European Journal of Food Science and Technology
Vol.7, No.3, pp.24-46, August 2019
Published by European Centre for Research Training and Development UK (www.eajournals.org)

EFFECT OF POST-HARVEST STORAGE AT AMBIENT TEMPERATURE ON NUTRITIONAL CONSTITUENTS, *IN VITRO* ANTIOXIDANT ACTIVITY AND GC-MS PROFILE OF KING TUBER MUSHROOM (*PLEUROTUS TUBER-REGIUM*)

Agoreyo, B.O.* and Oseghale, E.I.^
Department of Biochemistry, Faculty of Life Sciences, University of Benin,
P.M.B. 1154, Benin City, Nigeria

ABSTRACT: *King tuber mushroom (Pleurotus tuber-regium (Fries) Singer) is found in the continents of Africa, Asia and Australia. It is a macro-fungus with edible sclerotium, used both as food and herbal medicine. It grows naturally on decaying wood in the forest. When cultivated, it provides good source of income to farmers. It aids in transforming agricultural wastes to biomass due to its ability to grow on them. It is usually kept by local farmers and traders for long duration under ambient temperature during post-harvest storage. This research aimed at determining the effect of post-harvest storage at ambient temperature for long duration on the nutritional constituents, amino acid profile, antioxidant activity and biologically active constituents profile of the king tuber mushroom in order to ascertain the best time for its use as food or herbal medicine. Nutritional and medicinal properties of the sclerotia of the mushroom were analyzed when freshly harvested, at 8 and 16 weeks of post-harvest storage at ambient temperature. The nutritional constituents of the mushroom were determined by proximate analysis, employing the Standard Methods of Association of Official Analytical Chemists (AOAC), while the amino acid profile was determined by gas chromatography – flame ionization detector (GC-FID) technique. In vitro antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, hydrogen peroxide scavenging assay ferric reducing antioxidant potential (FRAP) assay and by monitoring the degree of lipid peroxidation. Gas chromatography – mass spectrophotometric (GC-MS) technique was employed for the identification of compounds with nutritional and medicinal properties present in the mushroom. Results show that the best time to use the mushroom as food is when it is freshly harvested; however it can be used as herbal medicine after long post-harvest storage.*

KEYWORDS: *Pleurotus tuber-regium*, King tuber mushroom, post-harvest storage, nutritional constituents, *in vitro* antioxidant activity, GC-MS profile

INTRODUCTION

King tuber mushroom (*Pleurotus tuber-regium*) is a widely known edible fungi in the tropics. *Pleurotus tuber-regium* is a saprotrophic fungus that grows naturally on hard and soft woods, it breaks down the lignin in woods with its inherent ligninolytic enzymes. (Gregori*et al*., 2007; Apetorgbor *et al*., 2013).It has a mycelium that serves as an underground storage tuber, known as sclerotium that is used for food storage and for the cultivation of new fruiting bodies (Lebauer and Isikhuemen, 2004). Sclerotia of king tuber mushroom are irregularly shaped, dark brown on the outside and whitish on the inside (Lebauer and Isikhuemen, 2004; Peterson *et al*., 2011). All over the world, they are known as the King tuber mushroom, but amongst some ethnic groups in

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Nigeria, are called "uhenru" in bini, "osu" or "eronsu" in Igbo, "orlu" in Yoruba and "katala" or "rumbagada" in Hausa Languages (Ikwechi and Ikwechi, 2009). The sclerotia of *Pleurotus tuber-regium* are usually collected from the wild (forest) by farmers and hunters in the rural area and it used as food, food additive (bulking and flavoring agents) and in the preparation of herbal medicine. As food, sclerotia can be ground, mixed with water, molded into round shapes and allowed to cook inside different soups serving as an alternative to meat and also a cheap source of protein in the food. They are majorly and widely used by cooks as food additives in the preparation of sauces and soups such as "Egusi", "Achi", "Oha" and "Ofeakwu" in large, medium and small scale restaurants serving as thickeners, bulking and flavoring agents; to increase the quantity and enhance the taste of the food. These tuberous sclerotia can be used as partial replacement for melon seeds (*Cucumeropsis manni*) or groundnut (*Arachis hypogea*) cake in traditional preparation of soups, sauces and snacks in many parts of tropical Africa, where these sclerotia are milled with melon or groundnut seeds, seasoned and molded into patties for cooking or baking (Apetorgbor *et al*., 2013). The bulking effect of sclerotia of king tuber mushroom in food has been attributed to its ability to absorb fluid and swell up to three times its volume (Okoye and Onyekweli, 2016) and has been attributed to the presence of high level of dietary fibre (Isikhuemen *et al.,* 2016). The flavors of food are usually correlated to taste and aroma and studies have shown that flavors of some edible mushrooms have been attributed to the presence of some amino acids such as valine, leucine and isoleucine which are precursors of aroma volatiles (Kuo, 2006; Moliszewska, 2014).

Sclerotia of king tuber mushroom have been considered as profound health promoting edible tuberous mushroom for decades based on its rich nutritional qualities and medicinal properties. Traditional medical practitioners have for long used them in the preparation of herbal medicine for the treatment of stomach ailments, fever, headache, cough, cold, skin diseases, obesity, inflammation, childhood anemia, asthma, smallpox, high blood pressure, cancer, malnutrition and the management of diabetes (Zhang *et al.,* 2007; Wong *et al*., 2011; Huang *et al*., 2012; Ferreira *et al.,* 2014). It aids in transforming agricultural wastes to biomass due to its ability to grow on them and when cultivated, it provides a good source of income to farmers. It is usually kept by local farmers and traders for long duration under ambient temperature during post-harvest storage. This research aimed at determining the effect of post-harvest storage at ambient temperature for long duration on the nutritional constituents, amino acid profile, antioxidant activity and biologically active constituents profile of the king tuber mushroom in order to ascertain the best time for its use as food or herbal medicine. Nutritional and medicinal properties of the sclerotia of the mushroom were analyzed when freshly harvested, at 8 and 16 weeks of post-harvest storage at ambient temperature.

MATERIALS AND METHODS

SAMPLE COLLECTION

Freshly harvested sclerotia of the king tuber mushroom was collected from the forest at Ugbojobo village at Ovia North-East Local Government Area in Edo State, Nigeria.

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

IDENTIFICATION OF SAMPLE

The sclerotia of the king tuber mushroomwas identified and authenticated in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria and African Centre For Mushroom Research and Technology Innovation (ACMRTI), University of Benin, Nigeria, where a herbarium voucher specimen number **ACMRTI/ S.0011** was deposited in its reference.

POST-HARVEST STORAGE

Post-harvest storage of freshly harvested sclerotia of the king tuber mushroom was carried out by storing them at ambient temperature (25± 2 ^{0}C) for 16 weeks. Samples were obtained from the freshly harvested sclerotia (study group 1), at eight (8) weeks (study group 2) and sixteen (16) weeks post-harvest storage (study group 3). The samples from each study group were pulverized and used for proximate analysis, antioxidant activity assay, lipid peroxidation assay, amino acid profiling and GC-MS analysis.

PREPARATION OF PULVERIZED SAMPLE

The outer dark-brown covering of the sclerotia of the king tuber mushroom was carefully removed with a clean knife and discarded, while the inside of the sclerotia was cut into small bits and ground with mortar and pestle. The pulverized sample was put into airtight containers and used for analysis.

