreement with what is

required in plants (Sutcliffe and Baker, 1974). Fe, Cu, Zn were all lower than the RDA levels (NRC, 1989). Pb (0.002), Cd (< 0.001) were low, this is good for a food source and their presence could be due to onset of pollution of the environment. Both Na and K are involved in many biochemical activities in the body but the Na/K value of 4.68 was much higher than 0.60 required to avoiding high blood pressure (Nieman et al.,1992). The value of Ca/P (5.80) showed that the sample had an excellent combination of Ca and P since the value of Ca/P (7.79-2.42) falls within the excellent group (Hathcock, 1985). The value of 18.2 for Ca/Mg is above the minimum level of 1.0 (NRC, 1989). The value of 0.032 for [K/(Ca +Mg)] milliequivalent was far less than 2.2. To prevent hypomagnesemia, Marten and Andersen (1975) reported that the milliequivalent of [K/(Ca +Mg)] must be < 2.2; this is the case in this report. Mn was low as it obtains in some animal sources (Adeyeye, 1996).

In Table 3, values of mineral safety index (MSI) showed the values of Ca, P and Na to be higher than their corresponding standard values. Those MSI levels less than the standards came from Fe, Cu, Zn, Mg and Se. The standard MSI for the elements are Na (4.8), Mg (15), P(10), Ca (10), Fe (6.7), Zn (33) and Se (14). The explanation of the MSI can be understood as follows taking Ca as example: the recommended adult intake (RAI) of Ca is1200 mg, its minimum toxic dose (MTD) is 12000 mg or 10 times the recommended daily average (RDA) which is equivalent to MSI of Ca. This reasoning goes for the other minerals whose MSI was determined. Minerals whose MSI values were higher than the Table MSI (TV) had calculated values (CV) of Ca (104 >10 TV), P (17.9 >10 TV) and Na (9.47 >4.80). The Ca CV > TV by a value of 10-104 or -94 (940 % difference), etc. For Na where CV>TV by a value of 4.80-9.47 or -4.67 (97.3 % difference), this meant that 9.47 times the RDA, hence more K would have to be consumed to compensate for the high Na intake from the sample. It is interesting to note similar values of 9.47 (CV) obtained in this result was also noticed in *Callinectes latimanus* (a commercially important crab occurring along the East and Gulf coasts of the USA) (Adeyeye et al.,2014). The following minerals have their TV > CV: Fe, Cu, Zn, Mg, Se giving positive differences with corresponding lower percentage difference having range values of 61.0-90.3. High levels of Ca and P might not cause deleterious diseases unlike Na. The general trends in MSI values of *P. tuber-regium* followed similar trend as seen in *Callinectes latimanus* (Adeyeye et al.,2014).

Antinutrient factors

Some anti-nutrient factors are shown in Table 4. All the anti-nutrient values

were very low and ND was even reported in saponin. In sclerotium of *P.tuber-regium*, Agomuo (2011) reported the following phytochemical levels (mg/100 g): saponins (0.14 ±0.05), tannins (1.75±0.01) and alkaloids (1.25±0.02). Tannins may decrease protein quality by decreasing digestibility and palatability (Butter, 1989). The tannin content of the sample was 0.060 mg/100 g (dw) and lower than other foods like lima beans (*Phaseolus lunatus*), 0.59 mg/100 g (Egbe and Akinyede, 1990); black specie of cowpea (*Vigna unguiculata*), 0.78 mg/100 g (Ologbobo and Fetuga, 1983). Saponins have been shown to possess a hemolytic effect on red blood cells. It also has cytotoxic effects and beneficial in its cholesterol lowering ability (Price et al., 1987). The alkaloids value of 0.040 mg/100 g was also lower than those of the species of *I. tetraptera* (0.70 mg/100 g), *M. myristica*(0.50 mg/100 g) and *Piper guineensis* (0.55 mg/100g (dw)(Agomuo, 2008). The oxalate result of 1.01 mg/100 g in *P. tuber-regium* was lower than (mg/100 g) in hull (1.98) and cotyledon (1.56) in *I. gabonensis*(Adeyeye, 2013). The presence of oxalate has undersirable effect on Ca absorption and utilization, its acid combines with Ca to form calcium oxalate, which passes through the intestineunabsorbed. The amount of oxalate formed depends on the amount of oxalic acid in the food (Fleck, 1976); the oxalate level in this sample was low. The flavonoid pigments are water-soluble, they are found dissolved in the cell-sap water. A subgroup called anthocyanins is responsible for reds, blues and violetsfound in a wide variety of fruits (Ihekoronye and Ngoddy, 1985). Flavonoids had a low value of 0.010 g/100 g in the sample. Total phenol in the sample was 0.030 mg/100 g; this is a heat-stable anti-nutritional factor which is not eliminated by simple soaking and heating but through germination or fermentation. The phytic acid level was 0.295 ±0.021 mg/100 g in the sample. The phytochemicals analysed for in *P. tuber-regium* had values below the established toxic levels(Nkafamiya et al., 2010).

