CHEMICAL COMPOSITION OF MARASMIUS OREADES: A WILD EDIBLE MUSHROOM AMONG KABBA-BUNU INHABITANTS OF NIGERIAN

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ABSTRACT
The use of mushroom as food ingredient has been practiced over years, though many species are consumed for their nutritional and health benefits, the chemical composition of most of these wild edible mushrooms in the tropics has been poorly studied. In this work, a wild mushroom Marasmius oreades; commonly eaten among inhabitants of Bunu land in Nigeria was assessed. The mushrooms were harvested from forests in three villages namely Aduratedo-Ape (AA), Okebukun (OK) and Aiyetoro-Kiri (AK). Proximate, phytochemical, mineral, fatty acid and amino acid constituents of the mushrooms were assessed using standard methods. The proximate on dry matter basis were; protein (12.06-14.54%), fat (7.73-8.38%), fibre (0.56-0.62%), total ash (10.15-14.90%) and carbohydrate (56.01-57.60%). There was no significant (P<0.05) difference in the parameters tested except the protein (14.54%) and ash (14.90%) of samples AK and OB respectively. The predominant phytochemical was flavonoids (12.052%) with others occurring at lower concentrations. Linoleic are most predominant fatty acids with an average result of 51.78%. The range of values obtained for both monounsaturated (28.77-29.34%) and polyunsaturated (51.68-53.20%) fatty acids were greater than saturated fatty acid (18.22-18.29%) in all samples. M. oreades contains great amount of potassium (1270.61 mg/kg), sodium (822.34 mg/kg), phosphorus (619.77 mg/kg), calcium (180.27 mg/kg) and magnesium (95.49 mg/kg). Some amino acids were significantly (P<0.05) affected by sampling locations such as threonine, lysine, cysteine, serine, aspartate and glutamate. The essential amino acids score (1.340) and essential amino acid index (1.261) indicated M. oreades as a good source of quality protein. However, it was limiting in tryptophan and leucine. This study concludes by recommending M. oreades as a good dietary supplement for adults.

Keywords: Marasmius oreades, proximate composition, phytochemicals, minerals, amino acid.
INTRODUCTION

Mushroom is a fungus typically produced on the soil or a suitable food substrate. It consists of a fleshy and spore bearing fruiting body. Mushrooms can be found in many forests of the tropical countries (Zoberi, 1985). Many species such as *Pleurotus* thrive well in moist conditions (Mirko, 1985). According to Miriko (1985), some mushrooms especially members of the genus *Amanita* are extremely poisonous. Wild edible mushrooms are widely consumed in many countries of West Africa especially Nigeria. Their culinary and commercial value are due to their organoleptic properties such as aroma and taste (Guedes et al., 2008), and also to their composition of other nutrients such as carbohydrate, fibre, vitamins and minerals (Mattila et al., 2000) and because of the high proportions of unsaturated fatty acids content of the well-known species (Pedneault et al., 2006). The high protein and low fat-energy contents of wild edible mushrooms make them an excellent food that can be used in low caloric diets (Barros et al., 2007). Lipids display an important role in human body acting like hormones or their precursors and constituting a source of metabolic energy. They also work as structural and functional components of bio-membranes, constituents of myelin sheath and as thermal insulators for the body (Burtis et al., 1996).

Fatty acids are the basic building blocks of most lipids. Polyunsaturated fatty acids from omega-6 and omega-3 families have intense biological properties in low concentrations (Gibney et al., 2002) and are therefore, biosynthetic precursors of the eicosanoids or prostaglandins. Prostaglandins are signalling molecules with complex control over many body systems which has positive effects on cardiovascular diseases, triglyceride constituents, blood pressure and arthritis (Voet and Voet, 2004). Diverse mineral concentrations in mushrooms are considerably higher than those in agricultural crops because numerous macro-fungi were reported to have possessed an effective mechanism that enables them readily absorb different minerals from their ecosystem when compared with green plants grown in similar conditions (Svoboda et al., 2000).

