



Pre-Treatments Effect on Proximate and Phytochemical Components of Flour Prepared from Oyster Mushroom

Abenaa A. Okyere^{1,*}, Emmanuel K. Gasu¹, Isaac K. Asare¹, Dora Duah-Bisiw¹, Shadrack Donkor² and Ernestina A. Ayeh¹

¹Radiation Technology Centre, Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon, Accra-Ghana.

²Applied Radiation Biology Centre, Radiological and Medical Sciences Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon, Accra-Ghana.

ARTICLE INFO

Article history:

Received: 18 February 2016;

Received in revised form:

9 April 2016;

Accepted: 14 April 2016;

Keywords

Mushroom,
Oven-drying,
Blanching,
Citric Acid,
Ascorbic Acid.

ABSTRACT

The effect of pre-drying treatments on the proximate and phytochemical quality of oven-dried mushroom was evaluated using conventionally accepted methods. The pre-treatments used were dipping in 10% citric acid solution, dipping in 10% ascorbic acid solution and a control. The pre-treatments resulted in significant differences in pH, protein, vitamin C, some trace elements and phytochemicals present. Control sample had a protein content of 7.4%, while a range of 19.4% and 14.3% was recorded for samples pre-treated with citric acid and ascorbic acid respectively. The total phenolic content was in the range of 6.39 – 32.26 mg GAE/g of dry weight. Thus the use of these pre-treatments can be used to effect changes in some proximate and phytochemical components of oyster mushroom powder.

© 2016 Elixir all rights reserved.

Introduction

Mushrooms have been consumed and appreciated for their flavour, economic and ecological values and medical properties for many years (1). Mushrooms have been used as food and food-flavouring material in soups and sauces for centuries, due to their unique and subtle flavour. The occurrence of high amounts of proteins, carbohydrates and fibres and low fat contents is often referred in the literature in relation to their nutritional value. Furthermore, they have significant levels of vitamins, namely thiamine, riboflavin, ascorbic acid and vitamin D2, as well as minerals (2). Regarding their medicinal value, mushrooms are effective as antitumor, antibacterial, antiviral and haematological agents and in immunomodulation treatments (3); (4).

Mushrooms, like other fruits and vegetables, respire, grow, mature and senesce after the harvest resulting in weight loss, veil-opening, browning, wilting and finally in spoilage which affect quality and shelf-life significantly (5). Owing to the short shelf life of mushrooms, they should be processed directly after harvest in order to maintain their supply to consumers throughout the year. The long term preservation of mushrooms can be achieved by freezing, drying and sterilisation. Dehydration combined with some pre-treatments appears to be a cost effective method of preservation (6). Pre-treatments such as chilling, freezing, blanching, osmotic dehydration and combination treatments have been investigated in order to improve the drying efficiency and product quality of fruits (7). Mushrooms pre-treated with sulphites or H₂O₂ and steam blanching had the best colour values (8).

This work therefore sought to pre-treat oyster mushroom with a chelating agent (citric acid), and antioxidant agent (ascorbic acid) before the flour preparation and subsequently investigates the magnitude of proximate and phytochemical components present in Ghanaian oyster mushroom. There is also scanty literature on the effect of pre-treatments on these components of mushroom flour produced in Ghana.

Material and methods

Sample preparation and treatment

Freshly harvested oyster mushroom was purchased from a local cultivator in Accra, Ghana. Fully-grown mushrooms cap were used throughout the study. The samples in triplicates collected from one lot of cultivation were prepared by rinsing with clean water and subjected to the following treatment;

- soaking in 10% citric acid for 5 minutes.
- soaking in 10% ascorbic acid for 5 minutes.
- control.

Prepared samples were placed in strainers to remove excess water. The samples were gradually dried (in a Gallenkamp oven) at 40-50°C. The dried samples were milled (using a laboratory blender) to a fine powder. The samples were then stored in polyethylene pouches (in desiccators) at room temperature for further analysis.

Physicochemical analysis

All the physicochemical analyses were done using the official methods of (9).

Atomic Absorption Spectrophotometer determination

Analysis of heavy metals of interest was performed using a Varian model AA 240 FS Atomic Absorption Spectrophotometer.

