Chemical Composition and Bioactive Compounds of Wild Edible Mushroom (*Agaricus bisporus*) from Al-Jabal Alakhdar in Libya

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ABSTRACT: Consumption of mushroom has increased remarkably because of their desirable aroma, taste and high nutritional content. Wild edible mushrooms are well known for their nutritious and antioxidant properties. The present study was conducted to evaluate chemical composition and nutritional values as well as evaluate the quality characteristics of sponge cake supplemented with Libyan wild edible mushroom (*Agaricus bisporus*). The results showed that wild edible mushroom contained high levels of proteins, fiber and total phenols and contained bioactive compounds as flavonoids, anthocyanins, tocopherols, ascorbic acid, β-carotene and lycopene. Wild edible mushroom contained considerably high amounts of minerals which the most abundant elements were potassium, calcium, sodium and magnesium followed by the iron, zinc and copper. Wild mushroom was rich source of essential and non-essential amino acids which eighteen amino acids were described. The major unsaturated fatty acids found in the studied samples were linoleic acid ($C_{18:2}$) and oleic acid ($C_{18:1}$), and the major saturated fatty acid was palmitic acid ($C_{16:0}$). Chemical composition and sensory evaluation of sponge cake supplemented with 20% mushroom powder showed that prepared cake contained more protein and fiber and cake rated the most acceptable. Due to the investigated bioactive compounds of Libyan mushroom and its well-balanced nutritional profile, this mushroom can be consumed as functional food and further used as a medicinal ingredient.
Keywords: Edible Mushroom, chemical composition, bioactive compounds, nutritional content

INTRODUCTION

Wild mushroom are found in arid and semi-arid zones of different countries all over the world (Mandeel and Al-Laith, 2007, Trappe *et al*., 2008, Abraheem, 2015 and Abugri *et al*., 2017). The edible wild mushroom growing in their natural habitats are traditionally appreciated for their health promoting attributes (Akyüz and Kirbağ, 2010, Akata *et al*., 2012, Alfaig, 2017 and Mocan *et al*., 2017). Wild mushrooms were recognized as prized source of nutrients with highly desirable flavor and aroma (Wang *et al*., 2014, Kalac, 2016 and Bhushan and Kulshreshtha, 2018), essentially because of being quiet rich in digestible proteins with significant amount of essential amino acids, carbohydrates, dietary fibers, vitamins and minerals (Liu *et al*., 2012, Atri *et al*., 2013, Kumari and Atri, 2014, Fernandes *et al*., 2016 and Vishwakarma *et al*., 2017).

Additionally, they represent an important sources of different bioactive compounds such as unsaturated fatty acids, phenolic compounds, tocopherols and carotenoids, and can be ultimately developed into functional foods, neutraceuticals or pharmaceutical products, taking advantage of their unique chemical features and the advantage of the additive and synergistic effects of all their panel of bioactive compounds (Pereira *et al*., 2012 and Taofiq *et al*., 2017). The usage of wild edible mushrooms in cooking has been maintained and developed in different cultures and civilizations around the world (Toledo *et al*., 2016 and Barroetaveña and Toledo, 2017). Moreover, the consumption of wild growing mushrooms is currently preferred to eating of cultivated fungi in many countries. Collecting them is very popular in numerous regions in the

mountain areas, representing an important component of the human diet, and the main proportion amongst other wild foods (Gąsecka *et al*., 2017).

Wild mushrooms are promising food that may overcome protein-energy malnutrition problem in the third world. The protein, fiber, mineral, carbohydrates and different bioactive compounds make them ideal food for diabetic, cancer and heart patients (Ferreira *et al*., 2010, Kumari and Atri, 2014 and Bhushan and Kulshreshtha, 2018). Partial replacements of wheat flour with other nutritional ingredients as mushroom to produce functional bakery products to improve the overall nutritional quality of cakes are in high demand (Sheikh *et al*., 2010, Verma and Singh, 2014, Salehi *et al*., 2016 Soković *et al*., 2017 and Arora *et al*., 2017). Wild mushrooms growing in East of Libya have not been completely investigated for their major components or for their bioactive compounds that would be a reliable indicator of the nutritional value. People living in Al-Jabal Alakhdar region of Libya consume wild edible mushrooms because of their abundance during the rainy season.

Therefore, the aim of the present work was to evaluate the chemical composition and nutritional values of wild edible mushrooms collected from the different area of Al-Jabal Alakhdar province of Libya. On the other hand the chemical composition and sensory evaluation of sponge cake supplemented with Libyan wild edible mushroom flour were evaluated.

MATERIALS AND METHODS

1. Materials and Chemicals

Fruiting bodies of wild edible mushroom (*Agaricus bisporus*) was harvested from different Alosita region of Al-Jabal Alakhdar, Libya. The collections were done during rainy season in Al-Jabal Alakhdar province from September to November, 2016. The habitat and morphological characteristics of mushroom found was recorded and photographed for diagnosis.

The collected mushrooms were cleaned and subsequently identified by Dr. Abdolmageed Kamara (May Allah have mercy on him), Department of Plant Pathology, Alexandria University, Egypt (Table 1).