Amino acids profile

The amino acids profile of *P. tuber-regium* are shown in Table 5. High levels of amino acid (AA) are seen in Gly, Ala, Ser, Pro, Val, Thr, Asp, Glu, Tyr and Arg. The total AA in *P. tuber-regium* was 93.6 g/100 g being greater than in *Pandalus borealis*: whole organism (92.7 g/100 g), endoskeleton (86.6 g/100 g) and exoskeleton (93.0 g/100 g) (Adeyeye, 2015) and *Callinectes latimanus* (94.5 g/100 g) (Adeyeye et al., 2014); both *P. borealis* and *C. latimanus* are shell fishes. The Lys content in this sample was 3.72 g/100 g being greater than 2.55 g/100 g in *C.latimanus* and 2.46-3.34 g/100 g

in *P. borealis*. Also the protein of *P. tuber-regium* (19.2 g/100 g) was higher than in *C. latimanus* (19.1 g/100 g) and *P. borealis* (17.2-19.1 g/100 g). Lys, Arg, Val, Met were higher in *P. tuber-regium* than in *C. latimanus* whereas Val, Lys, Met and Arg were more concentrated in *P. tuber-regium* than in *P. borealis*. In the scores determined with comparison to the whole hen's egg AA values, score values > 1.0 were in Gly, Ala, Pro, Val, Thr, Glu, Tyr and His; Cys being the limiting AA (0.25). In FAO/WHO (1973)comparison, Val, Thr, Phe+Tyr and total essential AA (EAA) had score values > 1.0 in each case. In comparison with pre-school child (2-5 years), these EAA had score values > 1.0: Val, Thr, Met + Cys, Phe +Tyr, His and total EAA. Whilst Ile (0.55) was limiting in the provisional amino acid scoring pattern, it was Trp (0.54) in pre-school child comparison. Gly recorded the highest score (1.93) in the egg comparison, Phe +Tyr recorded the highest score (1.78) in the provisional EAA score whereas Val recorded the highest score (2.26) in the pre-school comparison. To make corrections for the limiting amino acid (LAA) in the sample if it serves as sole source of protein food therefore, it would be 100/25.0 (or 4.0) x protein of sample (in hen's egg comparison), 100/55 (or 1.82) x protein of sample (provisional essential amino acid comparison) and 100/54 (or 1.85) x protein of sample (pre-school essential amino acid comparison).

Some amino acid quality parameters are shown in Table 6. The following values would show the position of the quality of the fungus sample: the EAA requirements across board are (values with His) (g/100 g crude protein cp): infant (46.0), pre-school (2-5 y) (33.9), school child (10-12 y) (24.1) and adult (12.7) and without His: infant (43.4), pre-school (32.0), school child (22.2) and adult (11.1) (FAO/WHO/UNU, 1985). From the present result based on these standards, we have EAA of 41.6 g/100 g (with His) and 38.0 g/100 g cp (no His). These results are not too far from the following literature values of the total EAA: egg, 51.2 (with His) and 49.0 (no His); cow's milk, 50.4 (with His) and 47.7 (no His); beef, 47.9 (with His) and 44.5 (no His) (FAO/WHO/UNU, 1985). The total sulphur AA (TSAA) of the sample was 2.66 g/100 g cp which is about one-half of the 5.8 g/100 g cp recommended for infants (FAO/WHO/UNU, 1985). The aromatic AA (ArAA) range suggested for infant protein (6.8-11.8 g/100 g cp) has the present value of 8.57 g/100 g cp being within the range. The percentage ratio of EAA to the total AA (TAA) in the sample was 44.4. This value is well above the 39 % considered adequate for ideal protein food for infants, 26 % for children and 11 % for adults (FAO/WHO/UNU, 1985). The EAA/TAA of 44.4 % was close to the value of 42.9-44.0 reported for the whole organism, flesh and shell of *Pandalus borealis*(Adeyeye, 2015) and 45.1 % in

Callinectes latimanus(Adeyeye et al.,2014). The percentage of total neutral AA (TNAA) result was 57.5 g/100 g indicating that it formed the bulk of the AA.

The other calculated parameters from the AA profile are further shown in Table 6. The predicted protein efficiency ratio (P-PER) were: $P\text{-}PER_1$ (1.10) and $P\text{-}PER_2$ (1.31). This may lead to low level of physiological utilization of the protein. In general, it has been found that the better the protein, the lower the level in the diet required to produce the highest P-PER. This is clear reflection of the importance of the proper nutritive balance of all of the amino acids to produce optimum metabolic efficiency. The Leu/Ile ratio was low at 2.23, hence no concentration antagonism might be experienced in the sample when consumed as protein source in food because 2.36 is the most ideal Leu/Ile (FAO/WHO, 1991). The essential amino acid index (EAAI) of 90.3 and its corresponding biological value (BV) of 86.7 depicted the high quality of the protein of *P.tuber-regium*. This is shown in the literature comparison: milk, cow (whole, non fat, evaporated, or dry), EAAI (88) and BV (84, predicted; 90, observed); human, EAAI (87) and BV (83); eggs, chicken (whole, raw or dried),EAAI (100), BV (97, predicted; 96, observed); whites (raw or dried), EAAI (95), BV (92, predicted; 93, observed); yolks (raw or dried), EAAI (93), BV (89, predicted); shellfish (shrimp, including prawns, raw or canned), EAAI (67), BV (61, predicted) (Oser, 1959). These literature results show the quality position of *P. tuber-regium* under discussion. The isoelectric point, pI was 5.40 showing the sample to be in the acidic medium of the pH range. Actually the minimum protein solubility of the sample was at a pH of 5.0 (Fig. I) showing a very close relationship with the pI of 5.40 between the observed and the predicted.