PROXIMATE ANALYSIS

This analysis was carried out according to the method of AOAC (2012), to determine the percentage moisture, crude protein, crude lipids, ash, crude fibre and carbohydrate contents of the pulverized sample.

Moisture content determination

The percentage moisture lost due to drying at a temperature of 105^{0}C was the moisture content of the sample.

Dry matter determination

The percentage dry matter was calculated by subtracting the percentage moisture content from 100%.

Ash content determination

The residue remaining after combustion of the sample in a furnace at a temperature of 600^{0}C for 3 hours was the ash content.

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Crude Fat determination
The crude lipid content of the sample was determined by soxhlet extraction.

Crude fibre determination
Two grammes (2 g) of pulverized sample was used to determine the crude fibre, after boiling for 30 minutes with 100 mL of 1.25% sulphuric acid.

Determination of crude protein content: This method is based on the principle of the transformation of the protein and other nitrogen containing organic compounds other than nitriles and nitrate into ammonium sulphate by acid digestion, using Kjeldahl method.

Determination of carbohydrates
The total proportion of carbohydrate, which is also known as nitrogen free extract (NFE) in the sample was obtained by calculation using the percentage weight method. It was calculated by subtracting the sum of the percentages of the food nutrients (% crude protein, % crude lipids, % crude fibre and % ash) from 100%.

ANTIOXIDANT ACTIVITY ASSAY
DPPH free radical scavenging capacity
DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity was carried out according to the method of Brad-Williams *et al.* (1995) with modifications.A dose response curve was plotted with percentage DPPH scavenging capacity against different concentrations of DPPH. Linear regression analysis was further carried out to calculate the effective concentration of methanol sample extract or standard (vitamin C) required to scavenge DPPH radical by 50% (IC_{50}).

Hydrogen peroxide scavenging (H_2O_2) Assay
The ability of sample extracts to scavenge hydrogen peroxide was estimated according to the method of Ruch*et al.* (1989). A dose response curve was plotted with various percentage of H_2O_2 scavenging capacity against different concentrations of H_2O_2. Linear regression analysis was further carried out to calculate the effective concentration of methanol sample extract or standard(vitamin C) required to scavenge H_2O_2 by 50% (IC_{50}).

Ferric reducing antioxidant Potential (FRAP)
Ferric reducing antioxidant potential (FRAP) was carried out according to the method of Benzie and Strain (1996) with modifications. Ascorbic acid (vitamin C) was used as the reference standard antioxidant. Increasing concentrations of $FeSO_4$ (0.2 mM - 1 mM) were used to plot a

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

standard calibration curve from which FRAP values were extrapolated for methanol sample extract and reference standard (vitamin C).

LIPID PEROXIDATION ASSAY

Lipid peroxidation assay was carried out according to the method described by Shaw (1995) to ascertain the level of free radicals in the samples by quantifying the concentration of malondialdehyde (MDA). MDA is an end product of lipid peroxidation. MDA formed from the breakdown of the membrane lipid polyunsaturated fatty acids (PUFAs) serves as a convenient index for determining the extent of peroxidation

AMINO ACIDS PROFILE USING GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTOR (GC-FID)

Gas chromatography-flame ionization detector (GC-FID) analysis of the amino acids present in the pulverized sclerotia of king tuber mushroom was carried out according to the method of Agoreyo *et al*. (2016) with slight modifications. Amino acids present in the pulverized sample were identified and quantified using Ethylchloroformate as the derivatizing agent. Specific GC conditions were employed to characterize all amino acids that were present in the sample. Retention time and peak area were compared with that of standards to elucidate the different types and concentrations of amino acids.

GAS CHROMATOGRAPHY - MASS SPECTROPHOTOMETRIC (GC-MS) ANALYSIS

Gas chromatography-mass spectrophotometric analysis was carried out according to the method described by Afieroho and Ugoeze, 2014. Methanolic extract of the pulverized sample were used to identify and quantify all bioactive compounds present in the sample. Identification and quantification were done according to the retention time and peak formation of the compounds.

STATISTICAL DATA ANALYSIS

All analyses were carried out in triplicates. All data presented as means ± standard error of mean (MEANS ± SEM) of three determinations. The results obtained were analyzed by employing the statistical software, SPSS Version 21.0, SPSS Inc., Chicago, USA. Statistical analysis was performed using one-way analysis of variance (ANOVA) and differences were considered significant at $p < 0.01$.

RESULTS

PROXIMATE COMPOSITION

The results of the proximate composition of the sclerotia of king tuber mushroom (*Pleurotus tuber-regium*) as shown in Table 1 below revealed a significant decrease at ($p< 0.01$) in the

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

percentages of the moisture content, ash content, crude fat and crude fibre contents during the duration of the post-harvest storage. Conversely, there was a significant increase at $p < 0.01$ in the percentage dry matter and carbohydrates with a slight increase in the crude protein content during the period of post-harvest storage. Moisture, ash, fat and fibre contents, however, decreased during the post harvest storage period.

Table 1. Proximate analysis of the sclerotia of king tuber mushroom during postharvest storage at ambient temperature (PHSAT)

PROXIMATE COMPOSITION (%)	**FRESHLY HARVESTED**	**8 WEEKS PHSAT**	**16 WEEKS PHSAT**
Moisture (%)	27.27± 0.57	11.07± 0.17*	5.13± 0.01*
Dry matter (%)	72.71± 0.57	88.93± 017**	94.87± 0.01**
Ash (%)	3.19± 0.04	2.97± 0.12	2.11± 0.0*
Crude fat (%)	0.56± 0.007	0.53± 0.03	0.37± 0.07*
Crude fibre (%)	1.23± 0.003	1.12± 0.003*	1.12± 0.005*
Crude protein (%)	4.38± 0.0	5.54± 0.58	6.75± 0.25
Carbohydrate (N.F.E) (%)	63.38± 0.55	78.75± 0.51**	84.46± 0.32**

Results are Means ± SEM for three determinations.
* denotes significant decrease at $p < 0.01$ compared to when freshly harvested.
** denotes significant increase at $p<0.01$ compared to when freshly harvested.

ANTIOXIDANT ACTIVITY

The results of the antioxidant activity assays of the sclerotia of the king tuber mushroom are shown in tables 2 to 6 below. The results of the assays for both the sample and the reference standard were in dose dependent manner. The results revealed that the antioxidant activity of the sclerotia of king tuber mushroom (*Pleurotus tuber-regium*) decreased with increased duration of post-harvest storage.

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Table 2. DPPHfree radical scavenging capacity of the sclerotia of king tuber mushroom during post harvest storage at ambient temperature (PHSAT)

DPPH CONCENTRATION	VITAMIN C (%)	FRESHLY HARVESTED (%)	8 WEEKS PHSAT (%)	16 WEEKS PHSAT (%)
0.2mg/ml	52.07 ± 7.79	33.82 ± 0.23	26.55 ± 0.21*	12.60 ± 0.77*
0.4mg/ml	70.40 ± 1.26	35.63 ± 0.48	28.47 ± 0.31*	14.13 ± 0.29*
0.6mg/ml	80.74 ± 2.01	40.14 ± 1.43	29.59 ± 0.09*	17.34 ± 0.39*
0.8mg/ml	92.59 ± 0.23	48.39 ± 1.03	30.64 ± 0.14*	21.81 ± 1.69*
1.0mg/ml	98.13 ± 0.06	54.23 ± 0.29	31.48 ± 0.20*	26.51 ± 1.21*

Results are Means± SEM for three determinations.
* denotes significant decrease at $p < 0.01$ compared to when freshly harvested.