Table (1). Scientific Classificati

Kingdom	Myceteae	**Order**	Agaricales
Phylum	Basidiomycota	**Family**	Agaricaceae
Class	Basidiomycetes	**Genus**	***Agaricus***
Subclass	Holobasidiomycetidae	**Species**	***A. bisporus***

The collected samples were cut into small pieces and freeze dried (LOUW KOELTECHNIEK BVBA with a standard program) and mushroom powder were stored in pre-cleaned polyethylene bottles at -8 °C until the analysis started. Ingredients for cake preparation included commercial wheat flour (12-13% moisture and 8-9% protein), sugar, butter, milk baking powder, egg and vanilla were purchased from local market at Alexandria. All chemicals, solvents and standards were of analytical grade and purchased from Sigma (St. Louis, MO, USA).

2. Methods

1. Analytical Methods

1. Proximate analysis and energy value

Moisture, protein, fat, ash, and crude fiber were evaluated in mushroom powder and prepared cake as described by AOAC (2006). Nitrogen Free Extract (NFE) was calculated by difference. Energy values (Kcal/100g) were calculated as reported by Greenfield and Southgate (1992) applying the factors, 4, 9 and 4 for each gram of protein, lipids and carbohydrate, respectively, according to the following equation:

Energy = 4 × (g protein + g carbohydrate) + 9 × (g fat).

2. Extraction procedure

The freeze dried mushroom samples (5 g) were extracted by stirring with 100 ml of absolute methanol at 25 ± 1ºC at 150 rpm for 24 hrs. and filtered through Whatman No. 4 paper. The residue was then extracted with two additional 100 ml portions of methanol. The combined methanolic extracts was evaporated at 40 ±ºC to dryness using Büchi R-210 evaporator and re-dissolved in methanol at a concentration of 50 mg/ml, and stored at 4 ±ºC for further bioactive compounds analysis (Kumari and Atri, 2014).

3. Total phenolic

Phenolics were determined by a Folin–Ciocalteu assay as described by Singleton and Rossi (1965). The extract solution (1 ml) was mixed with Folin–Ciocalteu reagent (5 ml, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/l, 4 ml). The tubes were vortex mixed for 15 seconds and allowed to stand for 30 minutes at 40±ºC for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (0.0094–0.15 mg/ml), and the results were expressed as mg of gallic acid equivalents (GAE) per g of dry weight (dw).

4. Total flavonoids

Total flavonoids were determined as described by Yoo *et al*. (2008). The extract sample concentrated at 2.5 mg/ml (0.5 ml) was mixed with distilled water (2 ml) and $NaNO_2$ solution (5%, 0.15 ml). After 6 min, $AlCl_3$ solution (10%, 0.15 ml) was added and allowed to stand further for 6 minutes. NaOH solution (4%, 2 ml) was added to the mixture, followed by distilled water until a final volume of 5 ml was obtained. The mixture was properly mixed and allowed to stand for 15 minutes. The intensity of pink colour was measured at 510 nm. (+)-Catechin was used to calculate the standard curve (0.015–1.0 mM) and the results were expressed as milligrams of (+)-catechin equivalents (CE) per gram of dry weight.

5. Total anthocyanins

Anthocyanin contents were determined for mushroom samples based on cyanidin-3-glucoside equivalent (CGEs) through a pH differential method adapted from Tajalli *et al*. (2015). In brief, a 0.025 M-solution of KCl was acidified by HCL (to reach pH = 1), while a 0.4 M-solution of $NaCH_3CO_2$ was adjusted to pH 4.5 by CH_3CO_2H. Then, 100 µL of each mushroom extract was mixed in methanol (at 10 mg/mL) with 900 µL of the KCl solution and the same procedure was performed with the $NaCH_3CO_2$ solution. Amounts of hundred µL of the mixture were then loaded into each well of the 96-well plate. Absorbance was read by the spectrophotometer at two levels; 510 and 700 nm and calculations were made for total anthocyanins using the following equation (mg/100 g dw):

$$A \times MW \times DF \times 10^3/\varepsilon x1$$

Where A = (A_{520} nm - A_{700} nm), MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; l = path-length in cm; ε = 26 900 molar extinction coefficient, in Lxmolxcm, for cyd-3-glu; and 10^3 = factor for conversion from g to mg. Final values were then expressed as mg of CGEs per 100 g of dw.

6. Tocopherols

The tocopherol content was assessed as described by Mocan *et al*. (2017) using an HPLC system (Knauer, Smartline system 1000; Berlin, Germany) coupled with a fluorescence detector (FP-2020; Jasco, Oklahoma City, OK, USA). Individual compounds were identified by comparison with authentic standards under the same chromatographic conditions. By using the internal standard method (tocol, IS), the quantification was based on the fluorescence signal response, and the tocopherol content was expressed in µg per 100 g of dry weight (dw).

7. Ascorbic acid

For ascorbic acid quantification, standard ascorbic acid solution (5 mL L-ascorbic acid in 3% phosphoric acid) was added to 5 mL of meta-phosphoric acid. A microburette was filled with dye (2,6-dichlorophenol indophenol), and the samples were titrated with the dye solution to a pink color, which persisted for 15 seconds. The dye factor (milligrams of ascorbic acid per milliliter of dye using formula: 0.5/titrate) was determined. A sample was prepared by taking 10 g of sample grounded in metaphosphoric acid, and the volume was increased up to 100 mL. It was titrated after filtration until a pink color appeared (Arti *et al*., 2013). The amount of ascorbic acid was calculated with the use of the following equation:

mg of ascorbic acid per100 g mushroom dry weight =

$$\frac{\text{Titrate} \times \text{Dye factor} \times \text{Vol. made}}{\text{Aliquot of extract} \times \text{wt. of sample}} \times 100$$

8. β- Carotene and Lycopene

For β-carotene and lycopene determination, the dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone– hexane mixture (4:6) for 1 minute and filtered through Whatman No. 4 filter paper (Barros *et al*.,

2007). The absorbance of the filtrate was measured at 453, 505 and 663 nm. Content of β-carotene was calculated according to the following equations:
β-carotene (mg/ 100 ml) = $0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}$
Lycopene (mg/100 ml) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806 \times A_{453}$.
The results were expressed as µg of carotenoid/g of dry weight.

