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Chemical Profile, Agaritine and Selenium Content of *Agaricus bisporus*

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ABSTRACT

Chemical profile of the Agaricus bisporus samples were analyzed using GC/MS method in ethanolic extracts. A total of 174 metabolic products were detected, which included 13 significant metabolites between 1.2 to 83 % (w/w); other 13 metabolites at 1% (w/w) and 148 metabolites less than 1% classified into 12 categories. These metabolites had many medicinal activities, which included anti-cancer, anti-cardiovascular diseases, anti-hypercholesterol, anti-microbial, hepatoprotective, human health supporting and immune enhancer. HPLC analysis of water extracts of the A. bisporus samples showed that the spores and gills in the fresh adult mushroom had higher percentage of agaritine mycotoxin than the other parts. These contents were decreased by different treatments especially by boiling, preservation and cooking. The selenium contents (ppb) was measured by Galvanometric analysis and highest content was recorded in fresh whole mushroom sample (0.97) followed by cooked preserved (0.4) and fried sample (0.11).

Key words: *Agaricus bisporus*, chemical profile, agaritine, selenium, anticancer, antihypercholesterol, aroma compounds, Galvanometric, GC/MS and HPLC

INTRODUCTION

Agaricus bisporus (Lange) Imbach is the most wild and cultivated edible mushroom and represents more than 40% of world production of mushrooms (Callac et al. 2000; Carluccio 2003). It is cultivated in over 70 countries and on every continent, except Antarctica. The global production in 1990s was more than $ 800 million/year (Andersson and Gry 2004) and increased to $ 12,250 in 2002 (UN 2010). *A. bisporus* has delicious taste, high nutritional value, high aroma or flavoring taste and is used as a food and in food industries (Mattila et al. 2010). It has high biological activity, low toxicity and is used in folk classical medicines, flavouring of food products, perfume, cosmetics and pharmaceutical industries, as defoaming agents and to improve the shelf life and safety of minimally processed fruits (Caglarırmak 2009; Dastager 2009; WFI, $2011_{c\&d}$). The wild *A. bisporus* were referred for cocustomer due to their flavor and texture (Sadiq et al., 2008).

A. bisporus has many medicinal metabolites responsible for the therapeutic activity for which the treatment and prevention of many human diseases act as anti-cancer agents such as polysaccharides, fatty acids and ergosterol, N,N,N-tris"hydrazine carbonyl" phosphoric triamide, selenium and vaccenic acid; anti-hyper cholesterol agents (fatty acids, glycoproteins, sterols and vaccenic acid); anti-microbial agents (agaritine and alcohols); anti-cardiovascular disease metabolites (fatty acids, sterols and pyran derivative); hepato protective agent (triterpenoids); human health supporting agent's (fatty acids,

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sterols and sugar alcohols); immune enhancer metabolites (fatty acids, glycoproteins, polysaccharides and sterols). These medicinal metabolites such as β-glucan and G-glucan; polysaccharide K or PSK "protein bound polysaccharide"; phenols; polyketides, triterpenoids and sterols (De Barros, 2008); PSK (UN 2010); triterpenoids, lectins glycoprotein's (WU et al. 2007; USCDC 2009; UN 2010); ergothioneine (Ey et al. 2007; Borchers et al. 2008); selenium (Shi et al. 2002; Gergely et al. 2005); pyran derivative (SCB, 2007-2010); essential fatty acids (Andersson and Gry 2004; Ji, et al. 2006) and eicosanoids (WFI 2011_e).

Essential fatty acids in *A. bisporus* act against aromatase enzyme which used in estrogen production. Estrogen responsible for the development of breast cancer. The women who consumed more than 10g of fresh *A. bisporus* daily, were 64% less likely to develop breast cancer, while those that combined a mushroom diet with regular green tea, reduced the risk of breast cancer by nearly 90% (Chen et al. 2006; Hong et al. 2008).

A. bisporus contains small amounts of the carcinogenic mycotoxin agaritine, which is a health hazard. It is water soluble, has antiviral and mutagenic activity on *Salmonella typhimurium.* It is metabolized into its highly reactive diazonium ion, which modifies DNA through a radical mechanism. Its LD_{50} toxicity is between 1 - 10 mg/kg. Agaritine are synthesized in mycelium and translocated into the fruiting body. The highest amounts were found in the fresh cap and the gills of the fruiting body, especially in the spores. It is synthesized from P-hydroxybenzoic acid absorbed from the lignin in the substrates, hydrazine formed by the oxidative coupling of two amines via a phenolic radical mechanism through shikimate pathway from glutamic acid. It oxidizes rapidly upon storage, however, totally degrades after 48 h in aqueous solution with exposure to air. The degradation of agaritine is affected by many factors, including the amount, heat treatment, preservation, time of storage and the decreases by cooking up to 90% or storage in water (Andersson and Gry 2004; Janak et al. 2006; Roupas, et al. 2010; WFI 2011_a).

