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Proximate, Amino Acid Compositions and Food Properties of Some Mushrooms from Igede Ekiti, Nigeria. Note 1

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Abstract

The proximate, amino acids and functional properties of the samples were determined using standard analytical methods. Such determinations were made in some mushrooms (*Psathyrella atroumbonata*, *Agaricus campestris*, *Pleurotus sp* and *Pleurotus ostreatus*). Results showed that mushrooms contained high amount of crude protein (39.8 - 41.9g/100g) and metabolisable carbohydrate (27.7 - 31.0g/100g) dry basis. The available energy was high (1.26 - 1.29 MJ/100g) and the proportion of energy due to protein (52.8 - 55.1%). Aspartic (one sample) and glutamic (three samples) acids (81.4 - 92.0 mg/g crude protein) were the most abundant amino acids. The amino acid composition showed that the mushrooms contained useful nutritional quantities of essential amino acids, although *A. campestris* was low in some essential amino acids. The predicted protein efficiency ratio was 1.1 - 2.0 and leucine-isoleucine ratio was 1.6 - 1.9. Their EAAI ranged from 84.4 - 92.3 with corresponding BV of 80.3 - 88.9. Food properties with high values included water absorption capacity (254 - 398%), fat emulsion capacity (68.4 - 81.6%) and low least gelation concentration (2.0 - 14.0%). The mushrooms would be useful as food supplements and in other food functionalities.

Keywords: *Nutritional Composition; Food Properties; Nigerian Mushrooms*

Introduction

The edible fungi of Africa south of the Sahara are ill-known. Basically, the knowledge of all mushrooms of Africa is very poor; this is due to the difficulties of collecting and of studying being partly responsible for this situation [1].

Most indigenous populations in Africa eat mushrooms. In certain regions, at least during some period of the year, mushrooms are very important as a food source. Mushrooms start to fruit immediately at the beginning of the rainy season, a period in which food reserves are often exhausted and the newly planted crops are not yet available for harvesting. This is the traditional hunger period. Already Livingstone has noted in 1867 that large amounts of mushrooms are eaten in this period in the Zambian Northern Province. Mushrooms play a role in traditional medicine and beliefs [2], as cosmetic [1]; as dye; fumigation of huts; used to tie jewelry and for their psychotropic substances [3].

Agaricus is a very large genus occurring worldwide. Numerous species have wide areas of distribution, which is partly linked with their saprophytic habit. *A. campestris* is widespread and are common in Africa. The carphophores are popular as food for Europeans in Africa. *Pleurotus* has been used for different genera, e.g. *Lentinus* and *Panus* have been placed under genus *Pleurotus* [1]. The genus contains some of the most valuable mushrooms, for instance *Psathyrella atroumbonata* is eaten in Malawi as Ndiwo which is cooked with salt but with very little water. It is also consumed in Nigeria [2].

There is paucity of information on the chemical composition, nutritional and food functional values of mushrooms. Abulude., *et al*. [4] studied the metal levels in mushrooms consumed in Akure South Local Government Area in Southwestern Nigeria. The study was on *Volvariella volvacea*, *Termitomyces robustus*, *Termitomyces mammiformis*, *Schizophyllum commune*, *Termitomyces globulus*, *Pleurotus squarrosulus* and *Auricularia auricular*. Adeyeye., *et al*. [5] studied the effects of some salts on water absorption, least gelation capacities and fat emulsion stability of the above mentioned edible mushrooms. The salts used were NaCl, Na_2SO_4, $NaNO_2$, $NaNO_3$ and NaOAc. Adeyeye., *et al*. [6] studied effects of some salts on foaming capacity/stability and protein solubility of the seven edible mushrooms. The salts used were NaCl, Na_2SO_4, $NaNO_2$, $NaNO_3$ and NaOAc. Adeyeye and Abulude [7] studied the chemical composition and some anti-nutritive factors of seven mushrooms consumed in Nigeria. They were evaluated for proximate composition, phytate phosphorus and phytate composition and their calculated Phytate: Zn, Ca: Phytate and [Ca][Phytate]/[Zn] molar ratios. Eissa [8] determined the effect of chitosan coating in fresh-cut mushroom preservation, including microbiological, enzyme activities, colour characteristics and chemical quality attributes. Falandysz., *et al*. [9] determined the total mercury concentrations of eleven species of common edible wild mushrooms of the genus *Suillus, Xerocomus, Bololus, Leccinum, Annillariella, Russula, Lactarius* and *Lycoperdon* collected from the Borecka Forest and the adjacent area in 1998 using the cold-vapour flameless atomic absorption spectroscopy (CV-AAS).

In the present report, the proximate, amino acid compositions and food properties of *Psathyrella atroumbonata, Agaricus compestis, Pleurotus sp*. and *Pleurotus ostreatus* were evaluated.

Materials and Methods

Sample Collection and Preparation

Mushroom samples were purchased from Igede Ekiti, Nigeria. The samples were labeled as *Psathyrella atroumbonata* (Mr_1), *Agaricus compesti* (Mr_2), *Pleurotus sp*. (Mr_3) and *Pleurotus ostreatus* (Mr_4) where Mr = mushroom and 1, 2, 3, 4, represented their serial numbers. This is shown in Table 1. The samples were collected once in the second week of June, 2014 from a market in Igede Ekiti, Nigeria. The samples were washed with distilled water, oven-dried (55°C) for 24h and screened to remove the rotten ones. The good parts were blended into fine flour.