Phytochemicals analysis

Extraction Process

Extraction was performed according to the method of (10) with some modifications. 0.5 grams of each sample were extracted in 10 ml of 60% ethanol. The mixture was placed in a conical flask (wrapped with an aluminium foil) and agitated at 200 rpm with orbital shaker for 1 hour at 25°C. Additional 10 ml of ethanol was used to re-extract the plant residue and the supernatants pooled. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper.

Determination of Phenolic Content

The polyphenolic contents (PC) were measured by the Folin-Ciocalteu (FC) method using Gallic acid as standard (11) with modifications. Briefly, 50µl of the extract was mixed with 3ml of distilled water (dH₂O) and 250µl of FC reagents. The mixture was allowed to stand for 5 minutes, and then 750µl of 20% Na₂CO₃ was added. The resulting mixture was vigorously shaken on Vortex 2 Genie shaker for two minutes. After incubation of the resulting reaction mixtures for 30 min at room temperature, absorbance values were measured at 760nm using a UV-VIS Spectrophotometer (Shimadzu, 1201, Japan). All determinations were performed in triplicate. A calibration curve was prepared using serial dilutions of 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/l from a stock solution of 1 mg/ml gallic acid dissolved in methanol. 50µl each of these solutions was treated like the samples and a calibration linear regression equation $y = 1.1353x + 0.0007$, $R^2 = 0.9993$ developed [y = absorbance and x = gallic acid conc (mg/ml)].

The polyphenolic content in each extract were calculated from the calibration curve and final results were recalculated and expressed as gallic acid equivalents per gram of dry mushroom sample (mg GAE/g_{dw}).

Determination of Flavonoid Content

The aluminum chloride colorimetric assay method (12) was employed to evaluate total flavonoid content (TFC) in the samples using quercetin as standard. 500µl of extracts were mixed with 1500µl of 99.9% ethanol (EtOH), 100µl of 1 M potassium acetate, 100µl of 10% aluminum chloride and 3000µl of distilled water. The mixture was shaken vigorously and left to stand in the dark at room temperature. The resulting mixtures were incubated for 30 minutes at room temperature and corresponding absorbance measured at 415 nm. All determinations were carried out in triplicates. A standard calibration curve was constructed using quercetin standard solutions of 12.5µg/ml, 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml. 500µl of each standard was treated in the same manner as the samples above and calibration linear regression equation $y = 0.0062x + 0.0027$ ($R^2 = 0.9991$) generated. Flavonoid content of each extract was determined from the curve and the final results recalculated and expressed as microgram quercetin equivalent per gram of dry mushroom sample (µg QE)/g_{dw}).

Determination of DPPH Free Radical Scavenging Activity

The free radical scavenging activity was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by (13). 200µl of extracts were each added to 3800µl of 0.004% DPPH in methanol. After 60 minutes of incubation at room temperature in the dark, the absorbance was measured at 517 nm. A blank sample containing only methanol was used to zero the spectrophotometer. Ascorbic acid (Vitamin C) was used for comparison. Each experiment was performed in

triplicate. Radical scavenging activity (I%) was calculated as follows:

$$I\% = [(Abs_0 - Abs_1) / Abs_0] * 100$$

Abs₀ = absorbance of 0.004% DPPH without analyte.

Abs₁ = absorbance of 0.004% DPPH plus the test compound.

Statistical Analysis

Analysis was performed in triplicate. Results are expressed as mean ± standard deviation or pooled standard error. Statistical analysis was done using Statgraphics version 16.1. One-way ANOVA was used to test whether there were significant differences in the various treatment conditions. A significant difference was considered at the level of $p < 0.05$.

Results and discussion

The present study was conducted to find the effects of various pre-drying treatments on quality of dehydrated oyster mushroom powder. The physical and chemical properties of the dried mushroom samples are presented in Table 1.

Sample pre-treated with citric acid had a lower pH (3.4±0.01) when compared with those pre-treated with ascorbic (4.3±0.01) and the control (6.1±0.01). Accordingly, among all organic acids used to pre-treat green mussel, *Perna viridis* with, the citric acid had a lower pH when compared with those pretreated with lactic and acetic acids (14). The use of chemicals (citric and ascorbic acids) that lower the pH of a product finds widespread in the control of enzymatic browning (15). Titratable acidity showed significant differences among the various treatments. The control had a TTA value of 0.3±0.01, the citric acid treated sample (1.5±0.01) and the ascorbic acid treated sample (0.4±0.01). It was also observed that as the pH of the samples increased the TTA value decreased.