The work of Mitchell (1959) showed that there exists good agreements of growth needs and tissue AA patterns. This agreement is particularly good for the Lys/Trp (L/T) and Met/Trp (M/T) ratios of muscle proteins which constitute approximately 75 % of the infant body proteins. This present result had L/T of 6.35 and M/T of 3.77. Mammalian tissue patterns have the following values: L/T, muscle (6.3), viscera (5.3), plasma proteins (6.2); M/T, muscle (2.5), viscera (2.0), plasma proteins (1.1) (Mitchell, 1959). The available evidence indicates that the utilization of dietary proteins increases as their Lys and Trp content approaches that of muscle tissues. This concept gains further validity from the fact that the nutritional value of some protein products with low Lys/Trp values can be enhanced by small additions of Lys. In the present study, the Lys/Trp of 6.35 was about of similar value with that of the muscle (6.3) whereas the Met/Trp of 3.77 was far

more than 2.5 in the muscle. It has also been suggested that for optimal protein synthesis, the optimal dietary ratio of Phe and Tyr in mass units is 60:40 (Pencharz et al., 2007). A reverse order was observed in this report with Phe: Tyr ratio being 40.8: 59.2. Most animal proteins are low in Cys, for example, we have literature values of Cys/TSAA % as: three different Nigeria fishes (23.8-30.1) (Adeyeye, 2009a); male fresh water crab body parts (13.3-15.9) (Adeyeye and Kenni, 2008); female fresh water crab body parts (27.3-32.8) (Adeyeye, 2008a). The present Cys/TSAA % was 16.9 showing the sample behaving like an animal protein. In contrast, many vegetable proteins contain substantially more Cys than Met, examples (Cys/TSAA) %: 62.9 in coconut endosperm (Adeyeye, 2004); *Anacardium occidentale*, 50.5 (Adeyeye et al., 2007), 58.9 -72.0 (raw, steeped, germinated sorghum) (Adeyeye, 2008b); 51.2-53.1 (raw, steeped, germinated millet) (Adeyeye, 2009b).

The various amino acid class groups are shown in Table 7. The concentration trend of the classes could be seen to follow as shown in g/100 g cp: class I (27.8) > class IV (22.5)> class VI (14.9) > class II (11.1) > class VII (5.28) > class III (2.66). It could also be seen that the percentage values were close to their individual principal values, e.g. value (percentage): class I, 27.8 (29.7); class II, 11.1 (11.9); class III, 2.66 (2.84);class IV, 22.5 (24.0); class V, 13.0 (13.9); class VI, 14.9 (15.9) and class VII, 5.28 (5.64). The percentage levels were close, ranging from 2.84-29.7. Literature values of this nature had been found in *Callinectes latimanus* (a lagoon crab) (Adeyeye et al.,2014) and *Pandalus borealis* (a lagoon shrimp) (Adeyeye, 2015).

Fatty acids profile

In Table 8 we have the fatty acids (FAs) profile of *P. tuber-regium* (in % of total fatty acids). In the saturated FAs, only C16:0 and C18:0 were of significant values in the sample: % total fatty acids, C16:0 (12.6) and C18:0 (8.17). The total saturated fatty acid(SFA) value was 21.0 %. Both 16:0 and 18:0 belong to the long-chain fatty acids group of 14-18 carbon atoms per molecule. Palmitic acid is usually considered the most abundant SFA in nature. It varies from 10-40 % in seed oils; however, the bull's head and hen's head brains contain no detectable level of 16:0 (Adeyeye, 2012). Stearic acid was also found as the second highest SFA in the sample, this is the usual trend in FAs. Considering the influence of 16:0 and 18:0 on the lipoprotein profile, 18:0 is said to be neutral in its effect on blood cholesterol when consumed in natural fats; 16:0 is intermediate, that is, it can be neutral when placed on a triglyceride molecule with monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) or

18:0, or cholesterol-raising when attached along with 12:0 +14:0. In high amounts, 16:0 can even raise total cholestrol (TC) and low density lipoprotein (LDL) when substituted for 18:0, MUFA or PUFA in people who already have elevated TC or who eat large amounts of cholesterol (Hayes, 2002). The SFA in the present sample was of low level (21.0 % total FAs). The total monounsaturated fatty acids (MUFAs) was 40.6 % made up mainly by C16:1cis-9 (6.99 %), C18:1 cis-6 (4.45 %), C18:1 cis-9 (13.6) and C20:1 cis-11 (15.6). The gadoleic acid-trivial name for cis-9-enoic acid (20:1 n-11) is known to be present in marine oils (from fish or sea mammals) (Beare-Roger et al., 2001), it was the most concentrated of the MUFAs, although C16:1 cis-9 and C18:1 cis-9 used to be the most common MUFAs. Minus MUFAs being obtained from the diet, MUFA can also be synthesized by elongase and desaturase enzymes from SFAs primarily derived from de novo lipogenesis (Mashek and Wu, 2015). 18:1 cis-9 is present in olive oil (about 78 %) and it is believed to have especially valuable nutritional properties as part of the Mediterranean diet; it is the biosynthetic precursor of a family of FA with the (n-9) terminal structure and with chain- lengths of 20-24 or more. Petoselinic acid (18:1 cis-6) occurs up to the level of 50 % or more in the seeds of Umbelliferae family (carrot, parsely and coriander). Palmitoleic acid (16:1 cis-9)has strong antimicrobial properties (Enig and Fallon, 2000); beneficial in reducing bad cholesterol (LDL) (Nestle et al., 1994); reduces fat deposition in blood vessels and reduces blood clot formation (Grundy, 1994).

The C18 FAs may be elongated and desaturated in adipose tissue to produce long chain FAs (C22 and C20), which are beneficial for human health (Burge, 2002) using molecular oxygen and a reduced pyridine nucleotide (NADH or NADPH) as cofactors.