In recent times, mushrooms have assumed greater importance in the diets of both rural and urban dwellers, unlike in the past decades when its consumption was confined to rural dwellers in Nigerian. Mushrooms are now marketed along major highways and urban centres in few West African countries. Presently, mushroom seems to be relatively cheaper compared with animal protein foods such as beef, pork and chicken. Yamac et al. (2007) drew the attention of several researchers to the occurrence and concentrations of the mineral contents of macro-fungi. Sesli (2006) reported that the concentrations of different minerals in most species were found to be dependent on the physiology of the mushroom specie and in particular, on the ecosystem pattern where it is grown. This study investigate the proximate composition, phytochemical attributes, nutritive macro and micro minerals constituents, fatty acid and amino acid profiles of the mushroom *Marasmius oreades* commonly eaten as condiments in soup preparation among the Bunu speaking rural dwellers in Kabba-Bunu Local Government Area, Kogi State, Nigeria.

MATERIALS AND METHODS

Sources and authentication of the mushroom sample

The wild edible mushroom specie was collected from different forest locations in Bunu section located at 8°03´46.0˝N 6°12´12.1˝E of Kabba-Bunu Local Government Area, Kogi State, Nigeria. Three villages among the inhabitants that consume the mushroom and the coordinate points of mushrooms collection were taken as follow: Adurated-Ape (N08°00´36.8˝, E006°09´47.1˝), Okebukun (N08°12´34.7˝, E006°09´56.5˝) and Aiyetoro-Kiri (N08°20´72.9˝, E006°16´58.8˝) Kogi State, Nigeria. The fresh mushroom samples were manually uprooted from the forest at the peak of rainfall season in the early month of September and carefully packaged in an ice pack using suitable plastic container before transporting to the University of Ilorin, Ilorin.
Nigeria for authentication and further study. The mushroom sample was authenticated at the Department of Plant Biology, University of Ilorin, Nigeria and labelled with a voucher specimen number UIL/PLB/KAY/2014/02 before deposited at the herbarium of the University of Ilorin.

**Proximate and phytochemical analyses of *Marasmius oreades***

Proximate analysis was conducted according to the standard methods of Association of Official Analytical Chemist (AOAC, 2006). The anti-nutritional constituents of the mushroom sample were quantitatively determined as described by Bohm and Kocipal-Abyaza (1994) and Kayode et al. (2013).

**Preparation of sample for gas chromatography analysis**

The modified AOAC methods 982.30 and 920.39 (2006) was followed in the extraction of mushroom sample for amino acid and fatty acid analyses respectively. Ten (10) grams of dried mushroom was weighed into a thimble and defatted using petroleum ether by soxhlet extraction method for 3 hours. The solvent was evaporated from the oil to obtain a dried product to be used for fatty acid analysis while, the residue was completely hydrolysed for amino acid profile. The amino acid constituents of the sample were recovered by extraction of the residue with three portions of 30 ml dichloromethane before concentrating to final volume of 1.0 ml. The concentrated extract was dried for volatility suitable for gas chromatography analysis.

**Assay for amino acid and fatty acid profiles using gas chromatography**

Amino acid analysis of the sample was performed using gas chromatograph; Model HP 6890, Powered with HP Chem Station Rev. A 09.01 [1206] software (30m×0.25mm×0.25μm) and column type HP 5). The injector port was heated to 250°C. Injections were performed in split mode in the ratio 20:1. Hydrogen was used as carrier gas at a constant flow of 1.0 mL/min. The oven and detector were set at 60°C and 320°C while compressed air and hydrogen pressure were set at 20psi and 35psi respectively (Kayode and Afolayan, 2015).