The moisture contents of the samples studied was in the range of 10.3±0.3% to 10.8±0.3% comparable to button mushrooms (both untreated and treated samples) moisture content in the range of 9-12% after tray drying for various temperatures of air drying by (16). The low moisture reported in this study is acceptable because according to (17), moisture content is an indication of shelf stability of flour. As such, low moisture content flours will have better shelf life stability.

Vitamin C content of the various samples was varied. The results showed the control, citric acid treated and the ascorbic acid treated samples had 2.4±0.1, 0.4±0.04 and 28.6±0.1 mg/100ml vitamin C respectively. The canning of banana slices in solution containing 0.2% of L-ascorbic acid induced increase 13 times in the level of vitamin C in the final product according to (18). The low vitamin C content reported after pre-treatment with citric acid is due to the fact that citric acid prevents the oxidation degradation of vitamin C to other dehydroascorbic acid derivatives by scavenging oxygen according to (19). Furthermore, citric acid provides a low pH which inhibits enzymatic degradation of vitamin C. Vitamin C of a produce is also subject to degradation during processing and cooking (20).

Control sample had a protein content of 7.4% while a range of 19.4% and 14.3% was recorded for samples pre-treated with citric acid and ascorbic acid respectively. Although the sample is a good source of protein, the pre-treatments resulted in significant ($p < 0.05$) increase in their protein level. According to (21), mushroom samples steeped in citric acid, irrespective of drying methods used, exhibited higher contents crude protein as compared to control powder.

Table 1. Effect of different chemical treatments on proximate properties of dried oyster mushroom powder

Sample	pH	TTA (% lactic acid)	Moisture (%)	Vit C (mg/100ml)	Protein
Control	6.1±0.01 ^c	0.3±0.01 ^a	10.3±0.3 ^a	2.4±0.10 ^b	7.4±0.02 ^a
Citric acid	3.4±0.01 ^a	1.5±0.01 ^c	10.8±0.3 ^a	0.4±0.04 ^a	19.4±0.03 ^c
Ascorbic acid	4.3±0.01 ^b	0.4±0.01 ^b	10.4±0.4 ^a	28.6±0.1 ^c	14.3±0.05 ^b

a-c: Means in a column with different letters are significantly different ($p < 0.05$) from each other. Values are mean \pm SD of three replicates (n = 3)

Table 2. Effect of different chemical treatments on essential elements in dried oyster mushroom powder

Sample	Essential elements (mg/kg)				
	Fe	Cu	Mn	Zn	Cr
Control	18.2±2.0 ^b	1.6±0.4 ^b	15.6±1.0 ^a	5.3±0.7 ^b	0.2±0.2 ^{ab}
Citric acid	11.3±2.1 ^a	0.4±0.3 ^a	16.2±0.9 ^a	3.8±0.1 ^a	0.1±0.1 ^a
Ascorbic acid	9.1±0.3 ^a	5.0±0.6 ^c	15.6±0.6 ^a	4.6±0.1 ^{ab}	0.5±0.2 ^b

a-c: Means in a column with different letters are significantly different ($p < 0.05$) from each other. Values are mean \pm SD of three replicates (n = 3)

Table 3. Effect of different chemical treatments on heavy metals present in dried oyster mushroom powder

Sample	Heavy metals (mg/kg)				
	Pb	Cd	Ni	As	Hg
Control	9.7±3.1 ^a	Nd	Nd	0.3±0.02 ^c	Nd
Citric acid	9.3±1.6 ^a	Nd	Nd	0.1±0.02 ^b	Nd
Ascorbic acid	5.8±0.1 ^a	Nd	Nd	0.08±0.00 ^a	Nd

a-c: Means in a column with different letters are significantly different ($p < 0.05$) from each other. Values are mean \pm SD of three replicates (n = 3)

The entire samples contained nutritionally significant amounts of copper, iron, manganese, zinc and chromium (Table 2). Of those elements and trace minerals not required by humans, it is also noteworthy that all the samples contained quantities of arsenic and lead. Iron had the highest concentration of all the metals analysed. Iron is an essential trace element for humans due to its necessity in haem proteins such as hemoglobin, myoglobin and cytochromes. The Recommended Daily Intake of iron is 15mg/day. Consumption of these mushrooms will therefore serve as a very good source of iron supplementation particularly in low income countries where iron deficiency anaemia is a serious health challenge. Reported values for iron in mushrooms are usually high (22; 23; 24). This may be due to its abundance on earth.