SN	Local Yoruba name	English language name	Systematic name	Code number
1	Osun wowo	Wowo mushroom	*Psathyrella atroumbonata* Pegler	Mr_1
2	Absent in Nigeria	-	*Agaricus campestris* L.	Mr_2
3	Erirokiro	Leather-like nushroom	*Pleurotus squarrosulus*	Mr_3
4	-South African species-	-	*Pleurotus ostreatus* (Jacq.; Fr) Kumm	Mr_4

Table 1: *Names and Code Numbers of the Mushrooms Analysed.*

Sample Analysis: Proximate Composition

The moisture, ash, crude fat and crude fibre were determined by the AOAC [10] methods, whilst nitrogen was determined by the micro-Kjeldahl method and the percentage of nitrogen was converted to crude protein by multiplying by 6.25. Carbohydrate was determined by difference; all determinations were in duplicate. The proximate determinations were carried out after oven drying.

Sample Analysis: Amino acid Composition

About 2.0 g of sample was defatted with chloroform/methanol mixture (2:1 v/v). Between 30 - 35 mg of defatted sample was put in glass ampoule, 7 cm^3 of 6 M HCl was added and oxygen expelled. The sealed ampoule was put in oven at 105 ± 5°C for 22h and later allowed to cool before the content was filtered. The filterate was evaporated to dryness at 40°C under vaccum. Residue was dissolved with 5 cm^3 acetate buffer (pH 2.0) and stored in the freezer pending analysis. The method of amino acid analysis was by ion exchange chro-

matography (IEC) [11] using a Technicon Sequential Multisample Amino Acid Analyser (TSM) (Technicon Instruments Corporation, New York). The period of analysis was 76 min, with gas flow rate of 0.50 cm^3/min at 60˚C and the reproducibility was ± 3 %. The amino acid values were the average of two determinations. Tryptophan was not determined because of cost. The method used will need to determine tryptophan separately. This means the cost in the determination of seventeen (17 x4) parameters will be spent on one (1x4) parameter.

Determination of Samples Functional Properties

The method described by Adeyeye., *et al*. [12] was used for the determination of protein solubility at room temperature (25˚C). The protein solubility (PS) was examined from pH 2 - 12. The sample (0.2 g) was thoroughly stirred with distilled water (10 cm^3) and the pH was adjusted using either 0.1M HCl or 0.1 M NaOH and a supernatant was obtained whose protein content was determined by the Wiechselboven method [13]. The water absorption capacity, fat absorption capacity, foaming capacity and stability and least gelation concentration of the samples were determined according to the methods of Sathe., *et al*. [14] and Lin., *et al*. [15]. The water absorption capacity (WAC) was determined as follows: 1.0 g flour was added, mixed with 10 cm^3 of distilled water in a mixer and kept at room temperature for 30 min. It was later centrifuged for 30 min and the supernatant was noted in a 10 cm^3 graduated cylinder. The density of water was assumed to be 1 g/ cm^3. The excess absorbed by the flour was expressed as the percentage of water bound by 100g sample.

Fat absorption capacity (FAC) was determined by using 0.5g of the sample flour added to 3.0 cm^3 of Avop vegetable oil in 10 cm^3 graduated centrifuge tubes. The mixture was vortexed to disperse the flour in the oil. After holding for 30 min, centrifugation was carried out for 30 min and the volume of separated oil was noted. The excess oil absorbed was expressed as the percentage oil bound by 100 g sample. The density of the oil bound was determined by means of a specific gravity bottle. Foam capacity (FC) and foam stability (FS) were determined as follows: 1.0 g sample was whipped with 50 cm^3 of distilled water for 5 min in a Kenwood Major blender at speed setting 'fast' and was poured into a 100 cm^3 graduated cylinder. Total volume at intervals between 25 min and 1500 min was noted to study the foaming stability. To obtain the foaming capacity, volume increase (%) was calculated according to the following equation:

$$Volume\ increase\ \% = \frac{Volume\ after\ whipping - volume\ before\ whipping \times 100}{Volume\ before\ whipping}$$

Least gelation concentration (LGC) was determined as follows: appropriate flour suspensions of 2, 4, 6, 8, 10, 12, 14, 16 % (w/v) were prepared in 5 cm^3 distilled water. The test tubes containing these suspensions were heated for 1 h in boiling water followed by rapid cooling under running tap water. The test tubes were then cooled for 2h at 4˚C. The lowest gelation concentration was determined as the concentration in which the flour from the inverted test tube did not fall down nor slip. Fat emulsion capacity was determined by the procedure of Inklaar and Fortuin [16] and fat emulsion stability by the method of Beuchat [17]. In determining the fat emulsion capacity (FAC), 1.0g of flour was made into slurry in 20 cm^3 of distilled water in an Erlenmeyer flask by stirring at 100 rpm for 15 min with a small magnetic bar. 5 cm^3 of Avop vegetable oil was then added over a period of 5 min while stirring at 1000 rpm and stirring was continued for an extra min. The system was transferred to a centrifuge tube, heated in a water bath maintained at 85˚C for 15 min with occasional stirring and then cooled for 15 min in a water bath maintained at 25˚C. The tube was finally centrifuged at 3500 rpm until the volume of oil separated from the emulsion was constant. Results were expressed as the percentage of that emulsified oil after separating the upper layer from the emulsion.