Table 3. DPPH IC_{50} values of the sclerotia of king tuber mushroom during post harvest storage at ambient temperature (PHSAT)

ASCORBIC ACID (mg/ml)	FRESHLY HARVESTED (mg/ml)	8 WEEKS PHSAT (mg/ml)	16 WEEKS PHSAT (mg/ml)
0.096	0.882	1.588	2.310

Table 4. Hydrogen peroxide scavenging ability of the sclerotia of king tuber mushroom during post harvest storage at ambient temperature (PHSAT)

HYDROGEN PEROXIDE CONCENTRATION	VITAMIN C (%)	FRESHLY HARVESTED (%)	8 WEEKS PHSAT (%)	16 WEEKS PHSAT (%)
2µg/ml	18.53± 0.24	10.74± 0.54	10.27± 0.62	9.97± 0.33
4µg/ml	33.63± 1.31	16.29± 0.47	15.76± 1.99	16.15± 1.38
6µg/ml	57.44± 0.89	25.37± 0.49	24.78± 0.26	21.95± 0.14
8µg/ml	81.68± 0.44	36.14± 0.58	39.79± 0.88	30.72± 0.34
10µg/ml	97.16± 0.16	61.71± 0.89	55.89± 0.27	43.52± 0.30*

Results are Means± SEM for three determinations.
* denotes significant decrease at $p < 0.01$ compared to when freshly harvested

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Table 5. Hydrogen peroxide IC_{50} values of the sclerotia of king tuber mushroom during post harvest storage at ambient temperature (PHSAT)

ASCORBIC ACID (µg/ml)	FRESHLY HARVESTED (µg/ml)	8 WEEKS PHSAT (µg/ml)	16 WEEKS PHSAT (µg/ml)
5.25	9.28	9.59	12.22

Table 6. Ferric reducing antioxidant potential (**FRAP**) of the sclerotia of king tuber mushroom during post harvest storage at ambient temperature (PHSAT)

FRAP CONCENTRATION	VITAMIN C (mMFe(II)/g)	FRESHLY HARVESTED (mMFe(II)/g)	8 WEEKS PHSAT (mMFe(II)/g)	16 WEEKS PHSAT (mMFe(II)/g)
100 µg/ml	0.61± 0.0009	0.09± 0.001	0.04± 0.001*	0.014±0.0003*
200 µg/ml	0.66± 0.0007	0.16± 0.002	0.05± 0.0007*	0.018± 0.001*
300 µg/ml	0.67± 0.013	0.24± 0.01	0.06± 0.0008*	0.022±0.0006*
400 µg/ml	0.70± 0.0002	0.30± 0.002	0.08± 0.0001*	0.03± 0.0006*
500 µg/ml	0.74± 0.0005	0.39± 0.001	0.083± 0.002	0.04± 0.0005*

Results are Means± SEM for three determinations.

* denotes significant decrease at $p<0.01$ compared to when freshly harvested.

LIPID PEROXIDATION

Table 7 shows the levels of lipid peroxidation in the sclerotia of the king tuber mushroom during post harvest storage. The concentration of malondialdehyde, which shows the extent of lipid peroxidation, increased during the period of harvest storage.

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Table 7. Lipid peroxidation levels of the sclerotia of king tuber mushroom during post harvest storage at ambient temperature (PHSAT)

LIPID PEROXIDATION	**FRESHLY HARVESTED**	**8 WEEKS PHSAT**	**16 WEEKS PHSAT**
Malondialdehyde (M.D.A) concentrations in mM/gF.W	$2.14 \times 10^{-4} \pm 0.0$	$3.99 \times 10^{-4} \pm 0.0$	$6.98 \times 10^{-4} \pm 0.0$

Results are Means ± SEM for three determinations.
** denotes significant increase at $p < 0.01$ compared to when freshly harvested.

GC-FID AMINO ACIDS PROFILE

All essential and non-essential amino acids present in the sclerotia of the king tuber mushroom were identified and their different concentrations were quantified by GC-FID during the period of post-harvest storage at ambient temperature (PHAST) as shown in figure 1 below. Aspartate and glutamate had the highest concentrations, while the concentrations of cysteine and tryptophan were the lowest.

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

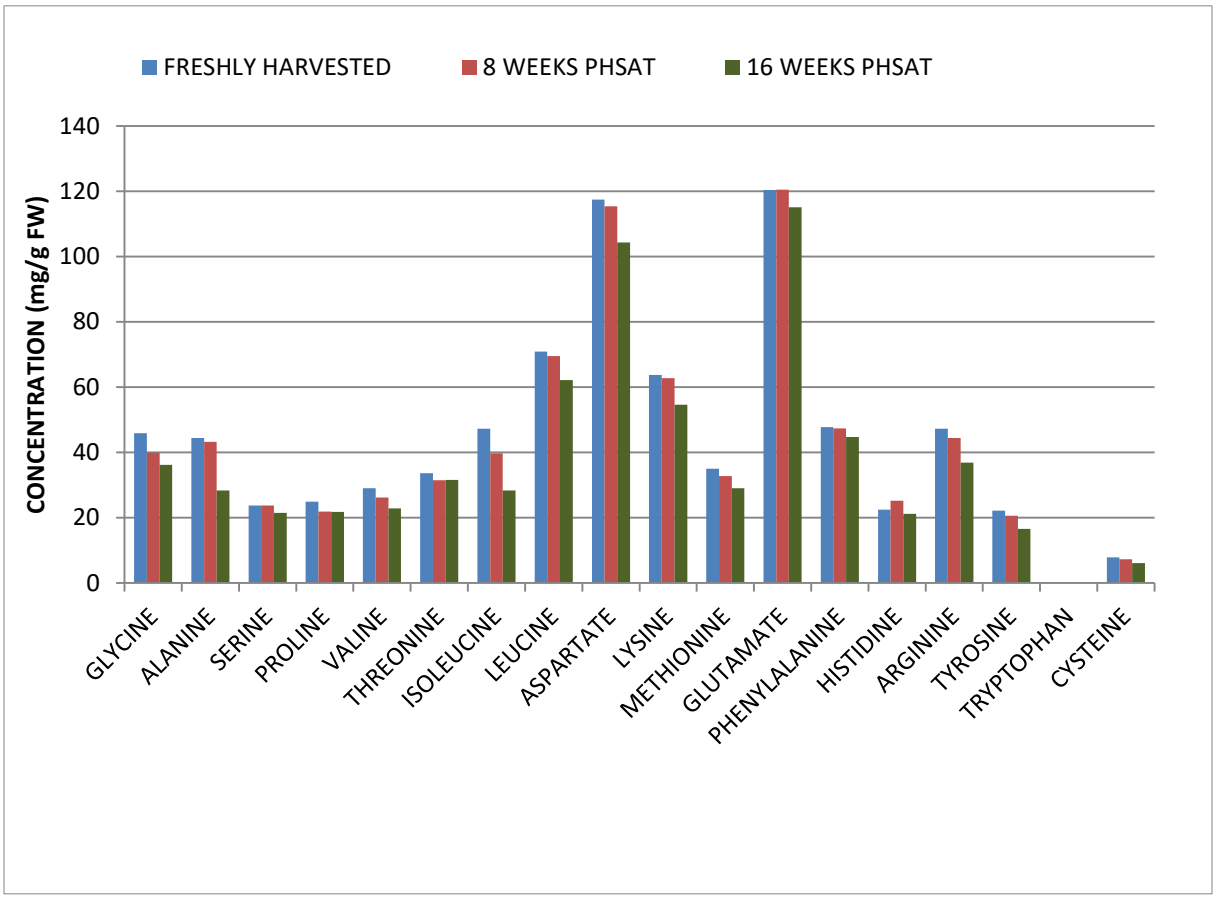


Figure1. Amino acids profile of the sclerotia of king tuber mushroom during post-harvest storage at ambient temperature (PHSAT)

GAS CHROMATOGRAPHY – MASS SPECTROPHOTOMETRIC (GC-MS) ANALYSIS

The GC-MS analysis of the sclerotia of the king tuber mushroom were carried out with the methanol extract of the sample during post-harvest storage. Methanol extract of the sclerotia of the king tuber mushroom revealed the presence of six (6) compounds in the freshly harvested samples, four (4) compounds at 8 weeks post-harvest storage at ambient temperature (PHSAT) and eight (8) compounds at 16 weeks post-harvest storage at ambient temperature (PHSAT) (Figure 2 and Table 8).