The EU maximum permitted level for lead in cultivated mushrooms is 0.3 mg/kg wet weight (25). The values (9.7±3.1, 9.3±1.6 and 5.8±0.1 mg/kg in Table 3) thus obtained are unacceptable as they all fall above the limits and therefore should be of public health concern. Pb finds its way into the environment as constituent of pesticides and industrial waste release into the environment, such as used car batteries, alloys, solder, broken ceramics and plastics. Lead has no benefit to human metabolism. Gradual accumulation can lead to lead poisoning. The ranges detected for lead fall outside what is generally detected from mushrooms in literature. These include (26) who reported Pb concentration of 0.04mg/kg in *P.ostreatus*. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) further gives the established Provisional Tolerable Weekly Intake (PTWI) of 0.015 mg/kg body weight allowing 0.9 mg of arsenic to be consumed weekly by a person of 60 kg in weight. As such, no significant risk of excess arsenic intake is seen to arise from the consumption of the studied mushroom species here (27). Arsenic values of the samples are 0.3±0.02, 0.1±0.02 and 0.08±0.00 mg/kg for the control, citric acid and ascorbic acid treated sample respectively.

The profile of total phenolic contents of pretreated mushroom samples was expressed as gallic acid equivalents

per gram of dry mushroom sample (mg GAE/g_{dw}) in Table 4. The highest total phenolic content of 32.26± 0.77 mg GAE/g_{dw} was in the ascorbic acid treated mushroom. This was followed by control 11.73±0.77 mg GAE/g_{dw} and citric acid 6.84±0.77 mg GAE/g_{dw}. There was significant difference ($p \leq 0.05$) among the various treatments and the control. Between the two treated mushroom samples, the total phenolic content of the ascorbic acid treated mushroom is almost five times higher than that of citric acid treated. The higher phenolic content can be partially explained by the treatment with ascorbic acid which tends to have higher antioxidant activity and correlates well with total phenolic content (28) The total phenolic content in a study by (29) was 8.85 mg GAE/g of dry weight, while the range in a literature by (30) was 8.00 -10.65 mg GAE/g of dry weight. In our study the total phenolic content was in the range of 6.39 – 32.26 mg GAE/g of dry weight. This may be due to the pre-treatment of our samples used.

Phenols are known as important plant constituents due to their scavenging ability provided by the hydroxyl groups (31). Phenolic compounds may have direct contribution to antioxidative action. In addition phenolic compounds were reported to be associated with antioxidant activity and play important roles in the stabilizing of lipid peroxidation (32). Natural phenolics are able to provide antioxidative function through various ways, such as intercepting singlet oxygen, decomposing primary products of oxidation, preventing continued hydrogen abstraction from substances, etc. In addition, total polyphenols are known to be the major naturally occurring antioxidant compounds in the wild edible mushrooms (33).

It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g is ingested daily from a diet rich in fruits and vegetables (34). Conclusively, numerous studies have shown that consumption of foods high in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis, because they act as

antioxidants (35;36). Therefore, edible mushrooms may have potential as natural antioxidants in food.

Total flavonoid contents of the mushroom are shown in Table 4. Ascorbic acid treated mushroom samples had the highest value of $24.77 \pm 0.11 \mu\text{g QE/gdw}$ and the citric acid treated sample had the lowest value of $1.84 \pm 0.11 \mu\text{g QE/gdw}$. The difference between the two samples may be due to ascorbic acid which is presumed to have higher antioxidant properties than citric acid.