This investigation was designed to study the chemical profiles of the *A. bisporus* and factors affecting its quality, (nutritional, medicinal, flavoring value). These factors included its consumption as fresh, preserved or cooked boiled, fried or heat treated. Also, the study was designed to detect and identify the most significant metabolites such as anti-cancer, anti-hypercholesterol, other human health supporting agents, and aroma compounds. The harmful effects of agaritine mycotoxin and how to avoid such effects are discussed. Selenium as antioxidant agent was also studied.

MATERIALS AND METHODS

The agaritine and ethanol of analytical grade were obtained from Sigma-Aldrich-Company. *I-Collection of A. bisporus samples*- Different fresh samples of *A. bisporus* were collected from the market (a maximum of 1 - 2 days old) in Assiut Governorate, Egypt. These samples were originated from the spores obtained from the Agricultural Egyptian Ministry. This strain was deposited in Assiut University Mycological Center Culture Collection with AUMC No. 8060.

II-Treatment and preparation of A. bisporus for chemical analysis-

a) Fresh samples of the whole mushroom, caps, gills and stalks were divided into 25g for each of the following treatment-

*Cooking either by frying for 5 min. in vegetable oil or boiling for 10 min. using the whole mushroom samples.

*Mushroom samples from whole, caps, gills and stalks were used without cooking.

b) Preserved samples (in water, NaCl and citric acid) of the whole *A. bisporus* were also collected from the markets and divided 25g each into uncooked (from two different brands) and cooked for 15 min an oven at 120°C (only one brand).

III-Extraction of the samples- From each sample, 25g were homogenized for 10 min. in a high-speed blender at 16.000 rpm with 500 ml ethanol and 25g with water. The extraction procedure was repeated three times. The ethanolic extracts were combined, washed, filtered, and concentrated to near dryness.

IV-GC/MS Analysis- The metabolites in the ethanolic extracts of all the samples were detected using GC/MS analysis: 250g fresh whole mushroom were also used for the GC/MS analysis (Mattila et al. 2010).

V-Agaritine content were determined in water extract by HPLC- As disscued by Nagadka et al., (2006).

VI-Galvanometric methods for determination of selenium was determined following Shi et al., (2002) and Gergely et al. (2005).

RESULTS AND DISCUSSION

Table 1 and Figures 1and 2 shows the chemical analysis of fresh, cooked and preserved ethanolic and water extracts of *A. bisporus* using different detection methods. There were 18 metabolites in the ethanol extract in addition to agaritine in the water extract; selenium was detected by the Galvanometric methods. Numerous studies showed that these metabolites have medicinal activity (Wang et al. 2007; WU et al. 2007; USCDC 2009; UN 2010), which include anti-cancer agents such as fatty acids poly saccharides, and ergosterol (Takaku et al. 2001; Ren et al. 2008; Koyyalamudi et al. 2009); N,N,N-tris (hydrazinecarbonyl) phosphoric triamide, selenium and vaccenic acid (Shi et al. 2002; Sosnovsky and Rao 2004; Gergely et al. 2005; De Barros 2008; Mattila et al. 2010); anti-hypercholesterol agents such as fatty acids, glycoproteins, sterols and vaccenic acid (Ey et al. 2007; Borchers et al. 2008; De Barros 2008); anti-microbial agents such as agaritine and alcohols (Janak, et al. 2006; Nagadka, et al. 2006; Roupas, et al. 2010); anti-cardiovascular disease agents such as fatty acids, sterols and pyran derivative (SCB, 2007-2010); human health supporting agent's such as fatty acids, sterols and sugar alcohols (Andersson and Gry 2004; Ji, et al. 2006); immune enhancer agents such as fatty acids, glycoproteins, polysaccharides and sterols (UN, 2010); hepato-protective agent such as triterpenoids (De Barros 2008) and food flavoring or aroma metabolites such as alcohols, aldehydes, amides, amines, carboxylic acid, esters, ketones, terponoids, thiols and mercapto (Mattila, et al. 2010). Linoleic acid was recorded at 12.2% in the whole fresh sample; 1.5 % in the stalks; 0.8% in the gills but was completely absent in the samples treated by heat and preserved. By GC/MS analysis of *A. bisporus*, Chen et al. (2006) recorded 0.21 linoleic, 0.28 linolenic and 0.39 mmol/L of oleic acid, which were active against the breast cancer cells but myristic (3.7%), palmitic (3.8%) and stearic acid (2.0%) had little activity. Amakura et al. ($2006_{a\&b}$) found that the linoleic acid was present in the water methanol extracts of mushroom by HPLC and spectroscopic analysis. Glycerol represented 5.8, 1.0, 0.5 and 0.3% in gills, caps, cooked preserved and fried, respectively. Caglarırmak (2009) has also reported the presence of glycerol in *A. bisporus* extract.