9-18:1 → 11-20:1 → 13-22:1 → 15-24:1 → etc

18:1(n-9) 20:1(n-9) 22:1(n-9) 24:1(n-9)

9-16:1 → 11-18:1 → 13-20:1 → 15-22:1 → etc

16:1(n-7) 18:1(n-7) 20:1(n-7) 22:1(n-7)

18:1 cis-6 can also be synthesised from 16:0 in the seeds of Umbelliferae:

16:0 desaturation 4-16:1 elongation 6-18:1(petroselinic acid)

In the n-6 PUFA FAs, linoleic acid (16.2 %), arachidonic acid (5.53 %) and docosadienoic acid (0.344 %) were of significant levels making the Σ n-6 PUFA of 22.2 %. Linoleic acid is required for the biosynthesis of

arachidonic acid, the precursor of the eicosanoids:

Linoleic → Arachidonic →Eicosanoids[prostaglandins, leukotrienes, thromboxanes]
(from diet) acid

The eicosanoids regulate blood clotting, the inflammatory response, the reproductive system, the gastrointestinal tract, the kidneys and the respiratory tract (Candela Gómez et al., 2011).

The significant levels of n-3 PUFA were observed in timnodonic acid, EPA (5.31 %) and cervonic acid, DHA (10.9 %) having Σ n-3 PUFA of 16.2 %. These FAs belong to the very-long-chain FAs and both are essential. Some people can make these FAs from EFAs (particularly from a-LA or C18:3 n-3), but others, particularly those whose ancestors ate a lot of fish, lack enzymes to produce them (Enig and Fallon, 2000). This sample contained a non important value of 0.000051 % for a-linolenic acid making the formation of EPA and DHA difficult from it. The "obligate carnivores" must obtain EPA and DHA from food sources and *P. tuber-regium* could be part of such food sources. The nutritional importance of EPA and DHA had earlier been highlighted (Laugharne, 1996).

The highlights of calculated quality parameters of the FAs composition can be seen in Table 9. The PUFA/SFA (P/S) was good at 1.83. The P/S is important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the dietary oil because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by SFA and PUFA fats (Honatra, 1974). The MUFA/SFA level was 1.93 which was more in favour of MUFA than SFA; it had a good comparison with P/S. cis-Monoenoic acids have desirable physical properties for membrane lipids in that they are liquid at body temperature, yet are relatively resistant to oxidation. Metabolites of n-6 are significantly more inflammatory [especially arachidonic acid(AA)] than those of n-3. This necessitates that n-3 and n-6 are consumed in balanced proportion, healthy ratios of n-6:n-3 range from 1:1 to 4:1 (Tribole, 2007). The n-6/n-3 value in the sample fell within the above range, it had a value of 1.37. The essential PUFA status index (EPSI) was 0.946 which was above average; the higher the EPSI, the better is the PUFA status (Benatti et al.,2004).The EPA/DHA was almost at about 50 % having a value of 0.487. No mead acid [20:3 n-9, cis-5, 8, 11] was detected in the sample implicating no general shortage of essential PUFA in the sample.

Phospholipidscomposition

The various phospholipids levels of the sample are shown in Table 10. The various phospholipids were generally low in value with a range of3.82 e-2 to 45.0 mg/100 g or 93.5 mg/100 g total weight value. As a constituent of all cells, phospholipids are also present at different concentrations in various foods. Phospholipids are used in the food industry as emulsifier or emulsion stabilizer; have positive effects on human health. Diverse beneficial health effects have been ascribed to the whole phospholipid mixture as well as to individual phospholipids. Gurr (1999) and Schneider (2001) had insisted that phospholipids exhibit well-documented nutritional and/or therapeutic benefits. Phospholipids account for approximately 20-25 % of the dry weight of an adult brain. Besides forming the backbone of the biomembrane, they also provide the dynamic membrane with a suitable environment, fluidity and ion permeability that affect cognition positively (Farooqui et al.,1988). The low value of total phospholipids in the sample (93.5 mg/100 g) was much higher than in the fast-food samples consumed in Nigeria with values of meat pie (22.9 mg/100 g), doughnut (27.1 mg/100 g) and cake (16.6 mg/100 g) (Adeyeye and Agesin, 2015).

The amounts of the various phospholipids in a membrane define the fluidity of the membrane and, consequently, the functions of the embedded proteins (Vance, 2008). Phosphatidylcholine (PC) is the most abundant phospholipid in mammalian cell membranes, comprising 40-50 % of total phospholipids; it was highest in the present sample 48.1%. The second most abundant mammalian membranephospholipid is phosphatidylethanolamine, which comprises 20-50 % of total phospholipids; this is not the same in the present sample as PE (cephalin) formed the third position at 20.3 mg/100 g (21.7 %) unlike in meat pie and doughnut that had PE in the second position in the phospholipid (Adeyeye and Agesin, 2015). In the brain, ~45 % of total phospholipids are PE whereas PE is only ~20 % in the liver (Vance, 2008). Phosphatidylserine (PS) was second (26.7 mg/100 g, 28.8 %) in the sample. There is a strong metabolic inter-relationships among PS, PE and PC (Vance, 2008). The relatively minor mammalian membrane phospholipids include phosphatidylinositol (PI) and lysophosphatidylcholine (LPC), as also demonstrated in *Pleurotus tuber-regium* with values of PI as 1.22 mg/100 g (1.31 %) and LPC as 3.82 e-2 mg/100 g (4.08e-2 %).