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

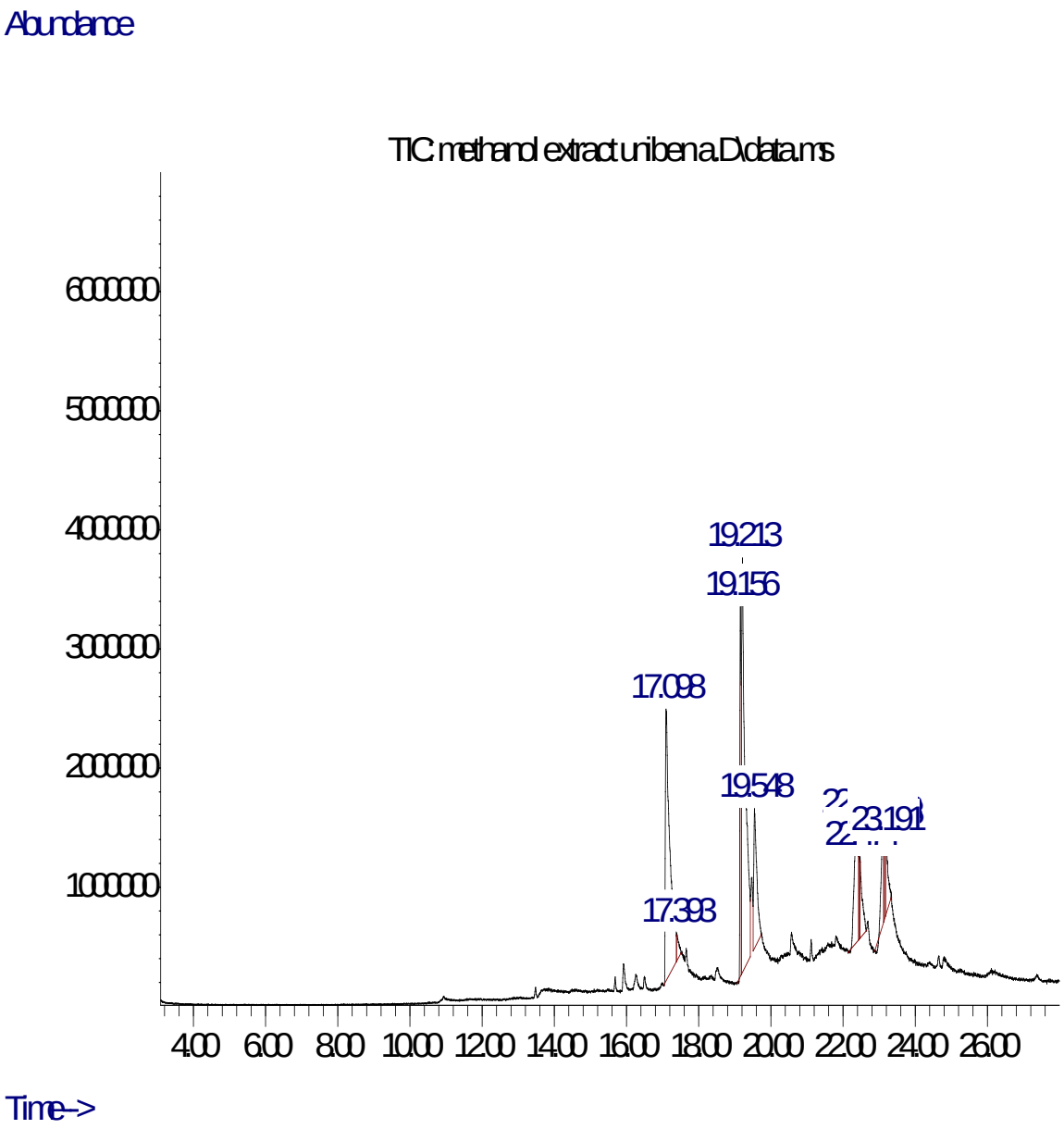
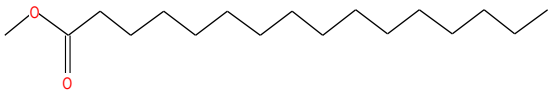
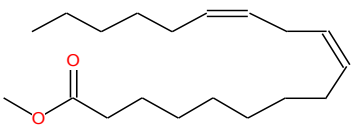
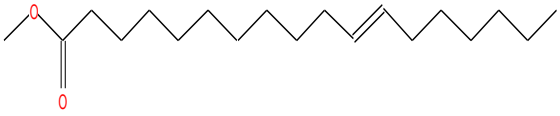


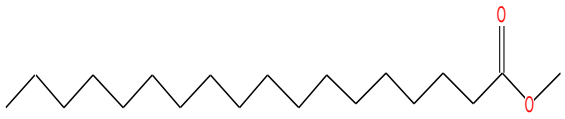
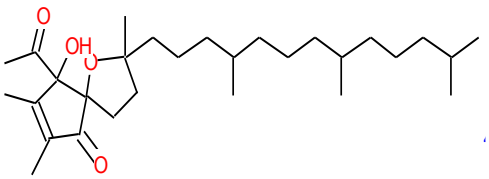
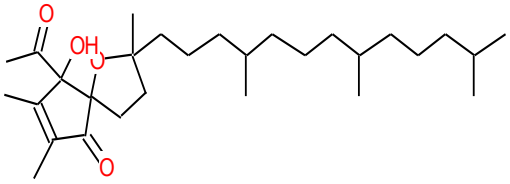
Figure 2. Total Ion Chromatogram (TIC) of methanol extract of freshly harvested sclerotia of king tuber mushroom (*Pleurotus tuber-regium*)

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Table 8. GC-MS compounds of the methanol extract of freshly harvested sclerotia of king tuber mushroom

Compound name; chemical formula	**Retention time (min)**	**Area**	**Common name; Molecular weight; Chemical structure; Biological activity**
Hexadecanoic acid, methyl ester $C_{17}Hc_{34}O_2$	17.098	24.57%	Methyl palmitate; 270.459g/mol  Flavouring agent, anti- inflammatory, anti-bacteria and antifungal (Chandrasekaran *et al.*, 2011).
9,12-octadecadienoic acid, methyl ester $C_{19}H_{34}O_2$	19.156	8.48%	Methyl linoleate; 294.479g/mol  Anti-hypertensive, antioxidant and anti-cancer (Yu *et al.*, 2005).
11- octadecenoic acid, methyl ester $C_{19}H_{36}O_2$	19.213	31.71%	Methyloctadecenoate; 296.495g/mol  Anti-cholesterolaemic and anti-carcinogenic (Asghar *et al.*, 2011).
Methylstearate $C_{19}H_{38}O_2$	19.548	8.75%	Stearic acid; 298.511g/mol

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

			 White crystal semi-solid ester; flavor component in food; Lubricant; used in the manufacture of pharmaceuticals, cosmetics and soaps; Surfactant and softening agent (Enas and Duha, 2014).
α -Tocospiro A $C_{29}H_{50}O_4$	22.387	9.30%	462.715g/mol  Cytotoxicity against human A549 and HL60 Lung cancer cell lines. Anti- mycobacterial activity against mycobacterium tuberculosis H37RV (Chen *et al*., 2010).
α- Tocospiro B $C_{29}H_{50}O_4$	23.097	4.18%	462.715g/mol  Cytotoxic activity against P-388 (leukamia) and HT-29 (human colon adenocarcinoma) cell lines invitro (Chen *et al*., 2006).