Three metabolites were detected only in the cooked preserved sample and included cyclobutyl alcohol at 64.7%; 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyrane-4-one at 10.3% and 2-chloropropane at 0.7%. Acetic, propionic, butyric and isobutyric acids stimulated the germination of *A. bisporus* spores. There are reports that these spores also contain phospholipids which includes 11.7% cardiolipin, 13.8% phosphatidie, 12.3% phosphatidyl ethanolamine, 39% phosphatidylserine, 10.5% phosphatidyl inositol and 12.6% others metabolites (Osullivan and Losel 1971).

In the present study the N,N,N-tris(hydrazine carbonyl) phosphoric triamide was found in the gills (5.9 %) and in the fried sample (0.5%). Sosnovsky and Rao (2004) studied that the structure and anti-cancer activity relationship among the various aliphatic and aromatic hydrazones which contained N,N,N′,N′-bis(1,2-ethanediyl) phosphoric diamide moiety. All the 19 compounds were active aganist murine lymphocytic leukemia.

Four compounds were detected only in the fresh whole sample and included penadecylamine (0.3%); 1-homoadamantane carboxylic acid (0.2%); 4-butyl aniline (0.04%) and ergosterol (5.7%). The highest percentage of alantoic acid was present in the cooked preserved sample (9.3%) followed by the boiled (1%), gills (0.8%) and fried (0.6%). The lowest amount of the acid was present in uncooked preserved samples (0.3% and 0.1%). 1,2,3,4,5,8,9,10,11,12,12,16-dodecadi-benzoid, di-[1,2,7,8] tetrathiacyclo dodecin was detected in all the tested samples except the samples of stalks, where it was 6.0% in the cooked preserved and 0.3 % in II uncooked preserved samples. 3-chloro-N-methylpropyl amine was detected only in the preserved samples which was 5.9% in the cooked preserved and 1.9% in the fresh sample. Glycinamide was detected in the all tested samples, except in the cooked and I uncooked preserved sample and fluctuated between 3.5 - 69.1%. The high levels were recorded in the different parts of mushroom which were 69.1% in the gills, 43.3% in the stalk, 39.5% in the fried, 24.6 % in the boiled, 14.2% in the caps, 5.6% in the whole fresh and 3.5% in the

preserved samples. 2R,3S-9-[1,3,4-trihydroxy-2tutoxymethyl] guanine was recorded in the fresh whole sample (1.4%) and fried samples (1.6%).

L-glutamic acid was recorded in the all tested samples except the cooked preserved sample and the highest percentage was in the caps sample (53.4%) followed by the gills (41.9%) and stalks (12.5%). But the levels of L- glutamic acid were decreased in the treated *A. bisporu*s samples (boiled 5%), uncooked preserved samples (3.0%, 1.9%), fresh whole (1.5%) and fried (0.7%). De Barros (2008) studied that the ethanolic extract of the fruiting bodies of *A. bisporus* and found that alanine, glutamic, proline and arginine were the predominant free amino acids. Also, ornithine, N-(γ-L-glutamyl)-4hydroxyaniline and γ-amino butyric were the most free amino acids. Pidolic acid had high percentage in the cooked preserved (19.9%) followed by the boiled (2.6%), fried (2.1%), uncooked preserved (1.9 and 1.5%) and whole fresh (0.7%).

D-mannitol was recorded in high amount in all the tested samples, representing 95.8% in the uncooked preserved I and 90.6% in II, boiled 73.7%, fried 68.9%, whole fresh 63.7%, cooked preserved 42.7% and stalk 16.6%. These results were similar with the results reported by De Barros (2008).

SH-indenol [1,2-b] pyridine-5-one (3.8%) and 1-methyl-2-phenoxyethylamine (0.9%) were recorded only in the caps sample.

Palmitic acid was recorded in 5.9% in the whole fresh, 0.3% in the gills and 0.29% in the stalk samples but it was completely absent in other tested samples. De Barros (2008) found that *A. bisporus* had high levels of polyunsaturated fatty acids such as palmitic, stearic and linoleic acid in most edible wild species. Linoleic acid is the precursor of 1-octen-3-ol. which is the principal aromatic compound and contributes to flavor. Carbonates from the alcohols, dialkylazodi carboxylate and tris (dimethylamino) phosphine ranged from 22–88% (Grynkiewicz et al. 1975).

Table 1 and Figure I showed the agaritine percentage in *A. bisporus* tested samples. They were 0.26, 0.22, 0.20 and 0.19% in the whole fresh sample, gills, caps and stalk, respectively. These results were in agreement with other studies on the agaritine content in different parts of *A. bisporus***.** Schulzová et al., (2002) tested 28 samples of fresh *A. bisporus* with a cup diameter of 4-6 cm and found that the agaritine content fluctuated between 165-457 mg/kg fresh weight. Andersson and Gry (2004) studied the presence of agaritine in various parts of the fruit body of the of the whole mushroom and found that the original agaritine levels in the fresh *A. bisporus* fluctuated between 228.2 - 720 mg/kg fresh weight, (12, 16, and 56% in the cap with its skin, skin of the cap and stalk and gills, respectively) 330 - 1730 mg/kg fresh weight in 14 lots from 10 different growers. Janak et al., (2006) recoded that the gills with the spores of *A. bisporus* had large percentage of agaritine fluctuating between 0.003 - 0.304% more than other parts.