9. Minerals

The elements were determined as described by Nakalembe *et al*., (2015). Element constituents comprising potassium (K), magnesium (Mg), iron (Fe), calcium (Ca), copper (Cu), manganese (Mn) and zinc (Zn) were determined by Atomic Absorption Spectrophotometry (Unicam Analytical system, Model 919, Cambridge, UK), whereas phosphorus (P) and sodium (Na) determination were determined using a flame photometer (Jenway, PF 7, Essex UK). For selenium (Se) analysis, digestion was done using an advanced Microwave digestion system (Milestone ETHOS, Labstation with easyWAVE) with nitric acid and hydrogen peroxide, and then analyzed using an inductively coupled plasma (ICP) spectrometer (iCAP 6000 series; Thermo Scientific). All minerals were expressed in mg/Kg dry weight basis.

10. Amino acids

The amino acid was determined according to the official method of the AOAC (2006). A sample of 2g ± 0.001g was weighed into a pre-dried and tarred dish. Then the sample was placed into an oven at 60±1°C for four hours as described by Alfaig (2017). The covered sample was transferred to desiccators and cooled to room temperature, after that the sample was digested with 30 ml of 6 M HCL for 24 hours at 110°C was digested again with 30 ml of 4.2 M NaOH for 24 hours at 110°C after the addition 25 ml of methanol (HPLC grade) of each sample extract was then filtered by (Whitman filter paper; No.42) into a round bottom flask before injection into the HPLC. Finally the volume was made up to 5ml and then injected to HPLC (Gilson), using an ion exclusion column (Nucleogel Ion 3exclusion column (Nucleogel Ion 300 OA; 300 × 7.7 mm), in conjunction with a column heating device at 30 °C.

11. Fatty acid composition

Total lipid was extracted from mushroom powder with a mixture of chloroform / methanol (2: 1 v/v) according to the method described by Folch *et al*. (1957). Fatty acids methyl esters (FAMEs) were obtained by the method described by Sudheepa and Sridhar (2014). Ten gram of the lipid extract was saponified with 0.5 mol/L NaOH in methanol followed by a methylation in 12% boron trifluoride in methanol (BF3/MeOH). The methylated sample was then extracted with n-hexane. All of these reactions were performed in quadruplicate for each sample. The resulting methyl esters were analysed by gas chromatography (GC) using an Agilent Technologies chromatograph 6890N (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID), a splitless injector and a polar INNOWAX 30 M silica capillary column (0.25 mm i.d. & 0.25 µm film thickness). The temperature of the injector and detector were 220 °C and 275 °C, respectively. Helium was used as a carrier gas with a flow rate of 1.5 ml/mn. Peaks were identified by comparison of their retention times with FAMEs standards (SUPELCO). The sequences of

fatty acids were ranged according to their chromatographic retention times and the values are given as percentages of the total fatty acids methyl esters.

12. Sensory evaluation of cakes containing Mushroom powder

Prepared cakes were evaluated organoleptically for colour, flavour, texture, and overall acceptability as described by Sheikh *et al*. (2010). A 1-9 point hedonic rating test was also performed to assess the degree of acceptability of cakes containing mushroom powder in different level. One slice from each lot of cake was presented to 10 panellists as randomly coded samples. The taste panellists were asked to rate the sample for colour, flavour, texture, and overall acceptability on a 1-9 point scale, where 9=Like extremely; 8=Like very much; 7= Like moderately; 6=Like slightly; 5= Neither like nor dislike; 4=Dislike slightly; 3= Dislike moderately; 2= Dislike very much; 1=Dislike extremely.

2. Technological Methods

1. Formulation of cake incorporated with mushroom powder

The basic formulation of sponge cake (Arora *et al*., 2017) as shown in Table (2). The additions of wheat flour in the formulations were made with 10%, 20%, and 30% of mushroom powder.

Table (2). Basic formulation of sponge cake on 300g flour basis

Ingredients	Quantity (g)	Ingredients	Quantity (g)
Wheat flour	300	**Milk (powder) or liquid milk**	40 g or 100 ml
Sugar (powder)	250	**Salt**	3
Butter	200	**Baking powder**	8-10
Egg	3-4	**Vanilla essence**	5 ml

2. Cake making procedures

Cakes were prepared by wheat flour with 10%, 20% and 30% of mushroom powder samples in the basic formulation of cake as described by Arora *et al*. (2017). The flour, mushroom powder and other ingredient for each cake were weighed accurately and the sugar and butter were mixed in a mixing machine for 20 minutes to produce a cream. In later stages, half of the milk, other ingredients, and finally the flour were mixed using at low speed (145 rpm) for 10 minutes to ensure even distribution of the components. All cakes were baked in National forced convection oven for 40 minutes at 170°C. Three cake samples were prepared for each trial.

RESULTS AND DISCUSSION

1. Proximate chemical composition and energy value of wild edible mushroom

The results of proximate chemical composition are shown in Table (3). The average moisture content in dried samples was 9.50%. This result was in agreement with Kumari and Atri (2014) who published that wild edible mushrooms in India contained from 8.5-10.0% moisture content in dried sample.