**Estimation of biological value of the protein in *Marasmius oreades***

The quality of the amino acids was assessed using chemical score of individual amino acid with reference to recommended values for preschool age (2-5yrs) children (Cavin et al., 1972; FAO/WHO/UNU, 1985 and Nielsen, 2002). The chemical score of the amino acids and the essential amino acid index were calculated as indicated below.

\[
\text{Amino acid score} = \frac{\text{Amino acid of Test Protein (mg/g)}}{\text{Amino acid of Reference Protein (mg/g)}}
\]

Essential amino acid index (EAAI) was obtained as described by Nielson (2002) using the formula given below.

\[
\text{EAAI} = \sqrt{\frac{\text{Lysine in Test Protein} \times \ldots \times \text{Other 8 Amino acids}}{\text{Lysine in Ref Protein} \times \ldots \times \text{Other 8 Amino acids}}}
\]

**Mineral analysis of *Marasmius oreades***

The mushroom sample was digested as described by AOAC (2006). Standard solution was prepared for each mineral before analysis using Atomic Absorption Spectrophotometer (Model-UNICAM 929 London) powered by Solar software.

**RESULTS AND DISCUSSION**

**Habitat, Physiology and Characteristics of *Marasmius oreades***

The mushroom is Saprobic, found in grass land area and occasionally on woodland edges. It is often found in season between July and October in the sampled area of Bunu inhabitants of Kogi State, Nigeria (Figure 1). The mushroom often produces fairy rings which are almost perfect circles, especially in its natural habitats; when there is no interception of animals or human path that could cause varying nutrient levels and soil densities which may result in differential growth rates of the underground mycelium. The cap produces about 2 to 5 cm across; initially convex, flattening with a
broad umbo; hygrophanous, orange-ochre or tan, drying buff or pallid cream; smooth and sometimes producing faint marginal grooves or striations.

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The gills are free, white at first and cream with age. They often ranged between 4 to 8 cm long and 2 to 6 mm in diameter, white or buff, darkening towards a white and downy base and cylindrical with the base slightly swollen. Representative sample of the wild edible *Marasmius oreades* is presented in Figure 2.

Figure 1: Sampling Location of *Marasmius oreades* in Kabba-Bunu Local Government Area of Kogi State, Nigeria

**Proximate composition**
The result of proximate composition of *Marasmius oreades* is shown in Table 1. The range of values obtained for the parameters tested were dry matter (89.89-90.48%), protein (12.06-14.54%), fat (7.73-8.38%), fibre (0.56-0.62%), total ash (10.15-14.90%) and carbohydrate (56.01-57.60%). There was no significant (P<0.05) difference in the parameters tested except the protein content (14.54%) and total ash (14.90%) of samples AK and OB respectively. The slight variation in proximate composition, especially on the significant (P<0.05) increase on ash and protein of the samples from different locations may be attributed to chemical composition of the soil ecosystems on which the *M. oreades* were harvested. Barua and Imamul-Haque (2010) noted that the nutrient composition within a single species of mushroom varies widely depends on habitat, growth medium and pre-harvest handling procedures.

**Phytochemical constituents**
The phytochemical constituents of *Marasmius oreades* harvested from different forest locations in Bunu land are presented in Table 2. The results indicated the presence and concentrations of tannin (0.548%), alkaloids (0.246%), phytate (0.076%), saponin (0.117%), oxalate (0.063%) and flavonoids (12.052%); which was most predominant in the mushroom. The forest locations from where samples were collected had no significant (P<0.05) difference on the concentration of phytochemicals, except flavonoids of samples collected from location AA that was significantly (P<0.05) lower than others as shown in Table 2. Flavonoids have been referred to as nature’s biological response modifiers, because of their inherent ability to modify body reactions to allergies and virus. Flavonoids are potential substance as anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Doughari, 2006). Saponins are known for their medicinal properties as a natural blood
Tannins may elicit antibacterial properties by certain mechanisms of action on microbial cells, such as inhibition of protein synthesis, rupturing of cell membrane, prevention of protease activities and microbial adhesions (Dulger et al., 2002). Barua and Imamul-Haque (2010) encouraged regular consumption of edible mushrooms because of their functional and medicinal properties within individual’s diet and stated that it could be used to prevent some life-style-related diseases in man. The extent of mushroom’s health beneficial effect in human, are often related to the variety, composition and concentrations of various inherent phytochemicals, quantity and regularity of consumption of the mushroom (Barua and Imamul-Haque, 2010).