Agaritine content were decreased by the heat treatments and preservation (0.26% in the whole fresh decreased by boiling to 0.2%; cooking and preservation to 0.12% and by frying to 0.1%). These results were similar with the results recorded by many other authors. Hashida et al., (1990) found that boiling at 100ºC significantly decreased the level of agaritine about 57 and 75% after 5 and 10 min, respectively but the percentage were reduced 55, 60, 75 and 90% after 5, 10, 60 and 120 min, respectively. Schulzová et al., (2002) found that the dry baking during pizza making, reduced the agaritine content by about 25-50%; frying at 100ºC for 5 min reduced 34 and 69% in the vegetable oils and butter, respectively but frying at 150 ºC for 10 min. reduced the content by 57%. Only 2.8 and 0.6% of the original content could be recovered in the butter and oil, respectively (Hajšlová, et al. 2002).

Agaritine percentage were decreased by preservation and fluctuated between 0.44-0.97% (Table 1 and Fig. 1). These results were in agreement with Andersson and Gry, (2004) who recorded that the agaritine percentage were decreased by 68% by preservation for five days. The storage at 4 ºC resulted the agaritine content of two batches as 440 and 720 mg/kg, respectively. After one week, one of the batches had lost 2% of its content, but another batch lost 47% and after two weeks were 36 and 76%, respectively (Ross et al., 1982). Storage at 5ºC reduced the content to 25, 40 and 50% after 6, 10 and 14 days, respectively (Schulzová et al. 2002).

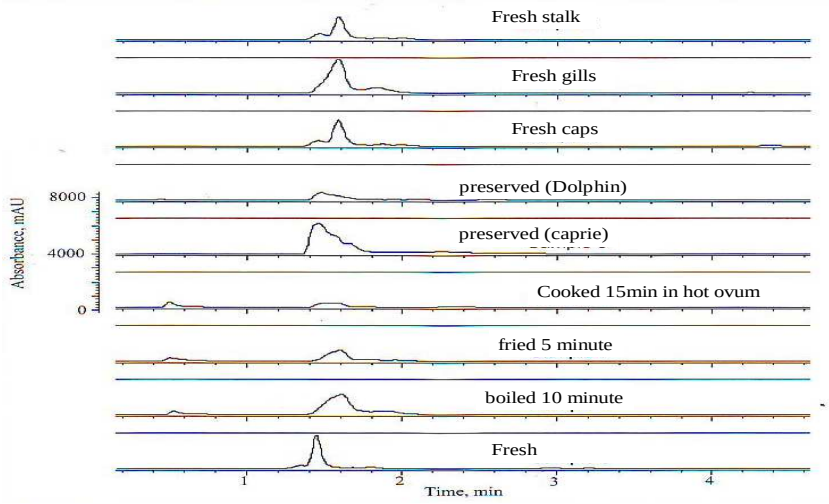

Figure 1- Agaritine mycotoxin (% w/w) detected by HPLC analysis in water extracts of *A. bisporus* samples.

Table 1- Detectable metabolites in the tested mushroom samples (% w/w) in ethanolic extracts, agaritine (% w/w) in water extract and selenium (ppb).

	a- Metabolites detected (as % w/w)	Mushroom samples treated								
		Fresh parts				Cooked		Preserved		
								Uncooked		Cooked
		Whole	Caps	Gills	Stalks	Fried	Boiled	I	II	
1	D-Mannitol	63.7			16.6	68.9	73.7	95.8	90.6	42.7
2	Glcinamide	5.6	39.5	43.3	69.1	24.6	14.2		3.5	
3	L-Glutamic acid	1.5	53.4	41.9	12.5	0.7	5.0	1.9	3.0	
4	Pidolic acid	0.7				2.1	2.6	1.9	1.5	19. 9
5	Linoleic acid	12.2		0.8	1.5					
6	2,3-Dihydro-3,5-2OH-6-CH3-4H-pyrane-4-one									10.3
7	Alantoic acid			0.8		0.6	1.0	0.1	0.3	9.3
8	1,2,3,4,5,8,9,10,11,12,12,16-dodecadibenzoid, [d,i][1,2,7,8]– tetra thiacyclododecin	0.9	1.5	1.1		0.8	3.6	0.3	1.1	6.0
9	N,N,N-tris(hydrazinocarbonyl) phosphoric triamide			5.9		0. 5				
10	Palmitic acid	5.9		0.3	0.3					
11	Glycerol		1.0	5.8		0.3				0.5
12	Ergosterol	5.7								
13	Cyclobutyl alcohol									4.7
14	2-Chloropropane									0.7
15	3-Chloro-N-methylpropylamine	1.9								5.9
16	2R,3S-9-[1,3,4-trihydroxy-2-tutoxymethyl] guanine	1.4				1.6				
17	5H-Indenol[1,2-b]pyridine -5-one		3.8							
18	1-Methyl-2-phenoxyethylamine		1							
b- Agaritine (% w/w)		0.26	0.20	0.22	0.20	0.10	0.20	0.1	0.04	0.12
c- Selenium (ppb)		0.97	0.08	0.1	0.10	0.10	0.03	< 0.01	< 0.01	0.40

Table 2- The chemical profile of the whole fresh *A. bisporus* samples.