For fat emulsion stability (FAS) determination 1.0g of sample was blended in a Kenwood Major blender with 50 cm^3 of distilled water for 30s at maximum speed. Avop vegetable oil was added in 5 cm^3 portions with continued blending. A drop in consistency (from a maximum judged objectively by a decrease in resistance to blending) was considered the point at which to discontinue oil addition. The emulsion so prepared was then allowed to stand in a graduated cylinder and the volume of water separated at intervals between 1h and 24h was noted in each case. Results were average of two determinations.

Other Calculations from the Samples Data

Some further calculations were done on the data obtained. Such calculations included the mean, standard deviation and coefficient of variation percent (CV %) using the data obtained for mushroom samples. The crude fat, crude protein and soluble carbohydrate values were used to calculate the energy contributed by the mushrooms and then the proportion of energy percent as contributed by fat, protein and carbohydrate. The energy values were calculated by adding up the values obtained for carbohydrates (x 17 kJ), crude protein (x 17 kJ) and crude fat (x 37 kJ) for each of the samples. Proportions of energy due to protein, carbohydrate and fat percent were also calculated as follows: energy due to a particular nutrient/total energy contribution by all the nutrients multiplied by 100; e.g. for protein = energy from protein/total energy x 100 (PEP %). The utilization energy due to protein percent (UEDP %) was determined as follows: 0.60 x value of proportion of total energy due to protein percent (0.60 x PEP %). The amino acid score was calculated using the following formula [18]:

$$Amino\,acid\,score \;=\; \frac{Amount\ of\ amino\,acid\ per\ test\ protein\left[mg\ /\ g\right]}{Amount\ of\ amino\,acid\ per\ protein\,in\,reference\ pattern\left[mg\ /\ g\right].}$$

Determination of the total essential amino acid (TEAA) to the total amino acid (TAA), i.e. (TEAA/TAA); total sulphur amino acid (TSAA); percentage cystine in TSAA (% Cys/TSAA); total aromatic amino acid (TArAA); total acidic amino acid (TAAA) and total neutral amino acid (TNAA) were carried out. The predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer., *et al*. [19], i.e. P-PER = -0.468+0.454 (Leu) -0.105 (Tyr). The leucine /isoleucine ratios were calculated. The theoretical isolectric point (pI) was estimated by the equation of Olaofe and Akintayo [20] of the form:

$$IPm \;=\; \sum_{i\,=\,1}^{n} IPiXi$$

where IPm is the isoelectric point of the mixture of amino acids, IPi is the isoelectric point of the i^{th} amino acid in the mixture and Xi is the mass or mole fraction of the i^{th} amino acid in the mixture. The essential amino acid index (EAAI) values were calculated due to Oser [21] using the egg protein amino acids as the standard. The computation of biological value (BV) was carried out following the equation of Oser [21] as follows:

$$Biological\,value \;=\; 1.09\left(EAAI\right) - 11.73$$

Results and Discussion

Results

The results of various analyses of the samples are shown under various headings in terms of various designations. All results were on dry weight basis.

The results in Table 2 present the proximate compositions of the mushroom samples. The moisture content was low (2.13-4.57 g/100 g) with a low value of coefficient of variation percent (CV %), 34.5; this would afford a long shelf life without microbiological spoilage of the samples. The CV % of the crude protein was low (2.76) with high value of crude protein (39.8 – 41.9 g/100 g). The result in Table 3 shows the various energy levels as contributed by protein, fat and carbohydrate.

Composition	Sample (g/100 g dry weight)[a]						
	Mr_1	Mr_2	Mr_3	Mr_4	Mean	SD[b]	CV %[c]
Moisure content	3.45	2.50	4.57	2.13	3.16	1.09	34.5
Dry matter	96.6	97.5	95.4	97.9	96.8	1.07	1.03
Crude protein	39.8	41.9	40.0	41.9	40.9	1.13	2.76
Crude fat	2.03	3.08	2.47	2.55	2.53	0.43	17.0
Total ash	12.4	13.2	12.7	13.5	12.94	0.48	3.69
Crude fibre	11.2	12.0	11.6	11.4	11.5	0.35	3.06
Carbohydrate	31.0	27.7	28.4	28.6	29.0	1.44	4.98

Table 2: *Proximate Composition of the Samples.*
[a]Determinations were in duplicate; [b]Standard deviation; [c]Coefficient of variation.

Parameter	Mr_1	Mr_2	Mr_3	Mr_4	Mean	SD	CV %
Total energy (kJ/100 g)	1278	1291	1260	1294	1281	15.5	1.21
PEP %	52.8	55.1	53.9	55.0	54.2	1.08	2.0
PEF %	5.88	8.83	7.24	7.30	7.31	1.21	16.5
PEC %	41.3	36.0	38.8	37.7	38.5	2.21	5.74
UEDP %	31.7	33.1	32.4	33.0	32.5	0.64	2.0

Table 3: *Energy Values as Contributed by Protein, Fat and Carbohydrate in the Mushroom Samples.*
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 = Utilisable energy due to protein.