Edible mushrooms are highly valued as a good source of protein. The average crude protein in dried mushroom samples was 30.44%. This result

was in agreement with Johnsy *et al*. (2011) who found that protein in wild edible mushroom ranged from 28.93% to 39.1% of dry weight. Protein contents of mushrooms were reported to vary according to the genetic structure of species and physical and chemical differences in growing medium (Agrahar-Murugkar and Subbulakshmi, 2005). The obtained values were almost similar to crude protein contents of wild edible mushrooms reported by Abdolgader *et al*. (2016). They found that crude protein in edible wild Libyan mushroom ranged from 29.64 to 43.28% according to the different regions. The average insoluble fiber in dried mushroom samples was 8.32%. This result was in agreement with Mallikarjuna *et al*. (2012) and Wandati (2013) who found that insoluble fiber ranged from 4.5-16.3% in wild *Agaricus bisporus*. The average ether extract in dried mushroom samples was 1.60%. In the literature crude fat concentration in wild mushrooms samples have been reported in the range of 0.92 to 4.88% in Portugal (Barros *et al*., 2008a), 0.5 to 4.7% in India (Kumari and Atri, 2014), 1.0 to 6.7% in China (Wang *et al*., 2014) and 1.02 to 2.82% in Libya. In general, wild mushrooms were a richer sources of protein and had a lower amount of crude fat than commercial mushrooms making it an ideal snack material (Agrahar-Murugkar and Subbulakshmi, 2005).

The average ash in dried mushroom samples was 9.25 %. Ash content is highly influenced by the growth substrate (Wandati, 2013). Agrahar-Murugkar and Subbulakshmi (2005) reported that ash content varied between 6.3% to 13.9% in seven wild mushroom species, which regularly consumed in India. Abdolgader *et al*. (2016) found that ash contents of Libyan wild edible mushroom samples in different regions ranged from 13.77 to 22.08%. Libyan wild edible mushroom contained 40.89% of nitrogen free extract (Table 3). Edible mushrooms are highly valued as a good source of carbohydrates and their contents usually, range from 40.6% to 53.3% of dry weight (Johnsy *et al*., 2011). Abdolgader *et al*. (2016) found that Carbohydrates contents of Libyan wild edible mushroom samples in different regions ranged from 33.86 to 53.21%.

Energy value was estimated based on its content of crude protein, carbohydrate (each one g gives 4 K cal), fat (one g gives 9 Kcal), energy value in studied samples was found to be 299.72 Kcal/100g. Kumari and Atri (2014) reported that energy was 364.7 Kcal/100g of *M. rhacodes*, on an average, which was found higher as compared to other wild edible mushrooms.

Table (3). Proximate chemical composition and energy value of wild edible mushroom collected from Al-Jabal Alakhdar, Libya

Component (%)	*Agaricus bisporus*
Moisture (Dry matter)	9.50 ± 0.4
Crude Protein	30.44 ± 1.8
Insolube fiber	8.32 ± 0.4
Ether Extract	1.60 ± 0.2
Ash	9.25 ± 0.2
Nitrogen Free Extract	40.89 ± 1.3
Energy (Kcal/100g)	299.72

All values are expressed as means± SD on dry weight basis of triplicates, n=3

2. Some bioactive compounds in wild edible mushroom

Phenolic and flavonoids are considered one of the major groups of nonessential dietary components which have been suggested to be beneficial for human health (Celestine *et al*., 2013 and Upadhyaya *et al*., 2017). The results of bioactive compounds in studied wild edible mushroom are shown in Table (4). Mushroom samples exhibited high amounts of total phenol content TPC (6.22 ± 0.4 mg GAEs/g dw). The TPC observed in Libyan wild edible was lower than that of Iranian wild *A. bisporus* with 9.61 mg GAEs/g (Tajalli *et al*., 2015) and Turkish wild *A. bisporus* with 13 mg GAEs/g (Elmastas *et al*., 2007). The level of TPC in this study was higher than those for wild *A. bisporus* isolates from Portugal (Barros *et al*., 2009) and China (Liu *et al*., 2013) with amounts of 4.49 and 6.18 mg GAEs/g, respectively.

Abraheem (2015) found that TPC was 4.37±0.05 mg GAE/g dw in mushroom from Alosita region at Al-Jabal Alakhdar, Libya during winter 2014, while in this study, mushroom was collected from the same region but in winter 2016 showed higher amount of TPC (6.22 mg GAE/g dw). It has been reported that phenolic compounds are known to be powerful chain-breaking antioxidants and possess scavenging ability due to their hydroxyl groups (Liu*, et al*., 2013).

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. The total flavonoid content of mushroom methanolic extracts in wild edible mushroom collected from Al-Jabal Alakhdar, Libya was found as 3.11 ± 0.2 mg CE/g dw (Table 4). Flavonoids belong to phenolic compounds and research has shown their antioxidant activity (Sesseira *et al*., 2009). However, there is limited information on flavonoid contents of well-authenticated wild *A. bisporus*. In comparison to cultivated strains of *A. bisporus*, the findings of this study indicate higher flavonoid contents than those reported for cultivated strains of *A. bisporus* from Portugal (Barros *et al*., 2008b) and Malaysia (Gan *et al*., 2013). Flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides in food systems (Roedig-Penman and Gordon, 1998).