No.	Detectable metabolites	% w/w	No	Detectable metabolites
1	Ergosterol	83.0	47	1-Butanamine,2-methyl-n-(2-methlbutylidene)-
2	D-Mannitol	63.0	48	13-Octadecenal, Octadecane,1-(ethenyloxy)-
3	Glcinamide	39.4	49	Cyclododecane
4	L-Glutamic acid	27.3	50	7-Pentadecyne
5	2-mono-linolein,	10.0	51	3-(1,3-Di-OHisopropyl)-1,5,8,11,14,17-hexaoxacyclononadecane
6	N,N,N-tris(hydrazine ocarbonyl) phosphoric triamide	6.0	52	3-[N-[3-Diethylaminopropyl]-1-cyclopentenylamino] propionitrile
7	Linoleic acid	4.2		**4] Amides**
8	5H-Indenol[1,2-b]pyridine -5-one	4.0	53	(Z)-13-Docosenamide,
9	Glycerol	3.4	54	2-Myristynoyl-glycinamide
10	Palmitic acid	2.2	55	9-Octadecenamide
11	3-Chloro-N-methylpropyl amine	2.0	56	N,N-bis(2-hydroxyethyl)-dodecanamide,
12	2-mono-palmitin	2.0	57	N-(2-hydroxyethyl)-decananamide,
13	1,2,3,4,5,8,9,10,11,12,12,16-dodecadi benzoid, di[1,2,7,8]–tetrathiacyclo dodecin	1.2	58	N,N-di(isopropyl)-O-ethyl phosphonodiamide,
			59	N-allyl-hexanamide,
14	Alantoic acid	1.0		**5] Amines**
15	1-Methyl-2-phenoxyethylamine	1.0	60	Acetyl-leucine
16	Ergosta-5,8-dien-3-ol,(3β)-	1.0	61	Agaritine
17	7,22-Ergostadienol	1.0	62	4-Butyaniline
18	γ–Ergostenol	1.0	63	D-alloisoleucine
19	N-hexadecanoic acid	1.0	64	2-chloro-N,N-dimethyl-ethanamine,D_l-norleucine
20	9,12-Octadecadecanoic acid, ethyl ester	1.0	65	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitro benzyli dene)tyramine
21	Carbonic acid,2-dimethylaminoethylpropyl ester	1.0	66	2,5-Diisopropylpiperazine
22	2R,3S-9-[1,3,4-trihydroxy-2-tutoxymethyl] guanine	1.0	67	D-norleucine
23	16,28-Secosol anidan-3-ol,(3β,5*a*)-	1.0	68	4-Fluorobenzyl(3-(4-luorobenzylsulfanyl) -[1,2,4] triazol-4-yl]amine
24	Pidolic acid	1.0	69	Indolizine
25	Propan-1-one,3-nitro-1-phenyl-	1.0	70	Isoleucine
26	1,5-Anhydro-d-mannitol	1.0	71	2R,3S-9-[1,3,4-trihydroxy-2-tutoxymethyl]guanine
	The following metabolites were detected at less than 1%		72	2-Hydrazino-8-hydroxy-4-phenylquinoline
	1] Alcohols			
27	1,5-Anhydroglucitol		73	5H-indeno[1,2-b]pyridine
28	1,5-Anhydro-d-mannitol		74	N-ethyl-2-carbomethoxyazetidine
29	Benzyl alcohol		75	1-Methyl-2-phenoxyethylamine
30	Cholesterol		76	Penadecylamine
31	(3.β, 22E)-ergosta-5,8,22-trien-3-ol		77	Piperine
32	8(14),22-Ergostadienol			**6] Carboxylic acids**
33	(3.β.)-ergosta-5,24(28)-dien-3-ol		78	1,2-Benzenedicarboxylic acid,
34	4,4-dimethyl-(3.β, 5x)-cholesta-7,14-dien-3-ol,		79	Benzoic acid
35	Ergost-4,7,22-trien-3.□.-ol		80	Carpic acid=Decanoic acid
36	(3.β.)-Ergosta-5,8-dien-3-ol,		81	Cis-Vaccenic acid
37	(3.β-a.5□)16,28-Secosolanidan-3-ol		82	2-Dodecene-1-yl(-)succinic anhydride
	2] Aldehyde		83	Hexadecanoic acid=Palmatic acid
38	4-Hydroxy-2-methylbenzaldehyde		84	Oleic acid
39	2-Heptadecenal		85	3-Methyl-decanoic acid,
40	9-Octadecenal,		86	Pentadienoic acid
41	2-octyl-cyclopropaneoctanal,		87	Tridecanoic acid
42	Trans-13-Octadecenal,			**7] Esters**
	3]Alicyclic compound		88	1-Benzazirene-1-carboxylic acid,2,2,5a-trimethyl1a
43	(1-octylnonyl)-cyclohexane,		89	[3-oxo-1-butenyl] perhydro-methyl ester
44	Ethanol, 2-(octadecyloxy)-		90	1Bis(2-ethylbutyl) ester
45	Oxime-,methoxy-phenyl-		91	Bis(4-methylpentyl) ester
46	1-(diethylboryl)-cyclooctane,		92	Butyl 9,12-octadecadienoate