**Fatty acid profile**

The Fatty acid profile of the wild edible *Marasmius oreades* is given in Table 3. Oleic is the most predominant fatty acids in the samples with an average result of 51.78%. The range of values obtained for both monounsaturated (28.77-29.34%) and polyunsaturated (51.68-53.20%) fatty acids were greater than saturated fatty acid (18.22-18.29%) in all samples. This observation is in agreement with the findings of Barros et al. (2007) and Nakalembe and Kabasa (2013) who noticed significantly higher values of linoleic and oleic acids compared with saturated fatty acids composition of two edible mushroom species namely; *Agaricus arvensis* and *Leucopaxillus giganteus*. Indeed, the result revealed higher amount of polyunsaturated fatty acid than monounsaturated fatty acid. This may be an advantage to consumers of the mushroom, since monounsaturated fatty acid which are lower compared to the polyunsaturated fatty acids have been reported to raise total cholesterol and low density lipoproteins (LDL) cholesterol (“bad” cholesterol) while simultaneously lowering the high density lipoproteins (HDL) cholesterol (“good” cholesterol) levels in the body (Chang and Huang, 1998). *Marasmius oreades* has been shown in this study to contain considerable amount of oleic acid. Oleic acid fraction is technically non-essential since the body cannot synthesize it; although there has to be supply of other fatty acid fractions needed for its formation in the body system (Nakalembe and Kabasa, 2013). Oleic acid is known to be resistant to insulin; it has the capacity to lower blood cholesterol, improves on immune function and aid in some cancer prevention (Carrillo et al., 2012). Oleic acid has been reported to exert cytotoxic effects on cancerous cells (Mei et al., 2006), as well as possesses strong antimicrobial properties (Seidel and Taylor, 2004). Linoleic is an essential fatty acid fraction that has to be supplied in the diets. This fatty acid (Linoleic) has been reported implicated as a precursor of 1-octen-3-ol ‘known as alcohol of fungi’ and a principal aromatic compound of most fungi contributing to mushroom flavour (Maga, 1981). In addition to the nutritional value of linoleic acid, it has been found to be a potent cytotoxic agent against HeLa cell (Mei et al., 2006) and possesses some antibacterial activity (Lee et al., 2002).

**Amino acid profile**

The amino acid profile of *M. oreades* is given in Table 4. The total amino acid constituents of the mushrooms harvested from the three sampling locations varies from 995.5 to 997.9 mg/g protein and were not significantly (P<0.05) different from each other. Most of the constituents amino acids where not significantly (P<0.05) affected by sampling locations except threonine, lysine and cysteine (essential) and the non-essentials amino acids namely serine, aspartate and glutamate (Table 4). The predominant essential and non-essential amino acids in the mushroom specie are threonine, arginine, leucine and glutamate (Table 4). Nakalembe and Kabasa (2013) similarly obtained predominant glutamic acid composition in *Termitomyces globules* and *Termitomyces eurrhizus*. The chemical score of essential amino acids in *Marasmius oreades* are indicated in Table 5. The result shows that the mushroom is adequate to meet the demands for different essential amino acids needed for growth and maintenance of the body system for different human age groups, especially children in the preschool age (2-5yrs), school children (10-12yrs) and adults as recommended by WHO/FAO (1990). From the chemical scores of 1.28 obtained for the sulphur...
containing amino acids, it is noteworthy to observe that the mushroom is not limiting in methionine and cysteine which are most limiting in many plant materials.