Anthocyanin content in wild edible mushroom (*A. bisporus*) collected from Al-Jabal Alakhdar, Libya was found as 4.12 ± 0.4 mg CGEs per 100 g of dw (Table 4). Anthocyanins are flavonoids commonly found in fruit and vegetables and research has confirmed their antioxidant activity (He and Giusti, 2010 and Vamanu and Nita, 2014). Similar results were determined with several Iranian and Turkish wild edible mushrooms (Sankara-Rao *et al*., 2013 and Tajalli *et al*., 2015). Thus, high amounts of anthocyanins found in the tested wild mushrooms are important for conferring antioxidant activity.

Tocopherol contents in wild edible studied mushroom samples are detailed in Table (4). α-tocopherol (0.85 ± 0.16 µg/g), β- tocopherol (1.12 ± 0.6 µg/g) and γ- tocopherols (0.46 ± 0.4 µg/g) were found to be present in Libyan wild edible mushroom. β-tocopherol was found in higher amounts as compared to α-tocopherol. γ-tocopherol was detected in low level. Similar findings were made in other wild and cultivated species with higher α- and β-tocopherol than γ-tocopherol (Barros *et al*., 2008b). The high levels of these two compounds

correspond with a higher oxidative activity, which is associated with cardiovascular protection (Reis *et al*., 2011). Ascorbic acid (0.92 ± 0.1 mg/100g dw) was found to be present in Libyan wild edible mushroom. These result was in agreement with results of Sharma and Gautam (2015) who found that ascorbic acid varied from 0.32 - 0.99 mg/100g dw in twenty wild mushroom species β-carotene and lycopene were found in low levels (8.22 ± 0.32 and 4.52 ± 0.22 µg/g) as described in Table (4). These results are in agreement with different publications (Tajalli *et al*., 2015 and Upadhyaya *et al*., 2017).

Some authors have already reported a direct correlation between mushrooms antioxidant activity and total phenolic content, although the antioxidant action is raised by other substances such as tocopherols, ascorbic acid and β-carotene (Cheung *et al*., 2003 and Barros *et al*., 2008b).

Table (4). Some bioactive compounds in wild edible mushroom collected from Al-Jabal Alakhdar, Libya

Bioactive component	*Agaricus bisporus*
Total phenols (mg GAE/g dw**)**	6.22 ± 0.4
Total flavonoids (mg CE/g dw)	3.11 ± 0.2
Anthocyanins (mg CGEs per 100 g)	4.12 ± 0.4
Total tocopherols (µg/g)	2.43 ± 0.16
α-	0.85 ± 0.05
β-	1.12 ± 0.06
γ-	0.46 ± 0.04
Ascorbic acid (mg/100 g dw)	0.92 ± 0.1
β-Carotene (µg/g dw)	8.22 ± 0.32
Lycopene (µg/g dw)	4.52 ± 0.22

All values are expressed as means± SD on dry weight basis of triplicates, n=3

3. Elements in wild edible mushroom

In general, Libyan wild edible mushroom contained considerably high amounts of elements (Table 5). The most abundant elements were potassium, calcium, sodium and magnesium followed by the iron, zinc and copper. These were in good agreement with the report of the analysis of cultivated mushrooms, such as *Agaricus bisporu*s and *Pleurotus ostreatus* (Mattila *et al*. 2002). The potassium content (27490 mg/kg dw) was higher than the level of other minerals. Gençcelep *et al*. (2009) and Kalac (2016) reported that potassium contents of wild edible mushrooms being between 12600 and 29100 mg/kg dw. Abdolgader *et al*. (2016) reported that potassium level varied in Libyan wild mushroom samples among different regions of Al-Jabl Alkhader province, ranged from 17336.6 mg/kg dw to 26311.0 in cap mushroom. The calcium content was found in high level (2505 mg/kg dw). Abdolgader *et al*. (2016) reported that calcium level varied in Libyan wild mushroom samples among different regions of Al-Jabl Alkhader province, ranged from 797.04 mg/kg dw to 3049.80 mg/kg dw in cap mushroom. Uzun *et al*. (2011) found different calcium concentrations (400 to 5720 mg/kg dw) of the same wild mushroom species during three years. They referred that environmental factors are very important for metal concentrations in mushrooms.

The sodium content was found in the level of 1988 mg/kg dw. This result showed higher content of sodium than cultivated *Agaricus bisporus* mushrooms from Australia (Koyyalamudi *et al*., 2013). They found that sodium contents ranged from 840 to 1640 mg/kg dw. The maximum sodium contents detected in stipe Libyan wild edible mushroom obtained from Ashnishen region followed by cap samples obtained from Alosita region (Abdolgader *et al*., 2016). The magnesium content was found in the level of 1450 mg/kg dw. These results were in agreement with the results of Uzun *et al*. (2011) who found that magnesium within the same range in wild mushroom species. The observation of different results can be attributed that the major elements (potassium, calcium, sodium and magnesium) profile of mushrooms has been affected by environmental factors such as climate, growing conditions, region and soil content (Colak *et al*., 2009). It is known that adequate iron in a diet is very important for decreasing the incidence of anemia. The iron content in Libyan wild edible mushrooms was found as 554 mg/Kg dw. This result was higher than found by Abdolgader *et al*. (2016). In literature, the iron content of the mushrooms ranged from 55 to 1930 mg/kg dw (Gençcelep et al. 2009, Uzun *et al*., 2011 and Nagy, 2016).