(Cont. ...)

Cont. Table 2

No.	Detectable metabolites	No.	Detectable metabolites
93	Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(4-frluoroanilino)propionate	134	1H-tetrazol-5-yl-pyrazine
		135	Oxirane,tetradecyl-
94	2-Butyric acid, 2-methoxy-3-methyl-,methyl ester	136	2-Pyridineacetic acide, hexahydro-
95	Butyric acid, 3-methyl-,hexadecyl ester	137	Methanone,(1,3-benzodioxol-5-yl)(4-methyl-6-phenyl-1,5,2-dioxazinan-2-yl)
96	Carbonic acid,2-dimethylaminoethyl propyl ester	138	2(3H)-Furanone,3-(2-bromoethyl)dihydro-
97	Ditridecyl ester	139	1,2,5-Oxadiazol-3-amino-e,4-(3-methoxyphenoxy)-
98	Ethyl 9-hexadecenoate	140	Benzaldehyde,4-methoxy-3-(8-quinolinyloxymethyl)-
99	Ethyl cyclohexanepropionate	141	1,4-Naphthalenedione,2-amino-3-chloro-
100	Ethyl acetoxycyanoacetate	142	1-Naphthalenemethanol,1,4,4a,5,6,7,8,8a-8H-2,5,5,8a-tetramethyl-
101	9(11)-Dehydroergosteryl benzoate	143	2-Oxabicyclo[4.4.1]dec-9-en-8-one,1,3,7,7-tetramethyl-,(-)-(1R,3S,6R)-
102	Fumaric acid,2-dimethylaminoethyl heptadecyl ester	144	Bacchotricuneatin c
103	Fumaric acid,pent-4-en-2-yl tridecyl ester	145	2-Oxabicyclo[4.4.0]dec-9-en-8-one,1,3,7,7-tetramethyl-,(-)-(1R,3S,6R)-
104	Hexadecanoic acid,bis (2-ethylhexyl) ester		**9] Ketones**
105	Hexadecanoic acid,2-3-di-OH-propyl ester	146	3-Chlorooxanilic acid N-(3-ethoxy-4-hydroxybenzylidene) hydrazide
106	Hexadecanoic acid,2-OH-1-(OH-methyl) ethyl ester	147	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyrane-4-one
107	Methyl 19-methyl-eicosanoate	148	2,3-Dihydro-3-methyl-4H-1,3-benzothiazine-2-thione-4-one
108	Mono(2-ethylhexyl) ester	149	5H-Indenol[1,2-b]pyridine-5-one
109	1-Naphthoic acids,2,4,6-trichlorophenyl ester	150	Octabenzone
110	Oleyl alcohol,heptafluorobutyrate	151	Oxacyclododecan-2-one
111	Oxalic acid, monoamide,N-allyl-,tetradecyl ester	152	1-(3-hydrohy-3-phenyl-1-triazenyl)-antra-9,10 quinone,
112	Oxalic acid,pentadecyl propyl ester	153	2-Methyl-cyclodododecanone
113	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid,4,7-dihydro-7-imino-,ethyl ester	154	5,24-Dimethyl-1,4,7,10,18,21-hexaoxa-15,24-diazacycloocta cosane-11,1425,28-tetrone
114	S-[2-[N,N-dimethylamino]N,N-dimethylcarbamoyl thiocarbo hydroximate		**10] Other metabolites**
115	1H-indole-2-carboxylic acid,6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-isopropyl ester	155	3-ChlorooxanilicacidN-(3-ethoxy-4-hydroxybenz ylidene)hydrazide
		156	2,4-Dimethyl-benzo[h]quinoline,
116	2,4:3,5-Dimethylene-1-iditol diacetate	157	2,4-Dimethyl-benzo[h],
117	9,12-Octadecadecanoic acid (Z,Z)-,2-OH-1-(OH-methyl)ethyl ester	158	2-amino-3-chloro-1,4-Naphthalenedione,
		159	1H-tetrazol-5-yl-pyrazine,
118	9,12-Octadecadecanoic acid, ethyl ester		**11] Pyrans**
119	Pentadienoic acid, 2-hydroxy-1-hydroxymethyl) ethyl ester	160	B-D-glucopyranoside,methyl
120	Undecanoic acid,11-bromo-,undecyl ester	161	2-H-pyran,2-(2-heptadecynyloxy)tetrahydro-
	8] Heterocyclic compounds	162	2H-pyran-2-acetic acid, tetrahydro-
121	Azetidine,2,2,3-tetramethyl-		**12] Terpenes**
122	Azetidine,3-methyl-1-(1-methylethyl)	163	(3.β)-lanost-8-en-3-ol,24-methylene,
123	Carbazol	164	2,4-difluoro-1-isocyanato-Benzene,
124	2,4-Dimethyl-benzo[h]quinoline,	165	1-Docosene
125	1-Docosene	166	Ethyl-3-octene
126	Eicosane	167	3-Ethyl-3-octene
127	Eicosane,9-cyclohexyl-	168	Methanamine,N-(1-methylbutylidene),
128	Ethyl-3-octene 3-Ethyl-3-octene	169	Glucitol,4,6-o-nonylidene-
129	Geranyl nitrile	170	(3.β)-lanost-8-en-2-ol,24-methylene-,
130	1,3,12-Nonadecatriene	171	1-Nonadecene
131	3-methyl-1-(1-methylethyl)- azetidine,	172	Molybdenum,tricarbonyl[(1,2,3,4,5,6- β)-H_3benzene]-
132	1,4-Dioxin,2,3-dihydro-	173	1,3,12-Nonadecatriene
133	1 H-indole,3-methyl-	174	Squalene