Sterol composition

In the sterol analysis results as shown in Table 11, only cholesterol was detected to a reasonable level of 44.8 mg/100 g (about 100 %). Other sterol values ranged from 0.00-9.21 e-3

mg/100 g. There are individuals who are sensitive to dietary cholesterol (Reiser and Shorland, 1990) and most authorities advise a general reduction in cholesterol intake for everyone. Bender (1992) had reported that dietary cholesterol should be reduced to around 300 mg or less per day; this is much higher than the sample cholesterol level (44.8 mg/100 g or 14.8 % of 300 mg/100 g).

Functional properties

Table 12 contains some functional properties of *Pleurotus tuber-regium*. The water absorption capacity (WAC) was high at 136 %. WAC is a critical function of protein of various food products like soups, gravies, doughs and baked products(Sosulski et al., 1976). *P. tuber-regium*flour could be useful in these functions. The WAC value in the sample was highly comparable to WAC in African yam bean (AYB) seed flours having 119-135 % in whole seed flours and 131-179 % in dehulled seed flours respectively (Oshodi et al., 1997]. The present value compared favourably with WAC reported for some seeds by Lin et al. (1974) and Adeyeye et al.(1994), but lower than the values reported for three varieties of melon (Ige et al., 1984). The oil absorption capacity (OAC) value was 97.8 %; this was comparable to 101-132 % for whole seed flours and 93.3-146 % for dehulled seed flours of AYB. The value of 97.8 % was higher than the values for pigeon pea flour (89.7 %) (Oshodi and Ekperigin, 1989), wheat flour and soy flour (84.2 %) and 84.4 % respectively (Lin et al., 1974), 82.3-91.5 % for three varieties of lima bean flours (Oshodi and Adeladun, 1993). The present result was lower than in the values of OAC in mucuna bean flours with value of 2.00-2.40 g/g for full fat and 2.10-2.60 g/g in defatted samples (Adebowale et al., 2005). Liquid retention is an index of the ability of proteins to absorb and retain oil/water which inturn influences the texture and mouth feel characteristics of foods and food products like comminuted meats, extenders or analogues and baked dough (Okezie and Bello, 1988). *Pletorus tuber-regium* would therefore be useful as a flavour retainer in some food products. The high values of WAC and OAC in *P. tuber-regium* could be attributed to the high value of crude protein (19.2 g/100 g). The extent of protein hydration correlates strongly with the content of polar residues and charge residues. Interaction between water molecules and hydrophilic groups occurs via hydrogen bonding (Chou and Morr, 1979). The high value of protein in the sample might be responsible for high hydrogen bonding and high electrostatic repulsion, both conditions facilitating binding and entrapment of water (Altschul and Wilcke, 1985). The high fat absorption in the sample flour is also closely related to protein content (Wolf and Cowan, 1977), the binding capacity

could have been enhanced by protein denaturation.

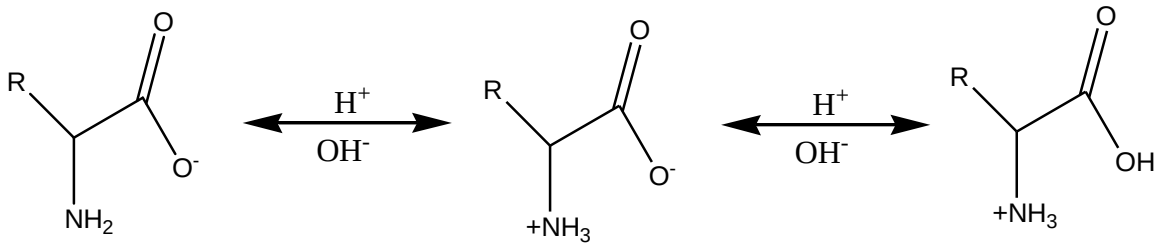
The oil emulsion capacity (OEC) value was 35.5 %. This is lower to the value of 40.0-90.0 % in whole seed flours of AYB but better than 10.0-20.0 % for dehulled seed flours of AYB (Oshodi et al., 1997). The present OEC (35.5 %) was also lower than 95.1 % in sunflower flour (Lin et al., 1974), 49.4 % for pigeon pea (Oshodi and Ekperigin, 1989) but higher than 7.00-11.0 % for wheat flour, 18.0 % for soy flour (Lin et al., 1974) and within 20-70 % reported by Adeyeye et al. (1994) for three other AYB whole seeds flours. *Pleurotus tuber-regium,* would be a useful additive for the stabilisation of fat emulsions in the production of sausage, soup and cake (Altschul and Wilcke, 1985). The capacity of protein to aid the formation and stabilisation of emulsions is important for many applications in cake batters, coffee whiteners, milks, mayonnaise, salad dressings, comminuted meats and frozen desserts (Kinsella et al., 1985). The flour formed a stable emulsion of 54.0 % for a reasonable period of time.

Both the foaming capacity (FC) (8.25 %) and foaming stability (FS) (1.50 %) were low. This is close to the value of FC in full fat *Macuna veracruz* white having a value of 9.60 % (Adebowale et al., 2005). It has been reported that foamability is related to the rate of decrease of the surface tension of the air/water interface caused by absorption of protein molecules (Sathe et al., 1982). Graham and Phillips (1976) linked good foamability with flexible protein molecules, which reduces surface tension. Low foamability on the otherhand can be related to highly ordered globular proteins, which resists surface denaturation. The basic requirements of proteins as good foaming agents are the ability to (i) absorb rapidly at air-water interface during bubbling, (ii) undergo rapid conformational change and rearrangement at the interface, and (iii) form a cohesive viscoelastic film via intermolecular interactions. The first two factors are essential for better foamability whereas the third is important for the stability of the foam (Adebowale et al., 2005). The success of whipping agents largely depends on how long the whip can be maintained. *Pleurotus tuber-regium* would be a poor aerating agent in whipped toppings, frozen desserts and angel food and sponge cakes (Adebowale et al., 2005).