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Methanol extract of the sclerotia of king tuber mushroom at 8 weeks post-harvest storage at ambient temperature(PHSAT)

The GC-MS components of the methanol extract of the sclerotia of king tuber mushroom at 8 weeks of post-harvest storage at ambient temperature (PHSAT) are shown below in figure 3 and table 9:

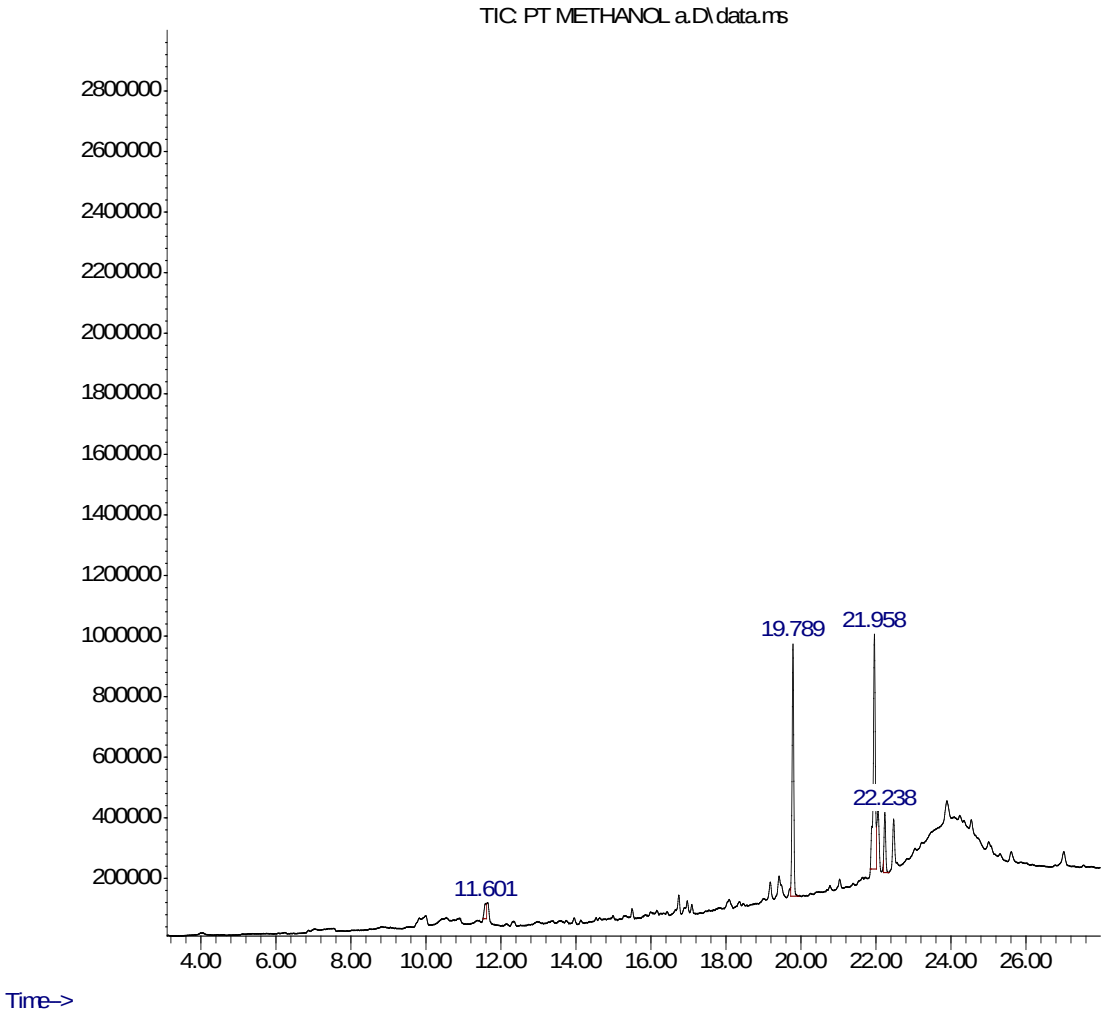
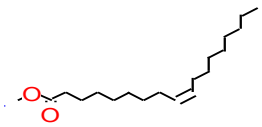
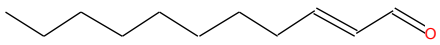
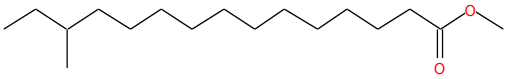
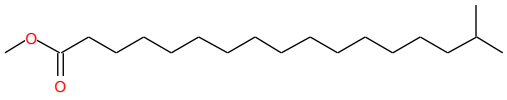


Figure 3. Total Ion Chromatogram (TIC) of methanolic extract of sclerotia of king tuber mushroom at 8 weeks PHSAT

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Table 9. GC-MS compounds of methanol extract of the sclerotia of king tuber mushroom at 8 weeks post-harvest storage at ambient temperature (PHSAT)

Compound name; chemical formula	**Retention time (min)**	**Area**	**Common name; Molecular weight; Chemical structure; Biological activity**
Udecenal; $C_{11}H_{20}O$	11.601	3.33%	Trans-2- undecenal; 168.28g/mol;  *Flavoring agent with antibacterial activity.
Pentadecanoic acid - 13-methyl-methyl ester; $C_{17}H_{34}O_2$	19.789	40.20%	270.457g/mol;  Antimicrobial, antioxidant (Arumugam and Vijisaral., 2013)
9-octadecenoic acid (z)-methyl ester; $C_{19}H_{36}O_2$	21.958	47.18%	Methyloleate;  Antibacterial, antiinflamatory, antiacne, antipruritics, anticarcinogenic activities. A food additive with bioremediating capacity (Asghar *et al*., 2011).
Heptadecanoic acid - 16-methyl ester; $C_{19}H_{38}O_2$	22.238	9.29%	Methylisosterate; 298.511g/mol  Antioxidant and antimicrobial activities (Vetha*et al*., 2016).