The phosphorus content was found in the level of 452 mg/kg dw. These results were in agreement with the results of Uzun *et al*. (2011). Dietary calcium and phosphorus intakes is often considered to be a primary factor in bone mineralization and metabolism (Nagy, 2016). Zinc was determined to be at low level (139 mg/kg dw) in Libyan wild edible mushroom. The reported literature showed that zinc content ranged between 22.1 and 214.3 mg/kg dw (Kalac and Svoboda, 2000, Kaya *et al*., 2011 and Kalac, 2012). The WHO permissible limit of zinc in foods is 60 mg/kg. The value for zinc in investigated mushroom samples were above the WHO's values (WHO, 1993).

Copper was the third most abundant trace element in the human body, with vitamin-like impact on living systems. Copper was determined to be at the low level (68 mg/kg dw) in Libyan wild edible mushroom. This result is higher than found by Abdolgader *et al*. (2016). Copper contents found in this study was comparable with those reported in literature. Copper concentrations, accumulated in mushroom species, are usually 100-300 mg/kg dw, which is not considered a health risk. Copper content in mushrooms was higher than those in vegetables should be considered as a nutritional source of the element. Nevertheless, for people, the bioavailability of copper in mushrooms was reported to be low, due to the limited absorption from the small intestine (Schellman *et al*. 1980). The manganese content of the mushrooms was determined to be at the low level (4.32 mg/kg dw) in Libyan wild edible mushroom. Uzun *et al*. (2011) found that manganese ranged from 0.2 to 80 mg/kg dw depending on mushroom species and environments. The manganese values in present study are in agreement with the results found in literature (Soylak *et al*. 2005 and Gençcelep *et al*. 2009). The selenium content of the mushrooms was determined to be at the lowest level (0.62 mg/kg dw) in Libyan wild edible mushroom. The content of selenium in mushrooms mostly ranges between 0.57 and 19.46 mg /kg depending on the type, age and place of finding mushroom (Bhushan and Kulshreshtha, 2018)

According to the EU Scientific Committee for Food Adult Weight parameter, 60 kg of body weight was used for intake calculations as the weight of an average consumer. In addition, for intake calculations, usually a 300 g portion of fresh mushrooms, which contains about 30 g of dry matter, per meal is assumed (Kalac and Svoboda 2000 and Svoboda *et al*. 2000).

Table (5). Elements in wild edible mushroom collected from Al-Jabal Alakhdar, Libya

Elements	*Agaricus bisporus* (mg/kg dw)
Potassium	27490
Calcium	2505
Sodium	1988
Magnesium	1450
Iron	554
Phosphorus	452
Zinc	139
Copper	68
Manganese	4.32
Selenium	0.62

4. Amino acids in wild edible mushroom

Fruiting bodies of Libyan wild edible *Agaricus bisporus* mushroom are a rich source of amino acids (essential and non-essential). Eighteen of these compounds were researched and described (Table 6).The amino acids found in *A. bisporus* in the highest amounts were leucine (7.65 g/100g protein), aspartic acid (7.23 g/100g protein), glutamic acid (6.63 g/100g protein), alanine (6.63 g/100g protein), lysine (4.66 g/100g protein), arginine (3.98 g/100g protein) and threonine (3.98 g/100g protein). Tseng and Mau (1999) reported that the arginine content of some wild edible mushrooms (*Agaricus bisporus*) was in (3.83 mg/100g protein). Arginine present in the *Agaricus bisporus* should be given special attention because it is a component used in dietary supplements for patients with cancer. Arginine delays tumor growth and metastasis, and also has a beneficial influence on the immunological system, body mass growth and the life-expectancy of oncological patients (Novaes *et al*., 2011). The amino acids found in *A. bisporus* in the highest amounts are alanine, aspartic acid, glutamic acid, arginine, leucine, lysine, phenylalanine, serine, proline, tyrosine and threonine (Bernaś *et al*., 2006 and Muszyńska *et al*., 2013).

Recently, Omer (2017) reported that *Agaricus bisporus* mushroom contained histidine (2.7 – 4.7 g/100g protein), lysine (5.1 - 9.1 g/100g protein), tryptophan (0.9 – 2.1 g/100g protein), phenylalanine (2.1 – 4.2 g/100g protein), methionine (4.1 -5.1 g/100g protein), threonine (4.1 – 5.5 g/100g protein), leucine (3.9 – 7.5 g/100g protein), isoleucine (4.5 – 5.5 g/100g protein) and valine (2.5 – 4.5 g/100g protein).

On comparison with FAO-WHO (1989) essential amino acids (EAAs) reference pattern for adults, Libyan wild edible *Agaricus bisporus* mushroom possess higher quantity of threonine, valine, isoleucine, leucine and histidine,

but the rest of essential amino acids were lower of them in mushroom. Supplementation of mushroom protein with phenylalanine and methionine would be necessary, when used as a sole source of protein in diet, to promote adequate growth. Since mushrooms are considered as delicacies, their supplementation with a cereal diet may help to overcome lysine deficiency (Longvah and Deosthale, 1998).

5. Fatty acid composition of wild edible mushroom

In the present study, fatty acid composition of Libyan wild edible mushroom namely *Agaricus bisporus* mushroom was analyzed. The results for fatty acid composition, total saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) of the studied mushrooms are shown in Table 7. The major unsaturated fatty acids found in the studied samples were linoleic acid ($C_{18:2}$) and oleic acid ($C_{18:1}$), meanwhile the saturated fatty acid was palmitic acid ($C_{16:0}$). Results revealed that the levels of unsaturated fatty acid were higher than saturated ones in *Agaricus bisporus* mushroom. These results were in agreement with the earlier findings that unsaturated fatty acids content was predominating fatty acids in different species of mushrooms as compared to saturated ones (Senatore, *et al*., 1988).