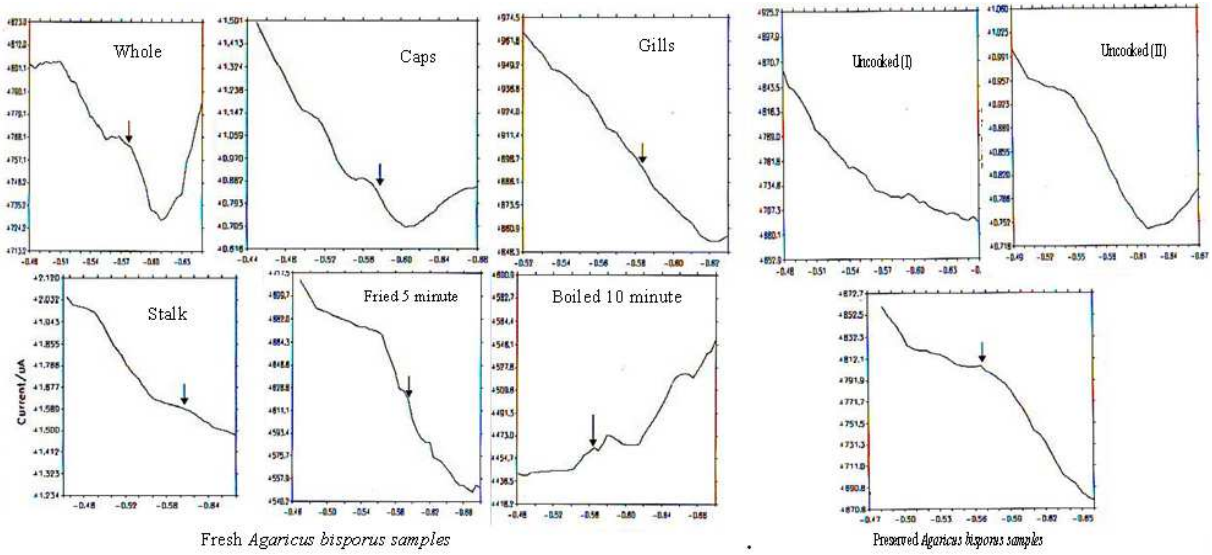

Figure 2 - Selenium (ppb) detected by Galvanometric method in *A. bisporus* samples.