The lowest gelation concentration (LGC) of *P. tuber-regium* was low at 6.00 % (w/v). This value is lower than 8.00-10.00 % (w/v) reported in AYB by Oshodi et al. (1997) and Adeyeye et al. (1994). This value of 6.00 % (w/v) is lower than the value obtained for other seeds reported earlier by Sathe and Salunkhe (1981), Sathe et al. (1982), Oshodi and Ekperigin (1989) and Oshodi and Adeladun (1993),

Adebowale et al. (2005). The ability of protein to form gels and provide a structural matrix for holding water, flavours, sugars and food ingredients is useful in food applications, and in new product developments, thereby providing an added dimension to protein functionality. The observed low-value of LGC of *P. tuber-regium* flour would lead to good setting of stews prepared from the sample. This property may also be useful in the production of curd or as an additive to other materials for gel forming in food products. Sathe et al. (1982) have associated the variation in gelling properties to the ratio of different constituents such as protein, lipids and carbohydrates present in a sample. Flemming et al.(1975) suggested a direct correlation between LGC and the level of globulin in legume seeds. Gelation properties are said to be related to WAC hence the high WAC recorded by the flour could explain the efficient gel formation capacity. Gelation takes place more readily at higher protein concentration because of greater intermolecular contact during heating (Adebowale et al., 2005). High protein solubility is always necessary for gelation as observed by Wilton et al. (1997). Low LGC in *P. tuber-regium* might be an advantage for its use in the production of curd and cheese (Altschul and Wilcke, 1985).

The pH dependent protein solubility profile of *P.tuber-regium* flour is presented in Figure 1. The flour showed high solubility in both the acid and alkali media. Minimum solubility was at pH 5.00. This result corroborated those of Ige et al. (1984). The high solubility of the sample in the acid medium of pH indicates that the flour may be useful in the formulation of acid food, for example, protein-rich carbonated beverages (Kinsella, 1979). The protein had a U-shaped pH-solubility curve. The protein solubility profile of dehulled defatted cowpea flour (DDCF) and cowpea protein isolate (CPIA) and micellization precipitation (CPIB) at pH value ranging from 2-9 were least soluble at pH 4-5 (their isoelectric point) (Khalid et al., 2012). Prevalent charge on the constituent amino acids of proteins at various pH values determine protein solubility as follows:



II I III

It is a zwitterion or dipolar ion which predominates at the region of isoelectric point in protein. At this pH, minimum solubility takes place because of minimum repulsion among the constituent amino acids. The balance in positive and negative charges minimised the electrostatic repulsion, and this reduced solubility of proteins at isoelectric pH. When pH of the solution is reduced further, cation III predominates while in alkaline medium, anion II takes preponderance. In both cases, electrostatic repulsion improved and this enhanced solubility as it is observed in pH 1 (75.4 %) and pH 12 (73.3 %). *P.tuber-regium* showed good solubility in both acid and alkaline pH regions which is most important characteristic for food formlation (Idouraine et al., 1991) since protein solubility largely affects other functionalities like emulsification, foaming and gellation (Kinsella, 1976).

Plate 1: Scloretium of *P.tuber-regium.*

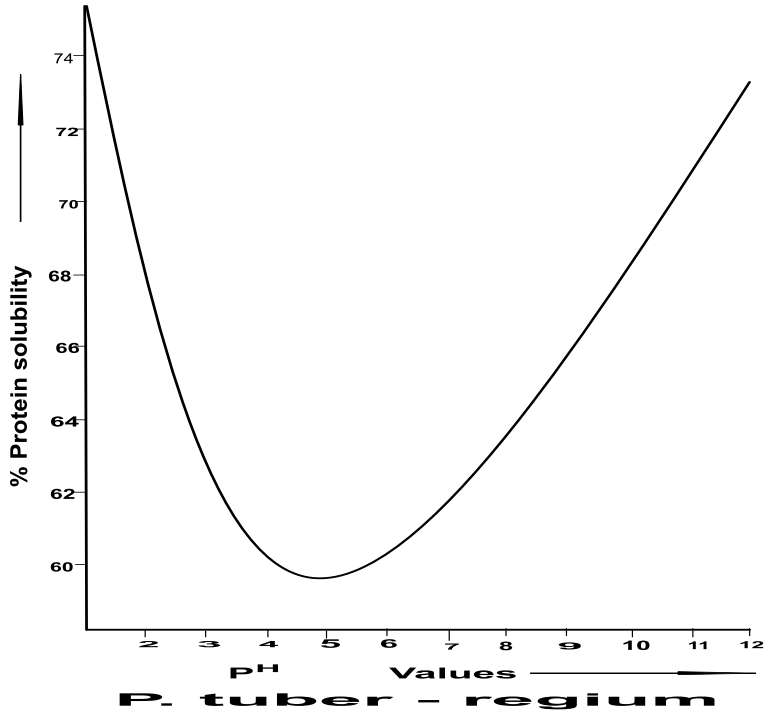


Figure 1. Protein solubility as a function of pH of *P. tuber - regium*.

Table 1: Proximate composition (g/100 g edible portion) of *Pleurotus tuber-regium* (dry weight)

Parameter*	**Value (g/100 g)**
Crude fat	0.83±0.01
Crude protein	19.2±0.11
Carbohydrate	46.5±0.11
Total ash	29.2±0.07
Crude fibre	0.01±0.00
Moisture	4.39±0.03
Energy (kJ/100 g)	1148
[Energy(kcal/100 g)]	270
PEP %	28.4
PEF %	2.68
PEC %	68.9
UEDP %	17.0

*PEP = proportion of total energy due to protein; PEF = proportion of total energy due to fat; PEC = proportion of total energy due to carbohydrate; UEDP = utilization energy due to protein.