As the result in Table 4 shows the amino acid composition of the mushroom samples, Asp was the highest (82.9 mg/g crude protein) in *P. atroumbonata* (Mr_1) but Glu was highest as 92.0 in *A. compestris* (Mr_2), 81.4 in *Pleurotus sp*. (Mr_3) and 81.5 in *P. ostreatus* (Mr_4). Glu was the highest concentrated amino acid (AA) in melon, pumpkin and gourd seeds [25] and in pigeon pea (*Cajanus cajan*) [26]. Leu was the highest concentrated essential amino acid (EAA) in all the mushroom samples. The variation in the AA composition as depicted by the coefficient of variation percent (CV %) showed that Glu was the most varied AA with a CV % of 53.8 whilst Pro was the least varied with CV % of 13.3. The various CV % showed that very little variation existed among the AA values of the mushroom samples.

The result in Table 5 shows the trend of the AA composition in the samples. It also contains some calculated parameters.

	Mushroom sample (mg/g amino acid, cp)[a]						
Amino acid	**Mr_1**	**Mr_2**	**Mr_3**	**Mr_4**	**Mean**	**SD**	**CV %**
Lys*	42.8	24.6	26.2	36.1	32.4	8.6	26.5
His*	14.1	20.2	15.9	23.1	18.3	4.1	22.3
Arg*	54.9	19.6	39.4	44.9	39.7	14.9	37.4
Asp	82.9	46.1	56.7	61.7	61.9	15.5	25.0
Thr*	44.0	18.2	20.9	20.6	25.9	12.1	46.8
Ser	24.7	18.1	19.5	32.3	23.7	6.4	27.1
Glu	13.5	92.0	81.4	81.5	67.1	36.1	53.8
Pro	24.4	19.8	17.8	20.7	20.7	2.8	13.3
Gly	43.6	23.2	45.1	20.2	33.0	13.1	39.8
Ala	47.6	34.7	26.8	28.2	34.3	9.5	27.7
Cys	22.0	7.1	15.9	24.3	17.3	7.7	44.4
Val*	45.0	27.0	24.0	36.9	33.2	9.6	28.9
Met*	22.2	9.4	9.2	9.1	12.5	6.5	52.0
Ile*	32.7	23.4	20.8	33.9	27.7	6.6	23.7
Leu*	61.9	40.9	40.4	52.6	49.0	10.3	21.1
Tyr	35.1	12.7	27.4	22.0	24.3	9.4	38.8
Phe*	47.4	27.9	27.5	38.4	35.3	9.5	27.0
Trp*	n.d[b]	n.d	n.d	n.d	n.d	n.d	n.d

Table 4: *Amino Acid Composition of Mushrooms.*

*[a]Crude protein; [b]Not determined; *Essential amino acid.*

	Mushroom samples						
Amino acid	**Mr_1**	**Mr_2**	**Mr_3**	**Mr_4**	**Mean**	**SD**	**CV %**
Total amino acid (TAA)	659	465	515	587	556	84.8	15.2
Total non-essential amino acid (TNEAA)	292	253	303	290	284	21.5	7.6
Total essential amino acid (TEAA) -with His	367	211	212	297	272	75.1	27.6
-no His	353	191	196	274	254	76.3	30.1
% TNEAA	44.3	54.5	58.8	49.4	51.8	6.3	12.1
%TEAA -with His	55.7	45.5	41.2	50.6	48.3	6.3	13.0
-no His	53.6	41.2	38.4	46.7	45.0	6.7	14.9
Total neutral amino acid (TNAA)	451	262	295	339	337	82.3	24.4
% TNAA	68.4	56.4	57.4	57.8	60.0	5.6	9.4
Total acidic amino acid (TAAA)	96.4	138	138	143	129	21.8	16.9
% TAAA	14.6	29.7	26.8	24.4	23.9	6.6	27.4
Total basic amino acid (TBAA)	112	64.4	81.5	104	90.5	21.6	23.9
% TBAA	17.0	13.9	15.8	17.7	16.1	1.7	10.3
Total sulphur amino acid (TSAA)	44.2	16.5	25.1	33.4	29.8	11.8	39.7

% TSAA	6.7	3.6	4.9	5.7	5.2	1.3	25.2
% Cys in TSAA	49.8	43.0	63.3	72.8	57.2	13.4	23.4
Total aromatic amino acid (TArAA)	82.5	40.6	54.9	60.4	59.6	17.4	29.2
% TArAA	12.5	8.7	10.7	10.3	10.6	1.6	14.7
P-PER*	2.0	1.3	1.1	1.7	1.5	0.4	26.9
Leu-Ile ratio	1.9	1.7	1.9	1.6	1.8	0.2	8.3
Leu-Ile (diff.)	29.2	17.5	19.6	18.7	21.3	5.4	25.3
% Leu-Ile (diff.)	47.2	42.8	48.1	35.6	43.4	5.7	13.6
Experimental pI[a]	3.0	5.0	3.0	4.0	3.8	1.0	25.5
Calculated pI	4.4	3.0	3.0	3.4	3.5	0.7	19.2
Exp. pI – cal. pI	1.4	2.0	0.0	0.6	1.0	0.9	87.9
EAAI[b]	84.4	92.3	89.8	86.0	88.1	3.59	4.07
Biological value (BV)	80.3	88.9	86.2	82.0	84.4	3.92	4.64