*Pubchem compounds (open chemistry database)

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Methanol extract of the sclerotia of king tuber mushroom at 16 weeks of post harvest storage at ambient temperature (PHSAT)

The GC-MS components of the methanolic extract of the sclerotia of king tuber mushroom at 16 weeks of post-harvest storage are shown below in figure 4 and table 10:

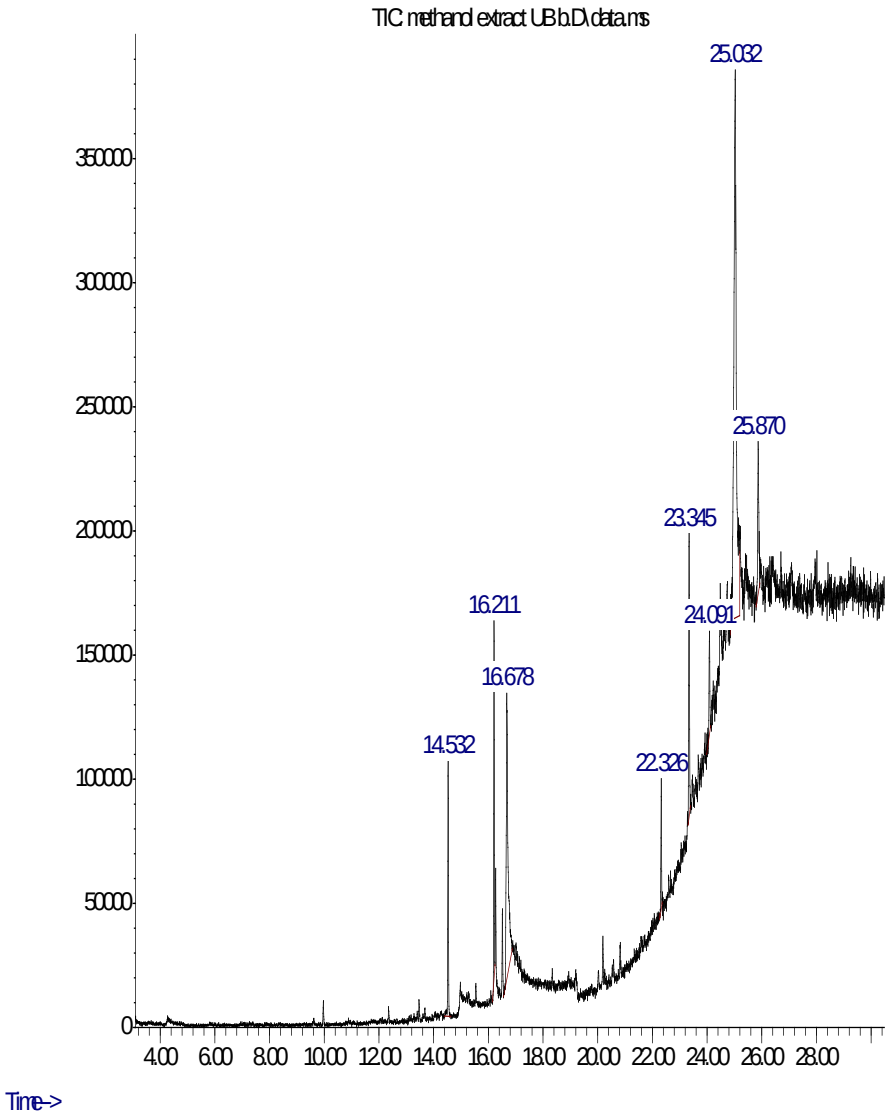
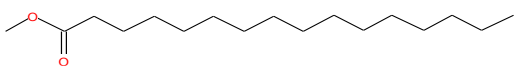
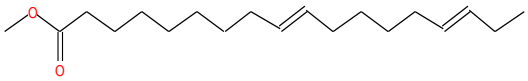
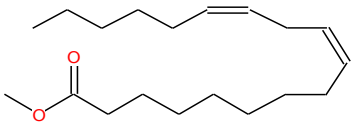
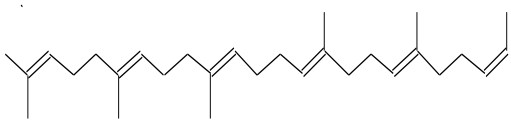


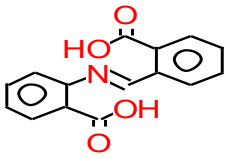
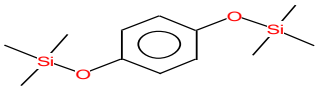
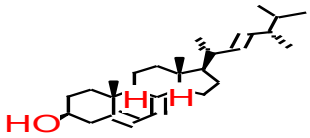
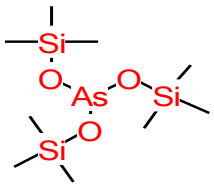
Figure 4: Total Ion Chromatogram (TIC) of methanol extract of the sclerotia of king tuber mushroom at 16 weeks PHSAT

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Table 10: GC-MS compounds of methanolic extract sclerotia of king tuber mushroom at 16 weeks post harvest storage at ambient temperature **(**PHSAT)

Compound name; chemical formula	**Retention time (min)**	**Area**	**Common name; Molecular weight; Chemical structure; Biological activity**
Hexadecanoic acid methyl ester; $C_{17}H_{34}O_2$	14.531	6.28%	270.459g/mol;  Antioxidant, antimicrobial, anti-inflamatory, haemolytic-5-α reductase inhibitor, antiandrogenic activities (Majinda and Abubakar, 2016; Ojekale *et al*., 2016).
9,15 – octadecadienoic acid methyl ester-(z-z); $C_{19}H_{34}O_2$	16.213	7.56%	Methyloctadecandienoate; 249.472g/mol  *Involved in cell signalling.
9,12 – octadecadienoic acid-(z-z); $C_{19}H_{34}O_2$	16.676	18.21%	Methyllinoleate; 294g/mol  Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, anticancer and anti-inflamatory properties (Majinda and Abubakar, 2016).
Squalene; $C_{30}H_{50}$	22.324	2.84%	410g/mol;  Antioxidant and anticancer activities (Enas and Duha, 2014; Rajani*et al*., 2015).

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Anthranilic acid N-(2-carboxyphenylmethylene)-; $C_{55}H_{11}NO_4$	23.343	8.22%	269.256g/mol;  *Anti-inflamatory agent.
Hydroquinone 2TMS derivative; $C_6H_4(OH)_2$	20.092	2.95%	110.11g/mol;  *reducing agent, antioxidant activity.
Ergosterol; $C_{28}H_{44}O$	25.031	47.87%	396.65g/mol;  Antifungal, biological precursor provitamin to Vitamin D_2 (Enas and Duha, 2014).
Arsenous acid tris (tri-methylsilyl); $CH_{27}AsO_3Si_3$	25.872	6.09%	342.489g/mol;  Antimicrobial, antioxidant activity (Omar and Kader, 2014).

*Pubchem compounds (open chemistry database)

DISCUSSION

The proximate composition of the sclerotia of king tuber mushroom, revealed a significant decrease at $p< 0.01$ in the percentage moisture, ash, crude fat and crude fibre during the period of post harvest storage at ambient temperature(PHSAT), however, percentage carbohydrate, protein and dry matter increased (Table 1). The reduced moisture, ash, crude fat and fibre contents that

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

were observed were similar to the report of Maleku *et al* (2014), which showed that storage caused a significant reduction on the nutritional composition of stored yam tubers. The report of Tessema *et al* (2015) on proximate composition of chickpea (*Cicer arietinum L.*) that was stored for six (6) months, showed a significant decrease in the moisture, crude fat, and carbohydrate content but an increase in crude fibre, protein and ash contents. The reduction in moisture content that was observed in the sclerotia of king tuber mushroom during PHSAT can be attributed to loss of water vapour due to transpiration and metabolic activities (Olayemi *et al.*, 2012). High moisture content is linked to susceptibility to microbial growth, fungal attack and shorter shelf life; therefore reduced moisture content that was observed is an indication of longer shelf life with post-harvest storage and most likely, a minimized fungal and bacterial attack occurrence (Muhammad *et al*., 2015). Decrease in fibre content could be attributed to the activities of endogenous glycanases that can degrade fibre (Guo *et al.*, 2015). Decrease in crude fat could be attributed to the susceptibility of fatty acids to oxidative degradation because when this is reduction in water as observed in the scletotia during post harvest storage, metal catalysts which promote the oxidation of unsaturated nutrients become more reactive, thereby accelerating the rate of oxidation (Pan *et al.*, 2017). The low fat and its reduction in the sclerotia during post harvest storage is also very beneficial to health. The increase in carbohydrate content showed that the sclerotia could serve as a good source of energy for the body in all the post-harvest storage period, however it will be advisable for those with conditions like diabetes mellitus (that require minimal amount of carbohydrate) to consume it when freshly harvested.. The increased protein content of sclerotia during post-harvest storage increased its nutritional value.