Table (6). Amino acids in wild edible mushroom collected from Al-Jabal Alakhdar, Libya

Amino acid pattern	*Agaricus bisporus* (g / 100 g protein)	Adult Requirements FAO/WHO (1989)
Essential Amino Acids (EAA)		
Threonine	3.98	3.4
Valine	3.57	3.5
Cystine	1.92	2.5
Methionine	1.44	
Isoleucine	2.82	2.8
Leucine	7.65	6.6
Tyrosine	2.53	6.3
Phenylalanine	2.64	
Tryptophan	1.45	1.1
Lysine	4.66	5.8
Histidine	1.98	1.9
Total EAA	34.64	
Non Essential Amino Acid (NEAA)		
Glutamic acid	6.63	
Aspartic acid	7.23	
Serine	3.22	
Proline	1.26	
Alanine	6.63	
Glycine	2.96	
Arginine	3.98	
Total NEAA	31.91	

Linoleic acid was the major fatty acid detected in *Agaricus bisporus* Libyan wild edible mushroom (68.36% total fatty acids) and it is one of the essential fatty acids which is not synthesized by man. Presence of linoleic acid is quite essential for the normal growth of human beings. It is known that linoleic acid is the precursor of 1-octen-3-ol, known as the alcohol of fungi, which is the principal aromatic compound in most fungi and might contribute to mushroom flavour (Magga, 1981). In addition to linoleic acid, oleic (10.48%), palmitic (12.20%), stearic acids (4.28%), linolenic (1.36%), myristic (1.35%) and palmitoleic (1.10%) acids were the other fatty acids present in the mushrooms examined. Some other fatty acids as lauric, behenic and arachidonic acids were in low levels. Similar observations have been observed in other mushrooms (Barros *et al*., 2007 and Lee *et al*., 2011).

In earlier studies, Cruz *et al*. (1997) the fatty acid concentration of two different strains of *Agaricus bisporus* at different stages of development reported that linoleic acid was major fatty acid whatever the strain or development stage. Rai *et al*., (1998) reported that mushrooms contained 70 % polyunsaturated fatty acids as linoleic acid. The results indicated that mushrooms were rich in polyunsaturated fatty acids, especially linoleic acid and therefore should be included in the diet. Libyan wild edible mushroom seems to be an excellent option as the food concerning content of fatty acids, since it has a lower percentage of SFA. Ozturk *et al*. (2011) revealed a slightly lower SFA content (20.28%) and higher levels of unsaturated fatty acids (79.72%) in a wild specimen of *A. bisporus* from Turkey. Reis *et al*. (2012) revealed different fatty acid compositions in a commercial sample of *A. bisporus* from Portugal, revealing higher PUFA (78.3%) and lower MUFA (1.4%) contents. The different fatty acid composition of mushroom species from place to another is supported by previous studies (Goyal *et al*., 2015 and Abugri *et al*. (2017) show that fatty acids tends to be influenced by environment factors as temperature, aeration, enzymatic activities and media component.

Table (7). Fatty acid composition in wild edible mushroom collected from Al-Jabal Alakhdar, Libya

Fatty acids (% of total FAs)		*Agaricus bisporus*
Lauric	$C_{12:0}$	0.22
Myristic	$C_{14:0}$	1.35
Palmitic	$C_{16:0}$	12.20
Stearic	$C_{18:0}$	4.28
Behenic	$C_{22:0}$	0.20
Total Saturated FAs		**18.25**
Palmetoleic	$C_{16:1}$	1.10
Oleic	$C_{18:1}$	10.48
Linoleic	$C_{18:2}$	68.36
Linolenic	$C_{18:3}$	1.36
Arachidonic	$C_{20:1}$	0.45
Total Unsaturated FAs		**81.75**

6. Chemical analysis of cakes supplemented with mushroom powder

Nutritional values of cake samples are depicted in Table 8. Moisture holding of the cake samples increased with increasing levels of mushroom powder, ranging from 23.2% in control to 31.6% in cake, with 70:30 blends of wheat flour and mushroom powder. The cake samples with 30% mushroom powder showed the highest contents of protein (12.32%), fiber (10.66%) and ash (5.49%) compared with other samples. There was no difference in fat content of samples, but values of protein, fiber and ash content increased with increasing levels of mushroom powder.

This increase may be due to inherent nutritional composition of mushrooms. These results were in agreement with those of Abdel-Kader (2001), Agu *et al*. (2010) and Okafor *et al*. (2012).The protein and ash content of sponge cake increased significantly with the increasing button mushroom powder level (Salehi *et al*., 2016).

Table (8). Nutritional parameters of sponge cakes fortified with mushroom

	Ingredient formulation* (Wheat: Mushroom powder)			
Parameter (%)	**100:0**	**90:10**	**80:20**	**70:30**
Moisture	23.2 ± 1.12	25 ± 1.24	27 ± 1.22	31.6 ± 1.32
Fat	14.2 ± 0.32	13.9 ± 0.24	14.1 ± 0.31	14.2 ± 0.33
Protein	7.3 ± 0.21	7.8 ± 0.23	8.2 ± 0.23	12.32 ± 0.35
Fiber	6.5 ± 0.11	7.66 ± 0.21	8.69 ± 0.23	10.66 ± 0.31
Ash	1.91 ± 0.05	2.95 ± 0.04	3.96 ± 0.04	5.49 ± 0.04

All values are expressed as means± SD on dry weight basis
of triplicates, n=3

7. Sensory evaluation of cakes containing mushroom powder

Sensory scores of cake samples are given in Table 9. The sensory evaluation of cake samples was done on 6 parameters (appearance, taste, color, aroma, texture and overall acceptability). There was no difference in individual organoleptic attributes of cake samples up to a level of 20 per cent replacement of wheat flour with mushroom powder. Overall acceptability score of 6.92 was obtained by sample contain 20 % mushroom powder against a score of 6.34 for control, so that on sensorial basis, 20 percent of mushroom powder in cakes can be optimized. Sensory evaluation based on appearance, crust color, crumb color, crumb texture, taste, chew ability, flavor and overall acceptability showed there were no significant ($p > 0.05$) difference between 10 per cent mushroom powder fortified bread and 100 per cent wheat flour bread (control) in all the attributes evaluated (Okafor *et al*., 2012).