Among the phenolic compounds are flavonoids which possess biological activities such as antioxidative, anti-inflammatory and antiatherosclerotic activities. The antioxidative properties of flavonoids can be linked to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS. The many pharmacological effects of phenolic compounds and flavonoids are linked to their ability to act as strong antioxidants and free radical scavengers, to chelate metals, and to interact with enzymes, adenosine receptors, and biomembranes (37).

Table 4. Effect of different treatments on the phytochemicals of dried mushroom powder

Treatments	Total Phenolics	Total Flavonoids	DPPH (%)
Control	11.73 ± 0.77^b	5.58 ± 0.11^b	10.74 ± 0.48^b
Ascorbic Acid	32.26 ± 0.77^c	24.77 ± 0.11^c	33.41 ± 0.48^c
Citric Acid	6.835 ± 0.77^a	1.84 ± 0.11^a	7.55 ± 0.48^a
	LSD = 3.480	LSD = 0.790	LSD = 2.180

Means \pm Pooled Standard error from three replicates

a-c different alphabets on the same column are significantly different ($P \leq 0.05$)

Plant tissue antioxidant capacity is clearly associated with the activity of “free radical scavenging enzymes” (superoxide dismutase, catalase, peroxidase, etc.) and with the contents of antioxidant substances, mainly phenolic compounds, carotenoids, tocopherol and ascorbic acid. It is evident that there is an increasing demand to evaluate the antioxidant properties of plant extract. DPPH radical is a widely used method to evaluate the free radical scavenging ability of natural compounds. The radical scavenging activity (RSA) of mushroom extracts was tested against the DPPH. DPPH is a stable free radical with a characteristic sorption at 517 nm. The effect of antioxidants on DPPH radical scavenging was conceived to be due to their proton-donating ability. As antioxidants donate protons to these radicals, the absorbance decreases. The decrease in absorbance is taken as a measure of the extent of radical-scavenging. Therefore, the antioxidant activity of a substance can be expressed as its ability in scavenging the DPPH free radical. In Table 4, the scavenging activities of the DPPH radical due to its reduction by different mushroom extracts are illustrated. The ascorbic acid treated sample showed the higher radical scavenging activity which was $33.41 \pm 0.48\%$ compared to citric acid treated sample which was $7.55 \pm 0.48\%$ (Table 4). There was significant difference ($p \leq 0.05$) among the various treatments and the control. The scavenging effects of mushroom extracts on the DPPH radical decreased in the order of ascorbic acid, control and citric acid treated samples. Comparatively, the radical scavenging activity range of 7.55 - 33.41 % in our study is lower than range of 82.39 - 85.44 % reported by (38). The

different scavenging effects may be due to different extraction procedure and mushroom species.

Conclusion

In general it can be concluded that the citric acid and ascorbic acid pre-treatments used in the study had significant effect on the pH, vitamin C, protein, iron, total phenolics, total flavonoids and DPPH contents of the mushroom powder studied. This study shows that there has been some heavy metals contamination in oyster mushroom in Ghana. Heavy metals are environmental hazardous and continuous monitoring of the trends of its concentrations in food and other biota is a requirement. Although heavy metals in samples do not pose any immediate risk to human health so far, a yearly monitoring program for heavy metals in food and other products is a necessity.

The quantitative nutritional information provided in this report should be regarded as provisional since the full value of the minerals in the food product analysed will necessarily be determined by the bioavailability of these minerals, which in turn will depend upon the efficiency of their digestion and absorption. Nevertheless, the data in the present report will provide public health officials in Ghana with nutritional information that should be helpful in advising local populations about the particular minerals value of oyster mushroom that are grown in the region.

Acknowledgement

Authors would like to appreciate the help provided by technicians and technologists especially Nash Bentil, Lebene Kpattah and C. Crabbe of the Nuclear Chemistry and Environmental Research Centre, NNRI, GAEC.