The results of the freshly harvested sclerotia showed that they are good sources of antioxidants as the results showed relatively close antioxidant capacity compared to ascorbic acid (vitamin C), the reference standard antioxidant.During post-harvest storage, antioxidant activity of the sclerotia of king tuber mushroom decreased significantly. Some phytochemicals such asphenols have been reported to be responsible for antioxidant activity, hence decreased antioxidant activity of the sclerotia of king tuber mushroom (*Pleurotus tuber-regium*) could be attributed to a reduced amount of total phenols in the sclerotia during the period of post-harvest storage. Lipid peroxidation showed that there were significant increase at $p< 0.01$ in the MDA concentrations during the storage period. This correlates with the decreased antioxidant activity of the sclerotia in this study. GC-FID showed a total of eighteen (18) essential and non-essential amino acids in the sclerotia. Aminoacids are of nutritional benefits and they provide important source of precursors for various aroma volatiles, therefore their concentrations are determinants of the quantity and quality of nutrients. Tryptophan. glutamate and aspartate are known for their uses as flavoring agents (Halpern, 2000). In this study they showed the highest concentrations; this scientifically explains the bases of the wide use of sclerotia of king tuber mushroom (*Pleurotus tuber-regium*) as food additive. The study also showed that the relatively high amount of aspartic

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

acid in the sclerotia was also in agreement with the reports of Tsai *et al.* (2008), who reported the presence of glutamic acid and aspartic acid in mushroom soup, proving that they were the main factor for the sweet taste in many species of mushroom. This could suggest why glutamic and aspartic acids are widely used as both sweetening and flavouring agents.

The GC-MS analysis of the methanol extract of the sclerotia of king tuber mushroom when freshly harvested, at 8 and 16 weeks of post-harvest storage was carried out to determine their bioactive components. Freshly harvested samples revealed the presence of six (6) compounds in the methanol extract. At 8weeks PHSAT, four (4) compounds were found in the methanol extract of the sclerotia. At 16 weeks PHSAT, eight (8) compounds were identified in the methanol extract. All the identified compounds have various characteristics such as anticancer, antioxidant, anti-cholesterolaemic, anti-diabetic, anti-inflammatory, nematicide, hepatoprotective, antihistaminic, anti-hypertensive, antimicrobial, hypocholesterolemic properties (Majinda and Abubakar, 2016). Some bioactive compounds which were in the freshly harvested sclerotia were not present in the subsequent stages of PHSAT, they may have undergone conversion to other compounds during the period of post-harvest storage (Oms-oliu *et al*., 2011). These bioactive compounds (secondary metabolites) identified in the sclerotia, give credence to the reasons for their trado-medical use, such as in the treatment of high blood pressure, high blood sugar levels, fever, headache, cough and catarrh, skin diseases, small pox, anaemia, stomach and digestive problems amongst others. Therefore the king tuber mushroom before and after post-harvest storage could be employed in the manufacture of products of pharmaceutical and therapeutic values (Huang *et al*., 2012; Zhang *et al.*, 2007; Ferreira *et al.*, 2014). The effect of post harvest storage especially for long duration on the sclerotia of king tuber mushroom was revealed in this study and the results obtained in the study will further increase the proper utilization of this mushroom as food or herbal medicine.

CONCLUSION

Post-harvest storage of the sclerotia of king tuber mushroom (*Pleurotus tuber-regium*) at ambient temperature resulted in increase in their protein and carbohydrate compositions and reduction in their fat, fibre, moisture and antioxidant compositions. The presence of amino acids in the sclerotia of king tuber mushroom gives credence to their unique taste and aroma in food, although some of them decreased during post-harvest storage. Post harvest storage increased the health promoting compounds in the sclerotia of king tuber mushroom. These health promoting compounds that were identified in the sclerotia of the king tuber mushroom also support the claims of their usefulness in the alleviation of common medical dysfunctions especially after long durations of post-harvest storage.

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Conclusively, the sclerotia of the king tuber mushroom are better used as food when they are freshly harvested and can be best used as herbal medicine even after long duration of post harvest storage.

REFERENCES

Afieroho, O.E., Ugoeze, K.C. (2014). Gas Chromatography-Mass Spectroscopic (GC-MS) Analysis of n-hexane Extract of *Lentinus tuber-regium*(Fr) Fr (Polyporaceae) Syn *Pleurotus tuber regium* Fr sclerotia. *Tropical Journal of Pharmaceutical Research* November, **13** (11): 1911-1915.

Agoreyo, B.O., Egharevba, M.E., Okpapi, P.E., Ogiugo, M.E. (2016). Phytosterol, phenolic acid, amino acid and sugar profiles of the unripe fruits of *Solanum melongena* L. (Round variety). *African Scientist*, **17** (2): 191-201.

Apetorgbor, A.K., Dzomeku, M. Apetorgbor, M.M. (2013). Growth factors and cultivation of *Pleurotus tuber-regium* on selected plant wastes. *International Food Research Journal*, **20** (6): 3387-3393.

.Arumugam, S., Vijisaral, E.D. (2014). GC-MS analysis of bioactive constituents of *Indigoferasuffruticosa* leaves. *Journal of chemical and pharmaceutical research*, **6**(8): 294- 300.

Asghar, S.F., Habib-ur-Rehman, Choudahry, M.I., Atta-ur-Rahman. (2011). Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bio-assays of crude extract of *Iris germanica*. *International Journal of Genetics and Molecular Biology*, **3** (7): 95-100.

Association of official analytical chemists (AOAC) (2012). *Official Method of Analysis of AOAC International*, 19th**ed**.; AOAC International: Gaithersburg, MD, USA.

Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, **239** (1):70-76.

Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und -Technologie/Food Science and Technology, **28** (1):25-30.

Chandrasekaran, M., Senthilkumar, A., Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from leaves of *Sesuvium portulacastrum* L. *European Review for Medical and Pharmacological Sciences*, **15** (7): 775–780.

.Chaudhary, S., Chandrashekar, K.S., Pai, K.S.R., Setty, M.M., Devkar, R.A., Reddy, N.D., Shoja, M.H. (2015). Evaluation of antioxidant and anticancer activity of extract and fractions of *Nardostachys jatamansi* DC in breast carcinoma. *BMC Complementary and Alternative Medicine*, **15** (50): 1- 13.

Chen, J.J., Lin, W.J., Shieh, P.C., Chen, I.S., Peng, C.F., Sung, P.J. (2010). A New Long-Chain Alkene and Antituberculosis Constituents from the Leaves of *Pourthiaea lucida*. *Chemistry & Biodiversity*, **7** (3): 717–721.