The essential amino acids total chemical score (1.340) and values obtained for essential amino acid index (1.261) further supported the fitness of the mushroom specie as a good source of quality protein in human diets. However, the result also revealed that *M. oreades* is limiting in two essential amino acids namely tryptophan and leucine having scored 0.19 and 0.93 respectively (Table 5). The presence of lysine in the mushroom specie indicated that it could be used as a supplement to cereal foods which are limiting in lysine. It may also be used as a substitute for animal protein foods such as beef, due to the presence and high concentrations of other essential amino acids like histidine, isoleucine, phenylalanine and tyrosine.

The reference pattern for preschool age children has been recommended for the evaluation of protein quality for all human age groups except infants; even though it may overestimate protein quality for adults and older children (WHO/FAO, 1990 and Nielsen, 2002). The recommended scoring pattern for infants is the amino acid composition of human milk (Cavins et al., 1972; Nielsen, 2002).

**Mineral constituents**

The mineral constituents of *Marasmius oreades* harvested from different ecological system are presented in Table 6. The values obtained for the micro minerals were not significantly (P<0.05) different. However, it was observed from the mean values that *M. oreades* contains great amount of five major nutritive minerals namely potassium (1270.61 mg/kg), sodium (822.34 mg/kg), phosphorus (619.77 mg/kg), calcium (180.27 mg/kg) and magnesium (95.49 mg/kg). These nutritive macro minerals were significantly (P<0.05) higher in sample obtained from location OB when compared with the values obtained in samples from other locations as presented in Table 6. The high concentrations of potassium in the mushroom may be of health benefits to consumers, especially adults that are potential victims of high blood pressure and stroke, heart and kidney disorder, anxiety and stress. Potassium has also been reported to be very useful ingredient for enhanced muscle strength, water balance, electrolytic function and nervous system (Saif, 2003; Kayode and Sani, 2010). The presence and concentration of sodium in the mushroom may help consumer muscles and nerves to function properly by assisting muscular contraction and transmission of nerve signal. It has also been useful element in the regulation of blood pressure and volume.

**CONCLUSION**

The findings from this study clearly showed that *Marasmius oreades* contain significant amount of nutrients necessary for proper body metabolism. The mushroom contains high amount of potassium, sodium, phosphorus, calcium, magnesium, trace minerals and health beneficial phytochemicals such as alkaloids, tannin and flavonoids; which was most predominant in the mushroom. The high amount of inherent lysine of the mushroom makes it a good supplement for cereal grains. *Marasmius oreades* contains numerous important fatty acids that may prevent different forms of cardiovascular diseases in human. For example, it contains minimal amount of low density lipoproteins; which is a precursor’s of bad cholesterol. The fatty acid constituents that contained lower concentrations of saturated fats with greater values of polyunsaturated fatty acids compared with the values of monounsaturated fatty acids indicated that the mushroom is not likely to produce much of low density lipoproteins (bad cholesterol) when consumed. Low density lipoproteins are substances known to have cause atherosclerosis and other cardiovascular diseases in many victims; thus making the mushroom a good dietary substance for adults. The study concluded by recommending commercial production of this mushroom specie in Nigeria and other countries where their climatic conditions favour mass production.

**ACKNOWLEDGMENTS**

The authors acknowledged the contributions of Mrs. Leah David and Mr. Emmanuel Kayode in Okebukun village, Kabba-Bunu Local Government Area of Kogi State in Nigeria, for leading us to the forests where the mushrooms were sampled. Much thanks to Prof. P.O. Fatoba and the Technologists of the Department of Plant Biology, University of...
AUTHORS CONTRIBUTIONS
R.M.O. Kayode conceived the idea of the research, conducted part of chemical analysis and drafted the manuscript. I.B. Kayode participated in samples collection and suggested the design principles. S.A. Laba, K.O. Salami and D.O. Opaleke were involved in sample collection, preparation and interpretation of some data.

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