On a nine-point hedonic scale, the sensory results of the control, 10 and 15 % were in the range of 5.8–7.3, indicating that these three cakes were moderately acceptable (Salehi *et al*., 2016). The sensory characteristics liking results pointed out that a partial replacement of cake flour with up to 15 % button mushroom powder in sponge cakes is satisfactory.

Table (9). Sensory evaluation of mushroom fortified sponge cake

	Ingredient formulation* (Wheat: Mushroom powder)			
Parameter	**100:0**	**90:10**	**80:20**	**70:30**
Appearance	7.5 ± 0.2	8.0 ± 0.3	7.8 ± 0.3	7.5 ± 0.3
Color	7.2 ± 0.2	6.8 ± 0.2	7.4 ± 0.3	6.5 ± 0.2
Aroma	6.2 ± 0.2	7.2 ± 0.3	7.6 ± 0.3	7.0 ± 0.3
Taste	5.4 ± 0.2	5.6 ± 0.2	5.9 ± 0.2	5.5 ± 0.2
Texture	5.4 ± 0.1	5.7 ± 0.2	5.9 ± 0.2	5.5 ± 0.2
Overall Acceptability	**6.34 ± 0.2**	**6.66 ± 0.3**	**6.92 ± 0.2**	**6.40 ± 0.2**

All values are expressed as means± SD on dry weight basis of triplicates, n=3

CONCLUSION

This research work has demonstrated that wild edible mushrooms obtained from Al-Jabal Alakhdar province, Libya were rich sources of essential nutrients as protein, carbohydrates, insoluble fiber and all the minerals required for a healthy body and had low amount of fat as well as contained biologically active compounds such as phenolics, flavonoids, anthocyanins, tocopherols, ascorbic acid, β-carotene and lycopene. The results of this work might be the basis that Libyan wild edible mushrooms can be used directly in the diet and promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present. This study has demonstrated that addition of increasing levels of mushroom powder in the cake affected the quality attributes. The findings of the present study may help in developing commercial processing technology for effective utilization wild edible mushroom powder especially for manufacturing of bakery products.

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الملخص العربي

التركيب الكيماوي والمركبات النشطة بيولوجياً لعيش الغراب البرى المأكول من الجبل الأخضر في ليبيا

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لقد ازداد استهلاك عيش الغراب بشكل ملحوظ بسبب الرائحة والطعم المرغوبين لدى المستهلكين وكذا المحتوى الغذائي العالي. وعيش الغراب البرى الصالح للأكل معروف بتراكيبه المغذية وخصائصه المضادة للأكسدة. أجريت هذه الدراسة لتقييم التركيب الكيميائي والقيمة الغذائية وكذلك تقييم خصائص الجودة الخاصة بالكيك الإسفنجي المدعم بعيش الغراب الليبي البرى المأكول من جنس *Agaricus bisporus*.

أظهرت النتائج أن عيش الغراب البرى المأكول يحتوي على مستويات عالية من البروتينات والألياف والفينولات الكلية ويحتوي على مركبات نشطة بيولوجيا مثل الفلافونويدات والأنثوسيانين والتوكوفيرولات وحمض الأسكوربيك والبيتا كاروتين والليكوبين. أحتوى عيش الغراب البرى المأكول على كميات كبيرة من العناصر المعدنية الضرورية وكانت العناصر الأكثر تواجداً فيه هي البوتاسيوم والكالسيوم والماغنسيوم يليها الحديد والزنك والنحاس. كما يعتبر عيش الغراب البرى المأكول مصدرا غنيا للأحماض الأمينية الأساسية وغير الأساسية حيث احتوى على ثمانية عشر حامض أمينى. تم فصل العديد من الأحماض الدهنية المشبعة وغير المشبعة من عيش الغراب موضوع الدراسة حيث مثل حمض البالمتيك ($C_{16:0}$) أعلى الأحماض الدهنية المشبعة تواجدا بينما كان كلا من حمض اللينوليك ($C_{18:2}$) وحمض الأوليك ($C_{18:1}$) هما أعلى الأحماض الدهنية غير المشبعة تواجداً.

أظهرت نتائج التركيب الكيميائي والتقييم الحسي للكيك الإسفنجي المدعم بمسحوق عيش الغراب بنسبة ٢٠٪ أن الكيك المحضر يحتوي على نسبة أعلى من البروتين والألياف وهو الأكثر قبولًا لدى المحكمين عن الكيك الإسفنجي المحضر من الدقيق فقط (الكنترول). نظراً لاحتواء عيش الغراب البرى المأكول الليبى على المركبات النشطة بيولوجياً والعناصر الغذائية المتوازنة بشكل جيد لذا يمكن استهلاك هذا الفطر كغذاء وظيفي وكذا يمكن استخدامه كعلاج طبى.