Table 5: *Essential, Non-essential, Acidic, Neutral, Sulphur, Aromatic Amino Acids (MG/G CP), P-PER, LEU/ILE of Mushroom Samples.*

**Predicted protein efficiency ratio; [a]Isoelectric point; Essential amino acid index*

As the result in Table 6 shows, the essential amino acid scores of the samples showed Lys has 0.78 score in *P. atroumbonata*; Lys has 0.45 score in *A. campestris*; Lys has 0.48 score in *Pleurotus* sp. whilst Thr has 0.52 score in *P. ostreatus*; these EAA were the limiting AA in the various samples.

Amino acid	Mr_1	Mr_2	Mr_3	Mr_4	Mean	SD	CV %
Ile	0.82	0.59	0.52	0.85	0.70	0.16	23.5
Leu	0.88	0.58	0.58	0.75	0.70	0.15	20.8
Lys	0.78	0.45	0.48	0.67	0.60	0.16	26.2
Met + Cys	1.26	0.47	0.72	0.95	0.85	0.34	39.6
Phe + Tyr	1.38	0.68	0.92	1.01	1.00	0.29	29.1
Thr	1.10	0.46	0.52	0.52	0.65	0.30	46.4
Trp	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Val	0.90	0.54	0.48	0.74	0.67	0.19	28.9
Total	1.01	0.55	0.56	0.78	0.73	0.22	30.0

Table 6: *Amino Acid Scores of the Mushroom Samples.*

Table 7 gives a summary of the amino acids profiles in the four samples. Column under Factor B means showed that the values were close with a range of 272 to 285. However, similar observation could not be made in Factor A column with amino acids profiles range of 232 to 330. It should however be noted that both columns A and B means terminated at 278.5. The various amino acid groups are shown in Table 8 [36].

Amino acid composition factor B	Samples (Factor A)				Factor B means
	M_1	M_2	M_3	M_4	
Total Essential Amino Acid	367	211	212	297	272
Total None Essential Amino Acid	292	253	303	290	285
Factor A means	330	232	258	294	278.5

Table 7: *Summary of the Amino Acid Profiles into Factors A and B.*

Class	Value in mg/g protein (% value)				Mean	SD	CV%
	Mr_1	Mr_2	Mr_3	Mr_4			
I [with aliphatic side chains (hydrogen and carbons) = Gly, Ala, Val, Leu, Ile]	231 (32.6)	149 (21.0)	157 (22.1)	172 (24.3)	177	37.1	20.9
II [with side chains containing hydroxylic (OH) groups = Ser, Thr]	68.7 (34.7)	36.3 (18.3)	40.4 (20.4)	52.9 (26.7)	49.6	14.6	29.4
III [with side chains containing sulphur atoms = Cys, Met]	44.2 (37.1)	16.5 (13.9)	25.1 (21.1)	33.4 (28.1)	29.8	11.8	39.7
IV [with side chains containing acidic groups or their amides = Asp, Glu]	96.4 (18.7)	138 (26.7)	138 (26.7)	143 (27.7)	129	21.8	16.9
V [with side chains containing basic groups = Arg, Lys, His	112 (30.9)	64.4 (17.8)	81.5 (22.5)	104 (28.7)	90.5	21.7	23.9
VI [containing aromatic rings = His, Phe, Tyr, Trp]	96.6 (31.0)	60.8 (19.5)	70.8 (22.7)	83.5 (26.8)	77.9	15.5	19.9
VII [imino acids = Pro]	24.4 (29.5)	19.8 (23.9)	17.8 (21.5)	20.7 (25.0)	20.7	2.76	13.4

Table 8: *Amino acid groups of the Mushroom Samples.*

The result in Table 9 presents the food properties of the mushroom flours.

	Sample (%)						
Food property	Mr_1	Mr_2	Mr_3	Mr_4	Mean	SD	CV %
Water absorption capacity	333	254	398	295	320	61.3	19.2
Fat absorption capacity	71.0	40.1	65.5	30.1	51.7	19.7	38.1
Foaming capacity	16.7	5.7	13.8	16.7	13.2	5.2	39.4
Foaming stability	20.0	33.3	12.5	10.0	19.0	10.5	55.2
Fat emulsion capacity	73.7	79.4	68.4	81.6	75.8	5.9	7.8
Fat emulsion stability (5 h)	45.0	50.0	43.0	46.0	46.0	2.9	6.4
Least gelation capacity (w/v)	6.0	14.0	4.0	2.0	6.5	5.3	80.9
Rate change of FS* (cm^3/min)	0.3	0.1	0.7	0.3	0.4	0.3	71.9

Table 9: *Food Properties of the Mushroom Samples.*

**Foaming stability*

The result in Figure 1 shows the mushrooms protein solubility as a function of pH. It provided a good index of the potential or limitation of a protein as a functional ingredient.