Table 2: Minerals (mg/100 g dw) and calculated mineral ratios of *Pleurotus tuber-regium*

Parameter	Value (mg/100 g)
Fe	5.83
Cu	0.443
Co	0.004
Mn	0.812
Zn	1.44
Pb	0.002
Ca	12445
Mg	685
K	211
Na	987
P	2146
Se	0.011
Cd	< 0.0001
Ni	< 0.0001
Na/K	4.68
K/Na	0.214
[K (Ca + Mg)]	0.032
Ca/P	5.80
Ca/Mg	18.2

dw = dry weight.

Table 3: Mineral safety index of Ca, P, Na, Fe, Cu, Zn, Mg and Se of *Pleurotus tuber-regium*

Mineral	CV	TV	D	% D
Ca	104	10	-94	940
P	17.9	10	-7.90	79.0
Na	9.47	4.80	-4.67	97.3
Fe	2.61	6.70	+4.09	61.0
Cu	4.87	33	+28.1	85.2
Zn	3.17	33	+29.8	90.3
Mg	25.7	15	+10.7	71.3
Se	2.28	14	+11.7	83.6

CV = Calculated value; TV = Table value; D = Difference.

No MSI standard for K, Mn, Co, Pb, Cd and Ni.

Table 4: Antinutrients composition of *Pleurotus tuber-regium* (dw)

Antinutrient	Value (mg/100 g)
Tannin	0.060 ± 0.014
Total phenol	0.030 ± 0.014

Phytic acid (mg/g)	0.295 ± 0.021
Oxalate (mg/g)	1.01 ± 0.014
Alkaloids (g/100 g)	0.040 ± 0.014
Flavonoids (g/100 g)	0.010 ± 0.00
Saponin (g/100 g)	ND*

*ND = not detected.

Table 5: Amino acid composition (g/100 g protein edible portion) and their scores of *Pleurotus tuber-regium* (dw)

Amino acid	**Value**	**Amino acid scores based on:**		
	(g/100 g)	**Whole hen's egg**	**FAO/WHO(1973)**	**Pre-school child (2-5 years)**
Gly	5.77	1.93	-*	-
Ala	7.03	1.30	-	-
Ser	5.74	0.72	-	-
Pro	5.28	1.39	-	-
Val	7.87	1.05	1.57	2.26
Thr	5.36	1.06	1.34	1.59
Ile	2.20	0.39	0.55	0.79
Leu	4.91	0.59	0.70	0.74
Asp	8.95	0.83	-	-
Lys	3.72	0.60	0.68	0.64
Met Cys	2.21 0.45	0.69 0.25	0.76	1.04
Glu	13.5	1.13	-	-
Phe Tyr	4.37 6.33	0.86 1.58	1.78	1.70

Trp	0.59	0.33	0.59	0.54
His	3.16	1.50	-	1.89
Arg	5.70	0.93	-	-
Total amino acid	93.6	0.94	1.15	1.23

* - = Not determined.

Table 6: Essential, non- essential, acidic, neutral, sulphur, aromatic amino acids and some other quality parameters (g/100 g) of *Pleurotus tuber-regium*

Amino acid	**Value (g/100 g)**
ΣAmino acid (TAA)	93.6
ΣNon-essential amino acid (TNEAA)	52.0
% TNEAA	55.6
ΣEssential amino acid (TEAA)	
with His	41.6
no His	38.0
% TEAA	
with His	44.4
no His	40.6
ΣEssential aliphatic amino acid (TEAIAA)	15.0
% TEAIAA	16.0
ΣEssential aromatic amino acid (TEArAA)	8.57
% TEArAA	9.16
ΣNeutral amino acid (TNAA)	57.5
% TNAA	61.5
% Cys in TSAA	16.9
Leu/Ile ratio	2.23
Leu/Ile difference	2.71

% (Leu-Ile)/TAA	2.90
% (Leu/Ile)/Leu	55.2
pI[#]	5.40
P-PER$_1$*	1.10
P-PER$_2$*	1.31
EAAI[†]	90.3
Biological value (BV)	86.7
Lys/Trp or L/T	6.35
Met/Trp or M/T	3.77

*Predicted protein efficiency ratio. [#]Isoelectric point. [†]Essential amino acid index.

Table 7: Amino acid groups of *Pleurotus tuber-regium*

Class	**Value (g/100 g)**	**%**
I [with aliphatic side chains (hydrogen and carbons) = Gly, Ala, Val, Leu, Ile]	27.8	29.7
II [with side chains containing hydroxylic (OH) groups = Ser, Thr]	11.1	11.9
III [with side chains containing sulphur atoms = Cys, Met]	2.66	2.84
IV [with side chains containing acidic groups or their amides = Asp, Glu]	22.5	24.0
V [with side chains containing basic groups =Arg, Lys, His]	13.0	13.9
VI [containing aromatic rings = His, Phe, Tyr, Trp]	14.9	15.9
VII [imino acids = Pro]	5.28	5.64

Table 8: Fatty acid composition of *Pleutotus tuber-regium* in % total fatty acids