References

- (1) C. Sanchez “Cultivation of *Pleurotus ostreatus* and other edible mushrooms”, Applied Microbiology and Biotechnology, 2010, vol 85, pp 1321-1337.
- (2) P. Mattila, K. Suonpaa, and V. Piironen, “Functional properties of edible mushrooms”, Journal of Nutrition, 2000, vol 16, pp 694-696.
- (3) S.P. Wasser and A.L. Weis, “Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (Review)”, International Journal of Medicinal Mushrooms, 1999, vol 1, pp 31-62.
- (4) J.H. Yang, H.C. Lin and J.L. Mau, “Antioxidant properties of several commercial mushrooms”, Journal of Food chemistry, 2002, vol 77, pp 229-235.
- (5) Rai and Arumuganatha, “Post-harvest technology of mushrooms”, Technical Bulletin, National Research Centre for Mushroom, Indian Council of Agricultural Research, 2008, pp 13
- (6) V. Rama and J.P. Jacob, “Effects of methods of drying and pre-treatment on quality of dehydrated mushroom”, Indian Food Packer, 2000, vol 54, pp 59-64.
- (7) C.K. Sankat, F. Castagine and R. Maharaj “The air drying behaviour of fresh and osmotically dehydrated banana slices”, Journal of Food Science and Technology, 1996, vol 31, pp 123-135.
- (8) H.A. Eissa, G.M. Fouad, and A.E.A. Shouk, “Effect of some thermal and chemical pre-treatments on smoked oyster mushroom quality”, International Journal of Food Science and Technology, 2009, vol 44(2), pp. 251-261. doi:10.1111/j.1365-2621.2007.01671
- (9) AOAC. Official methods of analysis (17th ed.). Gaithersburg, MD, USA: AOAC International, 2000.
- (10) J.H. Yang, H.C. Lin, J.L. Mau, “Antioxidant properties of