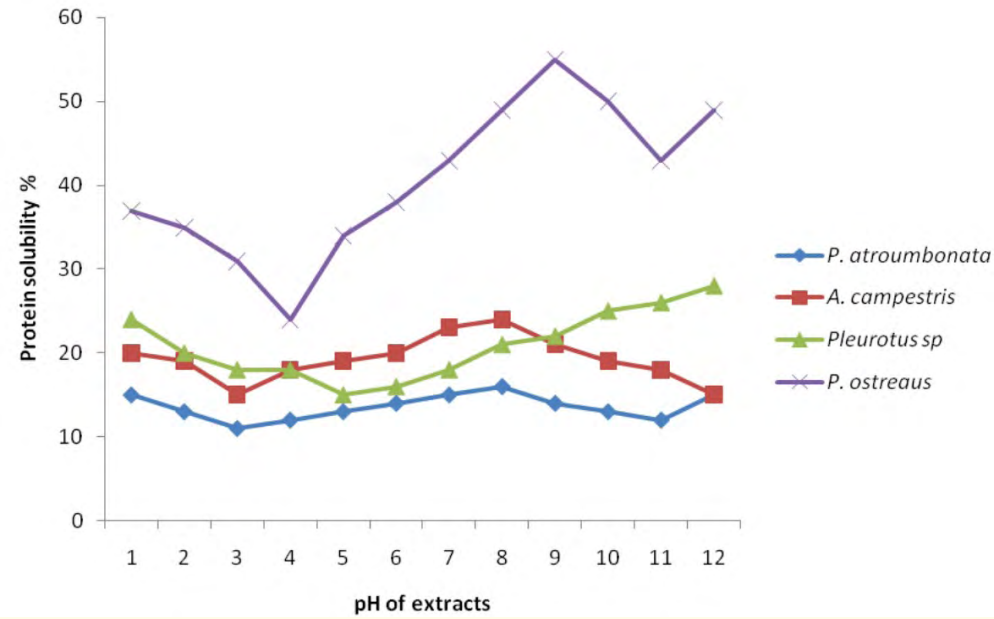

Figure 1: *Effect of pH on protein solubility (%) of mushroom samples.*

Discussion

The ash content of 12.4 - 13.5g/100g in Table 2 was higher than the value of 9.0g/100g earlier reported. The present crude fibre was also much higher (11.2 - 12.0g/100g) than the value of 6.8g/100g reported by Rammeloo and Walleyn [1] for South African mushroom. The crude fat of 2.03 - 3.08g/100g was low and the mushrooms are said to have hypocholesterolemic behaviour [10]. The carbohydrate levels of 27.7 - 31.0g/100g could not be said to be very high but it is good for the body since these might prevent ketosis, excessive breakdown of body protein, loss of cations, especially sodium and involuntary dehydration [22].

Mushrooms have been reported to contain 12 to 31% of protein on dry weight basis; which is about 1 - 3% of their fresh weight [1]. Even if mushrooms cannot supply the necessary daily amount of proteins, and can certainly not compete with beans (about 18 - 38g/100g crude protein), they can be considered as an important supplement as food proteins and other vital substances (e.g. vitamins) which are partly lacking in the often one-sided diet of local populations in Nigeria. Taking the large amounts of mushroom in consideration which is consumed in certain regions at a crucial time of the year, it is obvious that mushrooms play an important role in the nutrition of local people [1].

The utilisation of energy due to protein (UEDP %) in the samples was 31.7 - 33.1; this assumes 60 % protein energy utilisation. The UEDP % values were much higher than 2.4 - 6.0 reported for various ogi flours of corn, sorghum and millet [23] but lower than the value of 50.3 reported for *Gymnarchus niloticus* (Trunk fish) [24]. Our UEDP % values were higher than the recommended safe level of 8 % for an adult man who requires about 55 g protein per day with 60 % utilization. This implies that the protein concentration in the mushroom flours in terms of energy, may be able to prevent malnutrition in adults fed solely on mushroom flours as their main protein source.

The total AA trend shows *P. atrounbonata* (Mr_1) > *P. ostreatus* (Mr_4) > *Pleurotus sp*. (Mr_3) > *A. campestris* (Mr_2); similar trend was observed for the EAA. In % EAA (with His) the trend was $Mr_1 > Mr_4 > Mr_2 > Mr_3$ whilst % EAA (no His) trend was $Mr_1 > Mr_4 > Mr_2 > Mr_3$ meaning that only Mr_1 and Mr_4 kept constant positions. The literature values of EAA (mg/g cp) were: soybean, 444; cowpea, 426; *Cajanus cajan*, 436; pigeon pea, 452; pumpkin seed, 396; melon seed, 534; pumpkin seed, 383; gourd seed, 536. The present EAA can therefore be said to be able to contribute to the needed EAA in adult nutrition. Ile, Leu, Lys, Phe, Val and Met values in the mushroom samples were higher than or comparable to the FAO/WHO [18] reference values of 42, 42, 42, 28, 42 and 22 mg/g cp respectively. Some EAA literature values from animal sources (mg/g cp) were: Trunk fish, 355 [24]; variegated grasshopper, 351, reproductive termites, 350; *Limicolaria* sp., 428, *Archatina archatina*, 361 and *Archachatina marginata*, 450 [27]. The present results were close to many of these results. The percentage ratio of EAA to the total AA in the samples ranged from 41.2 - 55.7%; these values were well above the 39 % considered to be adequate for ideal protein food for infants, 26 % for children and 11 % for adults [28]. The present percentage of EAA/TAA is strongly comparable to that of egg, 50 %. The aromatic AA (ArAA) ranged between 40.6 - 82.5 mg/g cp which were very close to the range suggested for ideal infant protein (68 -118 mg/gcp) [28].