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Chen, J.J., Chou, E.T., Duh C.Y., Yang S.Z., Chen I.S. (2006). New cytotoxic tetrahydrofuran- and dihydrofuran-type lignans from the stem of *Beilschmiedia tsangii*. *Planta Medica*,**72**(4): 351-357.

Enas, J.K., Duha, A.A. (2014). Phytochemical Characterization using GC-MS Analysis of Methanolic Extract of Moringa oleifera(FamilyMoringaceae) Plant Cultivated in Iraq. *Chemistry and Materials Research*, **6** (5): 9-26.

Ferreira C.F.R., Obodai, M., Fernandes, A., Barros, L., Narh Mensah, D.L., Dzomeku, M., Urben, A.F., Prempeh, J., Takli, R.K. (2014). Evaluation of the Chemical and Antioxidant Properties of Wild and Cultivated Mushrooms of Ghana. *Molecules*, **19** (12): 19532-19548.

Gregori, A., Svageli, M., Pohleven, J. (2007). Cultivation Techniques and Medicinal Properties of *Pleurotus spp*. *Food technology and biotechnology*, **45** (3): 238-249.

Guo, P.P., Li, P.L., Li, Z.C., Stein, H.H., Liu, L., Xia, T., Yang, Y.Y., Ma, Y.X. (2015). Effect of post-harvest storage duration and variety on nutrient digestibility and energy content wheat in finishing pigs. *Asian Australas Journal of Animal Science*, **28** (10):1488-1495.

Halpern, B.P. (2000). Glutamate and the flavor of foods. *The journal of nutrition*, **130** (4): 910S-914S.

Huang, H.Y., Mallikarjuna, K., Chaing, Y.Y., Chien, T.Y., Tsai, Y.C. (2012). *Pleurotus tuber-regium* polysaccharides attenuate hyperglycemia and oxidative stress in experimental diabetic rats. *Evidence-Based Complementary and Alternative Medicine.* doi:10.1155/2012/856381.

Ikewuchi, C.C. and Ikewuchi J.C. (2009). Chemical Profile of *Pleurotus tuber-regium*(Fr) Sing's Sclerotia. *Pacific Journal of Science and Technology*, **10** (1): 295-299.

Isikhuemen, O.Y., Nwaoguala, C.N.C., Shittu, H.O., Odewale, J.O., Eke, C.R., Isikhuemen, E.M. (2016). Effect of phytohormones on callus induction and maintenance of *Dioscoreophyllum cumminsii* (Stapf) Diels cotyledon explant. *European International Journal of Science and Technology*, **5** (5): 1-11.

Kuo, M. (2006). "Morel data collection project: preliminary results". Retrieved 2009-05-26.

Majinda, R., Abubakar, M. (2016). Phytochemical constituents and antimicrobial activity of *Albizia adianthifolia. International Journal of Pharmacognosy and Phytochemical Research*. **3**(3): 1-7.

Muhammad M.M., Fai, F.Y., Wilson, L.D., Simon, Y.S. (2015). Chemical Composition and Nutritive value of *Leptadenia hastate* leaves. *International Journal of Innovation Sciences and Research*, **4** (10): 480-484.

Moliszewska, E. (2014).Mushroom flavour**.** *Folia Biologica et ecologica*, **10** (1): 80-88.

Nguyen, V., Lee, C.J., Wilson, L., Jordan, M.A. (2010). Hesperidin suppressed proliferations of both human breast cancer and androgen-dependent prostate cancer cells. *Phytotherapy research*, **24** (1): S15- S19.

Ojekale, A.B., Lawal, A.O., Lasisi, M.O. (2016).*Cyathula prostrata*: A potential herbal hope for hypertensives, an animal model study and its secondary metabolites assessent via GC-MS. *European Journal of Medicinal Plants*, **14** (2): 1-10.

Okoye, E.I., Onyekweli, A.O. (2016). Development and evaluation of *Pleurotus tuber-regium*-corn starch composite as a direct compression multifunctional excipient. *International Journal of Pharmaceutical Investigations*,**6**(1):10-22.

Olayemi, F.F., Raji, A.O, Oyelese, O.A, Oyewole S.N., and Omodara M.A. (2013). Effective smoking kiln for developing country. *International Journal of Scientific and Engineering Research*, **4** (1): 847-853.

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)

Oms-oliu, G., Hertog, M., Van de Poel, B., Ampofo-Asiama, J., Geeraerd, A. (2011). Metabolic characterization of tomato fruit during preharvest development, ripening and postharvest shelf-life. *Postharvest Biology and Technology*, **62** (1): 7-16

Pan, X., Zhang, W., Wang, C., Shi, B. (2017). Effect of storage temperature and time on the nutritional quality of walnut male inflorescences. *Journal of food and drug analysis*, **25** (1): 374-384.

Peterson, R.H., Hughes K.W., Psurtseva N. (2011). "Biological Species in *Pleurotus*". The University of Tennessee-Knoxville. Retrieved 2011-03-11.

Rajani, D., Haddad, S., Boudriga.S.,Akhaja, T.N., Raval, J.P., Porzio, F., Armand, S., Askri, M., Knorr, M., Rousselin, Y., Kubocki, M.M. (2015). A strategic approach to the synthesis of functiondizedspirooxindolepyrrolindine derivatives: *in vitro* antibacterial, antifungal, antimalarial and antitubercular studies. *New Journal of Chemistry*, **39** (1): 520-528.

Ruch, R.J., Cheng, S.J., Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, **10** (1):1003-1008.

Shaw,B.P. (1995). Effect of mercury and cadmium on the activities of anyopxidative enzymes in the seedlings of *Phaseolusaureus*. *Biology of plant*, **37** (1): 587- 596.

Tessema, K., Tadesse, F.T., Kurabachew, H. (2015). Effect of different treatments for the control of Callosobruchus chiensis L. on proximate composition of chickpea (Cicerarietinum L.), in Meskan district, Ethiopia. *Journal of Stored Products and Postharvest Research*,**6** (10): 83- 90.

Tsai, S.Y., Tsai, H.L., Mau, J.L. (2008). Non-volatile taste component of *Agaricusblazei, Agrocybe cylindracea* and *Boletus edulis*. *Food Chemistry*, **107** (1): 977-983.

Vetha, P., Fahad, A., Sami, A., Latifa, B.M., Saleh, A., Haysam, T., Ibrahim, B., Sameh, A. (2016). Guidelines for palivizumab prophylaxis in infants and young children at increased risk for respiratory syncytial virus infection in Saudi Arabia. *International Journal of Pediatrics and Adolescent Medicine*, **3**(1): 38- 42.

Wong, K.H., Lai, C.K.M., Cheung, P.C.K. (2011). Immuno-modulatory activities of mushroom sclerotial polysaccharides. *Food Hydrocolloids*, **25** (2): 150 -158.

Yu, F.R., Lian, X.Z., Guo, H.Y., McGuire, P.M., Li R.D., Wang, R. Yu, F.H. (2005). Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui (Euphorbiaceae*) and their inhibitory effects on the human SGC-7901 cells. *Journal of Pharmaceutical Science*,**8** (3): 528–535.

Zhang, M., Cui, S.W., Cheung, P.C.K., Wang, Q. (2007). Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science & Technology*, **18** (1): 4-19

Print ISSN: ISSN 2056-5798(Print)
Online ISSN: ISSN 2056-5801(online)