Fatty acids	**Value (% total fatty acid)**
Hexanoic acid C6:0	0.000
Octanoic acid C8:0	0.000
Decanoic (Lauric) acid C10:0	0.000
Dodecanoic acid C12:0	0.90
Myristic acid C14:0	0.111
Palmitic acid C16:0	12.6
Stearic acid C18:0	8.17
Arachidic acid C20:0	0.001
Behenic acid C22:0	0.000
Lignoceric acid C24:0	0.000
ΣSFA	21.0
Myristoleic acid C14:1 (cis -9)	0.000
Palmitoleic acid C16:1 (cis-9)	6.99
Petrolselinic acid C18:1 (cis-6)	4.45

Oleic acid C18:1 (cis-9)	13.6
Gadoleic acid C20:1 (cis-11)	15.6
Erucic acid C22:1 (cis-13)	0.006
Nervonic acid C24:1 (cis-15)	0.000
ΣMUFA (cis)	40.6
Trans-petroselinic acid C18:1 (trans-6)	0.000
Elaidic acid C18:1 (trans-9)	0.000
Vaccenic acid C18:1 (trans-11)	0.000
ΣMUFA (trans)	0.000
ΣMUFA (cis + trans)	40.6
Linoleic acid C18:2 (cis-9, 12)	16.2
Rumenic acid C18:2 (trans-9, cis-12)	0.000
Gamma-linoleic acid C18:3 (cis-6, 9, 12)	0.078
Eicosadienoic acid C20:2 (cis-11, 14)	0.000
Dihomo-gamma-linoleic acid (DGLA) C20:3 (cis-8, 11, 14)	0.000
Arachidonic acid C20:4 (cis-5, 8, 11, 14)	5.53
Docosadienoic acid C22:2 (cis-13, 16)	0.344
Σ n-6 PUFA	22.2
Alpha-linoleic acid C18:3 (cis-9, 12, 15)	0.000
Eicosatriennoic acid C20:3 (cis-5, 8, 11, 14, 17)	0.000
Timnodonic acid (EPA) C20:5 (cis-5, 8, 11, 14, 17)	5.31
Cervonic acid (docosahexanoic acid) (DHA) C22:6 (cis-4, 7, 10, 13, 16, 19)	10.9
Σ n-3 PUFA	16.2
Σ n-6 + n-3 PUFA	38.4
Σ n-6 + n-3 PUFA + Σ MUFA	79.0
Σ n-6 + n-3 PUFA + Σ MUFA + Σ SFA	100

Table 9: Highlight of calculated quality parameters of the fatty acids composition of *Pleurotus tuber-regium*

Parameter	**Value (% total fatty acid)**
Total SFA	21.0
Total MUFA (cis)	40.6
Total MUFA (trans)	0.00
Total MUFA (cis + trans)	40.6
Total n-6 PUFA	22.2
Total n-3 PUFA	16.2
Total PUFA (n-6 + n-3)	38.4
MUFA/SFA	1.93
PUFA/SFA	1.83
n-6/n-3	1.37
EPSI*	0.946
EPA/DHA	0.487

*Essential PUFA status index.

Table 10: Phospholipids level (mg/100 g) in *Pleurotus tuber -regium*

Phospholipids	Value (mg /100 g)	% value
Phosphatidylethanolamine (PE)	20.3	21.7
Phosphatidylcholine (PC)	45.0	48.1
Phosphatidylserine (PS)	26.9	28.8
Lysophosphatidylcholine (LPC)	3.82e-2	4.08e-2
Phosphatidylinositol (PI)	1.22	1.31
Total	93.5	-

Table 11: Sterol level (mg/100 g) in *Pleurotus tuber -regium*

Sterol	Value (mg /100 g)	% value
Cholesterol	44.8	100
Cholestanol	3.98e-4	8.87e-4
Ergosterol	4.75e-3	1.06e-3
Campesterol	9.21e-3	2.05e-3
Stigmasterol	0.000	0.000
5-Avenasterol	4.38e-4	9.76e-4
Sitosterol	7.62e-4	1.70e-4
Total	44.8	-

Table 12: Some functional characteristics of *Pleurotus tuber-regium*

Parameter	Value (percent)
Water absorption capacity (WAC)	136 ± 1.41
Oil absorption capacity (OAC)	97.8 ± 0.30
Oil emulsion capacity (OEC)	35.5±0.71
Foaming capacity (FC)	8.25±0.35
Foaming stability (FS)	1.50±0.71
Lowest gelation concentration (LGC, w/v)	6.00±0.00
Emulsion stability (ES)	54.0±0.00

CONCLUSIONS

The chemical composition and food properties of *Pleurotus tuber-regium* showed that it has many high nutritional qualities. It is very lowin fat but high in protein, carbohydrate, ash and proportion of energy due to protein. It is low to very low in trace metals but high to very high in the major minerals with good ratios of Ca/P and Ca/Mg. In the mineral safety index, Ca, P, Na were higher than the standard values but only the sodium was of nutritional concern. All the antinutritional components were extremely very low. The amino acids profile contained many amino acids close to or even greater than the values in whole hen's egg, FAO/WHO and pre-school child (2-5 y) standards. EAAI, BV, Lys/Trp and Met/Trp values established the high quality of the amino acids. The fatty acids were low in total SFA but high in MUFA and PUFA with MUFA + PUFA

being 79.0 % of total fatty acids; with good values for MUFA/SFA, PUFA/SFA, n-6/n-3, EPSI and EPA/DHA. The sample is low in cholesterol and total phospholipids. The functional properties showed the sample to be good in WAC, OAC, EC, ES and LGC with high protein solubility at both sides of pH regions which is most important characteristic for food product formulation. *Pleurotus tuber-regium* would be a friend of the heart with its low fat which is highly unsaturated.

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