Although tryptophan was not determined in our samples, it is seen from literature that mushroom may be low in Trp. Examples can be seen in the value of Trp in the various species of the genus *Boletus* as published by Dembitsky., *et al*. [10]. Detected free amino acids (with emphasis on Trp) of genus Boletus (µM/g dry wt basis): *B. aestivalis* (0.7); *B. aereus* (trace; t); *B. appendiculatus* (t); *B. badius* (t); *B. crocipodius* (0.9); *B. edulis* (t); *B. granulatus* (0.8); *B. impolitus* (0.6); *B. luridus* (t); *B. luteus* (t); *B. pinicola* (t); *Boletus* sp. (t); *B. queletii* (0.8); *B. scabe*r (t); *B. versipellis* (t).

Most animal proteins are low in Cys in TSAA, for examples: 36.3 % in *Macrotermes bellicosus* [29]; 25.6 % in *Zonocerus variegatus* [30]; 35.3 % in *A. marginata*, 38.8 % in *A. archatina* and 21.0 % in *Limicolaria* sp. [27]; 29.8 % in *G. niloticus* respectively. In contrast, many vegetable proteins contain substantially more Cys than Met, example, 62.9 % in coconut solid endosperm [31]. The present % Cys in TSAA was: 49.8 (*P. atroumbonata*); 43.0 (*A. campestris*); 63.3 (*Pleurotus* sp.) and 72.8 (*P. ostreatus*) respectively; these values were between animal and vegetable values. FAO/WHO/UNU [28] did not give any indication of the proportion of TSAA which can be met by Cys in man, for the rat, chick and pig, the proportion is about 50 % [11]. Cys has positive effects on mineral absorption, particularly zinc [32]. The protein efficiency ratios (PER) values from an animal assay vary from 0.00 for a very poor protein to maximum possible of just over 4 [33]. Our predicted PER (P-PER) results ranged from 1.1 - 2.0 which were favourably comparable to 1.21 (cowpea), 1.82 (pigeon pea); 1.62 (millet *ogi*), 0.27 (sorgum *ogi*) but much lower than 4.06 (corn *ogi*) [23]; 1.83 (Trunk fish) and reference casein of PER value of 2.50. The Leu/Ile ratio range was 1.6 - 1.9 or percentage difference of 35.6 - 48.1. It has been suggested that an amino acid imbalance from excess leucine might be a factor in the development of pellagra [34] and it has also been shown that high leucine in the diet impairs the metabolism of tryptophan and niacin. Further studies have shown that the biochemical and clinical manifestations of dietary excess of leucine could be counteracted not only in increasing the intake of niacin or tryptophan but also by supplementation with isoleucine [34]. These studies suggested that the leucine/isoleucine balance is more important than dietary excess of leucine alone in regulating the metabolism of tryptophan and niacin and hence the disease process. The CV % in the calculated pI and the experimental values were very close with respectively values of 19.2 and 25.5. Calculated pI is important in determining the minimum isoelectric point for the preparation of a protein isolate from a vegetable source.

The EAAI of 84.4 – 92.3 and their corresponding BV of 80.3 – 88.9 depict highly the quality of the protein of the mushroom samples. No literature values are available for the EAAI and BV of mushrooms. However, the protein quality of the mushrooms can further be compared with other EAAI and BV values available in literature: milk, cow (whole, nonfat, 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) [21]. EAAI is useful as a rapid tool to evaluate food formulation for protein quality, although it does not account for difference in protein quality due to various processing methods or certain chemical reactions [35].

Lys is the first limiting AA (LAA), followed by Met + Cys, Thr is the third LAA whilst Trp is the fourth LAA (which was not determined in the present report). To correct for the LAA in the samples the proteins must be multiplied by the following values: 100/78 or 1.28 in *P. atroumbonata*; 100/45 or 2.22 in *A. campestris*; 100/48 or 2.08 in *Pleurotus* sp. and 100/52 or 1.92 times as much respective protein values in the mushrooms if they serve as the sole protein sources.

The concentration trend of the classes could be seen to follow as shown in mg/g crude protein: class I (149 – 231) > class IV (96.4 – 143) > class V (64.4 – 112) > class VI (60.8 – 96.6) > class II (36.3 – 68.7) > class III (16.5 – 44.2) > class VII (17.8 – 24.4). The concentration range of the samples followed this trend: Mr_1 predominated in six classes (6/7 or 85.7%) of I, II, III, V, VI and VII whereas Mr_4 predominated in class IV. The amino acid in Table 8 shows that EAAs were distributed in the various classes as follows: class I (3EAA), class II (one EAA), class III (one EAA), class IV (no EAA), class V (3EAA), class VI (2EAA) and class VII (no EAA). This means in terms of essentiality, class I ≡ class V > class VI > class II ≡ class III > class IV ≡ class VII.