The results indicated that the samples tested contained relatively low amounts of selenium. The high amount was recorded in the whole fresh samples (0.97 pbb), followed by the fried (0.11), stalks (0.1), caps (0.08), gills (0.05), cooked preserved (0.04) and boiled (0.03). On the other hand, the two uncooked preserved samples contained selenium <0001 ppb. These results were similar to the results obtained by Shi et al. (2002) and Gergely et al. (2005), which showed 0.12-0.3 ppb selenium. The chemical profile of ethanolic extract of the fresh whole mushroom showed the presence of 174 metabolites out of which 13 had significant content from 1.2 - 83%; 13 as 1% and the remaining 148 metabolites were less than 1% (w/w). The significant significant metabolites were ergosterol (83%), D-mannitol (63%), glcinamide (39.4%), L-glutamic acid (27.3%), 2-mono-linolein (10%), N,N,N-tris(hydrazine ocarbonyl) phosphoric triamide (6%), linoleic acid (4.2%), 5H-Indenol[1,2-b]pyridine-5-one (4%), glycerol (3.4%), palmitic acid (2.2%), 3-chloro-N-methylpropyl amine (2%), 2-mono-palmitin (2%) and 1,2,3,4,5,8,9,10,11,12,12,16-dodecadibenzoid, di[1,2,7,8]–tetrathiacyclo dodecin (1.2%). Thirteen metabolites detected at 1% (w/w) included alantoic acid; 1-methyl-2-phenoxyethyl amine; ergosta-5,8-dien-3-ol,(3β)-; 7,22-ergosta-dienol; γ–ergostenol; N-hexadecanoic acid; 9,12-Octadecadecanoic acid ethylester; carbonic acid, 2-dimethylaminoethyl propylester; 2R,3S-9-[1,3,4-trihydroxy-2-tutoxy methyl]guanine; 6,28-secoso-lanidan-3-ol,(3β, 5*a*)-; pidolic acid; propan-1-one,3-nitro-1-phenyl- and 1,5-Anhydro-d-mannitol. Amakura et al. (2006_a) reported that the oleic acid was the main unsaturated fatty acid in mushroom and there were saturated long-chain fatty acids such as linoleic, palmitic, and stearic, with eleostearic acid. Amakura et al., (2006_b) reported that *A. bisporus* contained three novel conjugated long-chain fatty acids obtained from aqueous methanol extracts of mushroom together with nine known constituents of ostopanic acid as aroma compounds.

The remain 148 detected metabolites were classified into twelve categories according to the functional groups, which included alcohols 11, aldehydes 5, alicyclic 10, amides 7, amines 18, carboxylic acid 10, esters 33, heterocyclic 25, ketones 9, pyran 3, terponoids 12 and other metabolites 5. Tables showed that the five metabolites related to three sugar alcohol and its derivatives included glucitol, mannitol and sorbitol. Five amino acids glycine, leucine and their derivatives were detected. Forty seven metabolites were related to six fatty acids and their derivatives, which included butyric, carpic, linoleic, oleic, palmatic and pentadecyclic. Thirty two esters and sixteen metabolites related to four kinds of sterols (cholesterol, ergosterol, lanosterol and sitosterol) were detected. Eight metabolites contained thio group. These results were in

agreement with the findings of other authors, Mattila et al., (2010) could identified aroma metabolites includes 13 alcohols, 16 aldehydes, 7 ketones and 20 heterocyclic their concentration was 8-611, 3-4850, 7-150 and 0.1-236 μg/100 g, respectively. Caglarırmak (2009) studied the *A. bisporus* in three flushes and at two different harvest times and found volatile components includes octadecanoic acid, hexadecanoic acid derivatives, and other important volatiles like di-limonene, n-nonane, benzen dicarboxylic acid, and cis-linoleic acid esters. De Barros (2008) studied *A. bisporus* and other 80 mushroom species and recorded that the detected metabolites act as a source of powerful new pharmaceutical metabolites includes polysaccarides (β-glucan and G-glucans, PSK); phenolic metabolites (p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, chlorogenic acid; rutin and epicorazins); polyketides, triterpenoids and sterols (butylated hydroxytoluene, butylated hydroxy anisole and F-tocoferol, caffeic acid); vitamines (C, thiamine-B_1 and riboflavin-B_2,D_2). Since human civilization, fruiting bodies of mushrooms have been appreciated not only for texture and flavour but also for their chemical and nutritional properties. They are widely used as food flavouring ingredients in the soups. Their importance in the diet is not only for their nutritional and organoleptic values, but also for their pharmacological characteristics because they are rich in trace minerals, vitamins (C, thiamine-B_1 and riboflavin-B_2,D_2) and have high water, protein, fibre, carbohydrate, etc [(3-65% and non-starch dietary fiber 3-32% of dry weight), low mono- and disaccharides, glycogen, insoluble fraction chitin, and have low fats (2-8% of dry weight including, free fatty acids), sterols and their esters and phosphorus lipids]. These properties make them an excellent food for use in low caloric diets. Their protein contents is high (10 - 44% of the dry weight) and they are also proven to be a good source of essential amino acids such as (leucine and valine as 25 - 40% of the total amino acids content, threonine, lysine, methionine and tryptophan) and non-essential amino acids (alanine, arginine, glycine, glutamic, aspartic, proline and serine). They act as a good source of K, Mg, Ca, Cu, Fe, Zn, and P and have low content of Na. Several other compounds have also been detected from *A. bisporus* as reported by numerous workers (Shi et al. 2002; Andersson and Gry 2004; Gergely et al. 2005; Ji, et al. 2006; Ey et al. 2007; WU 2007; Borchers et al. 2008; De Barros 2008; Sadiq et al. 2008; USCDC 2009; UN 2010).

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WFI (Wikimedia Foundation, Inc.,): a) Agaritine; b) *Agaricus bisporus*; c) Glycerol; d) Aroma compound; e) Eicosanoids; f) Vaccenic acid. Available from: Wikipedia® is a registered trademark of the Wikimedia Foundation, Inc., a non-profit organization. 2012.

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