- several commercial mushrooms”, Journal of Food Chemistry, 2002, vol 77(2), pp 229-235.
- (11) V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós and P. Lester, “Methods in Enzymology”, ed., Academic Press. 1999, pp 152-178
- (12) J. Zhishen, T. Mengcheng and W. Jianming, “The determination of flavonoid contents in mulberry and their scavenging effects on Superoxide radicals”, J. Food chem., 1999, vol 64, pp 555-559.
- (13) W. Brand-Williams, M.E. Cuvelier, and C. Berset, “Use of free radical method to evaluate antioxidant activity”, Lebensm Wiss Technology, 1995, vol 28, pp. 25-30.
- (14) P. Masniyom, and O. Benjama, (2007) “Effect of lactic, acetic and citric acids on quality changes of refrigerated green mussel, *Perna viridis* (Linnaeus, 1758)”, Songklanakarin Journal of Science Technology, 2007, vol 29(4), pp 1123-1134.
- (15) J.D. Ponting “The control of enzymatic browning of fruits. In Food Enzymes”, H.W.Schultz, ed. AVI, Westport, 1960, pp 105-124.
- (16) V. Nour, I. Trandafir, and M.E. Ionica, “Effects of pre-treatments and drying temperatures on the quality of dried button mushrooms”, South Western Journal of Horticulture, Biology and Environment., 2011, vol 2(1), pp 15-24.
- (17) F.C.K. Ocloo, D. Bansa, R. Boatın, T. Adom, W.S. Agbemavor “Physico-chemical, functional and pasting characteristics of flour produced from Jackfruits (*Artocarpus heterophyllus*) seeds”, Agric Biol JN Am, 2010, vol 1(5), pp 903-908.
- (18) G. Jaworska, W. Kmiecik, and J. Słupski, “Effect of technological measures on the quality of canned banana desserts”, EJPAU, 2004, vol 7(1), pp 5.
- (19) G.M. Sapers and R.L. Miller “Heated ascorbic/citric acid solution as browning inhibitor for pre-peeled potatoes”, Journal of Food science, 1995, vol 60, pp 762-766.
- (20) S.K. Lee, and A.A. Kader “Preharvest and postharvest factors influencing vitamin C content of horticultural crops”, Journal of Postharvest Biology Technology, 2000, vol 20 (3), pp 207-220.
- (21) V. Dunkwal, S. Jood, and S. Singh, “Physico-chemical properties and sensory evaluation of *Pleurotus Sajor Caju* powder as influenced by pre-treatments and drying methods”, Br. Food Journal. 2007, vol 109, pp.749–759.
- (22) P. Nuorteva, S. Autio, J. Lehtonen, A. Lepistö, S. Ojala, A. Seppänen, E. Tulisalo, P. Veide, J. Viipuri, and R. Willamo, “Levels of iron, aluminium, zinc, cadmium and mercury in plants growing in the surroundings of an acidified and a non-acidified lake in Espoo, Finland”, Ann Bot Fenn., 1986, vol 23, pp 333–340.
- (23) J. Falandysz, K. Szymczyk, H. Ichihashi, L. Bielawski, M. Gućia, A. Frankowska and S. Yamasaki, “ICP/MS and ICP/AES elemental analysis (38 elements) of edible wild mushrooms growing in Poland”, Food Addit Contam, 2001, vol 18(6), pp. 503–513.
- (24) G. Tyler, “Metals in sporophores of basidiomycetes”, Transactions of the British Mycological Society, 1980, vol 74(1), pp 41–49.
- (25) European commission, Commission Regulation (EC) No 466/2001. Directive 2001/22/EC. European Commission, EU, 2001.
- (26) A. Quarcoo and G. Adotey, “Determination of heavy metals in *Pleurotus ostreatus* (Oyster mushroom) and *Termitomyces clypeatus* (Termite mushroom) sold on selected markets in Accra, Ghana”, Mycosphere, 2013, vol 4(5), pp 960–967, Doi 10.5943/mycosphere/4/5/9
- (27) P. Riina, A. Georg and J. Olli (2006) “Cadmium, lead, arsenic and nickel in wild edible mushrooms”, The Finnish Environment, 2006, vol 17, pp 32.
- (28) Y.S. Velioglu, G. Mazza, L. Gao and B.D. Oomah, “Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products”, Journal of Agric Food Chemistry, 1998, vol 46, pp 4113-4117.
- (29) I. Giannenas, E. Tsalie, E.F. Chronis, S. Mavridis, D. Tontis, and I. Kyriazakis, “Consumption of *Agaricus bisporus* mushroom affects the performance, intestinal microbiota composition and morphology, and antioxidant status of turkey poult”. Animal Feed Science and Technology, 2011, vol 165(3-4), pp218-29. <http://extoxnet.orst.edu/tibs/bioaccum.htm>.
- (30) N.J. Dubost, B. Ou and R.B. Beelman, “Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity”, Journal of Food Chemistry, 2007, vol 105(2), pp. 727–735.
- (31) P.D. Duh, Y.Y. Tu, and G.C. Yen, “Antioxidant activity of water extract of harnjyur (*Chrysanthemum morifolium Ramat*)”, Lebensmittel-Wissenschaft und Technologie, 1999, vol 32, pp. 269-277.
- (32) G.C. Yen, P.D. Duh and C.L. Tsai, “Relationship between antioxidant activity and maturity of peanut hulls”, J Agric Food Chem, 1993, vol 41, pp 67-70.
- (33) S.W. Choi and G.M. Sapers, “Effects of washing on polyphenols and polyphenol oxidase in commercial mushrooms (*Agaricus bisporus*)”, Journal of Agricultural and Food Chemistry, 1994, vol 42, pp. 2286-2290.
- (34) K.C.W Tanaka, Y. Nagashima and T. Taguchi, “Application of antioxidative maillard reaction products from histidine and glucose to sardine products”, Nippon Suisan Gakkaishil, 1998, vol 54, pp 1409-1414.
- (35) M.P. Kahkonen, A.I. Hopia, H.J. Vuorela, J.P. Raucha, and K. Pihlaja, “Antioxidant activity of plant extracts containing phenolic compounds”. Journal of Agric Food Chem, 1999, vol 47, pp 3954-3962.
- (36) C. Kaur, and H.C. Kapoor, “Anti-oxidant activity and total phenolic content of some Asian vegetables”, International Journal of Food Science Technology, 2002, vol 37, pp 153-161.
- (37) K. Mitra and N. Uddin, “Total Phenolics, Flavonoids, Proanthocyanidins, Ascorbic Acid Contents and *In-Vitro* Antioxidant Activities of Newly Developed Isolated Soya Protein. Discourse”, Journal of Agriculture and Food Sciences, 2014, vol. 2(5), pp 160 -168.
- (38) C.H. Gan, A.B. Nurul, and R. Asmah “Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*)”, International Food Research Journal, 2013, vol 20(3), pp 1095-1102.