The water absorption capacity (WAC) (253 - 398%) for the mushrooms was greater than that for oilseed flours (70 -120%) [25], pigeon pea (138 %) [37], soy flour (130 %) [15], defatted flours of oilseeds (100 - 266%) [38], cowpea flours (212 - 275%) [39], fluted pumpkin (85%) [40], trunk fish (275%), sunflower (107%) [15] and *Z. variagatus* (128%) [41]; so mushroom flours could be useful replacement in viscous food formulations such as soups or baked goods. The fat absorption capacity (FAC) (30.1-71.0 %) were highly comparable to FAC of wheat and soy flours (84.2, 84.4%) [15], pigeon pea 89.7%, *Z. variegatus* (33.3%), *Triticum durum* whole wheat flour in various salt concentrations (49.5 - 71.5%) [42] but much lower than that for defatted flours of oilseeds (98.5 - 302%), cowpea flours (281 - 321%) and fluted pumpkin seed flour (143%). FAC is important, as fat acts as a flavour retainer and improves the mouth feel of foods, so the mushrooms could be good samples for this property. Both the foaming capacity (FC) and the foaming stability (FS) were low with respective values of 5.7 - 16.7 % and 10.0 – 33.3%. The FS collapsed within 10 - 30 min with the rate of collapse at 0.1 - 0.7 cm^3/min. Since most commercial products have stable FS for more than 2h, then the mushrooms may not be useful for this function. The fat emulsion capacity (FEC) values of 68.4-81.6 % were better than in trunk fish (20.5 %), *Z. variegatus* (25.6%), wheat flour (7.0 - 11.0%), soy flour (18.0%) [15] and flour in various salt concentrations (49.5 - 71.5) [42] showing that mushrooms might be useful in the production of sausages, soups and cakes. The fat emulsion stability (FES) was 43.0 - 50.0 % at 5h which means they could be useful in the formation of products that depend on stable emulsions. The least gelation concentration (LGC) was 2.0 - 14.0% (w/v) with a wide variation of CV % of 80.9. The LGC were favourably comparable to trunk fish (8.0%), cowpea (10.0%), pigeon pea (10.0%), oilseed flours (12 - 18%), lupin seed flour and winged bean (14 and 18 %, respectively [14] and Great northern bean flour (10%) suggesting that the mushrooms would be better gelation agents than most literature results and may therefore be useful to provide good consistency to food and be useful in cheese and curd making. The LGC results appeared to be a function of the concentration of sulphur amino acids (SAA) (Table 5). In Table 5 the TSAA (and cystine) mg/g were: Mr_1, 44.2 (and 22.0); Mr_2, 16.5 (7.10); Mr_3, 25.1 (15.9) and Mr_4, 33.4 (24.3). Cystine is composed of two molecules of cysteine, both being made from methionine. Insulin has at least two peptide chains, which must be held together by other linkages; these are the S-S bonds of cystine [43]. Cystine is neutral since it contains two amino and two carboxyl groups; it is sparingly soluble in water. In Table 9, the LGC shows values (w/v %) of: 6.0 (Mr_1), 14.0 (Mr_2), 4.0 (Mr_3) and 2.0 (Mr_4). The Cys in TSAA followed this trend: Mr_4 (24.3 mg/g) > Mr_1 (22.0 mg/g) > Mr_3 (15.9 mg/g) and Mr_2 (7.1 mg/g) with corresponding values of LGC (w/v %): Mr_4 (2.0) < Mr_3 (4.0) < Mr_1 (6.0) < Mr_2 (14.0). While Mr_4 and Mr_2 maintained their positions of first and fourth respectively in their Cys and LGC values, Mr_3 and Mr_1 interchanged their positions. Methionine may not have such influence on the LGC value as shown in Mr_1 where it was mostly concentrated (22.2 mg/g). Therefore, the S-S bonds could have played an important role in maintaining a low value of LGC.

The minimum solubilities (pI) occurred at pH 3.0 (*P. atroumbonata*), 5.0 (*A. campestris*), 3.0 (*Pleurotus* sp.) and 4.0 (*P. ostreatus*). Asp and Glu have pI of 3.0 and 3.1 respectively whilst the pI of all other AA are between 5.0 - 10.8. The TAAA and TBAA (Table 5) did not show any significant influence on the experimentally determined pI under pH influence although the TAAA and TBAA values determined the calculated pI. The pI differences in the experimental and calculated versions are shown in Table 5 and it showed a high CV % of 87.9. The presence of only one major pI in each sample was an indication that the sample has only one major type of protein constituent. *P. ostreatus*

has the highest solubility in both acid and alkaline medium indicating that it may be the most useful of all samples in formulating carbonated beverages and very low-acid foods such as biscuit and meat products. It is interesting to note that only A. campestris did not reduce in its solubility after the pI whilst others decreased at pH 8.0 (*P. atroumbonata* and *Pleurotus* sp.) or pH 9.0 (*P. ostreatus*). This was probably due to the exposure of some hydrophobic group or electrostatic shielding at pH above 8.0, which caused the reduction in the solubility.

Conclusion

The present results showed that mushroom flours have good quality proteins, high in energy content, good substituent for human food because of good functional properties such as high water and fat absorption capacities, fat emulsion capacity and stability, good protein solubility and low gelating capacities which made mushroom protein suitable for food formulation and stabilising colloidal food systems.

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