



Sudan University of Science and Technology
College of Graduate Studies



**Chemical Composition and Nutritional Value of Some Types
of Wild Mushrooms in Blue Nile State**

التركيب الكيميائي والقيمة الغذائية لبعض أنواع المشروم البرية الصالحة للأكل في
ولاية النيل الأزرق

By

Ibrahim Ishag Ibrahim Omer

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Supervisor

Dr. Ebrahim Alfaig Alnoor Alfaig

Department of Food Science and Technology

College of Agricultural Studies

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا
مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنَ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ
وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انظُرُوا إِلَى
ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكَُمْ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ)

صدق الله العظيم

(سورة الانعام الآية 99)

Dedication

To the utmost knowledge lighthouse, to our greatest and most honored prophet Mohamed may peace and grace from Allah be upon him.

To the spring that never stops giving, to my mother who weaves my happiness with string from her merciful heart.

To whom he strives to bless comfort and welfare and never stints what he owns to push me in the success way who taught me to promote life stair wisely and patiently to the spirit of my dear father.

To whose love flows in my veins and my heart always remembers them to my brothers and sister.

To those who taught us letters of gold and words of jewel of the utmost and sweetest sentences in the whole knowledge who reworded to us their knowledge simply and from their thoughts made a lighthouse guides us through the knowledge and success path to our honored teachers and professors.

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Abstract

This study was conducted to determine the chemical composition and nutritional value for eight samples of edible mushrooms, including seven samples of wild edible mushrooms collected from Blue Nile State and one commercial sample. All samples were classified by mycokey software program and mushroom expert web site.

The mushrooms varieties found are: *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*). Also toxicity was detected using potassium hydroxide (4%) during collection in the field and for all samples the toxicity test was negative. The results showed that the samples containing varying moisture content between samples ranged from 5.26% to 11.11%. Fat content ranged from 0.94% to 2.99%. Protein content ranged between 19.41% to 34.14%. Ash content ranged from 0.22% to 1.47%. Fiber content ranged from 5.25% to 23.86%. Carbohydrate content ranged from 26.76% to 77.91%. The calcium content (mg/kg) ranged from 5.80 to 8.60. The magnesium content (mg/kg) ranged from 3.07 to 5.50. The phosphorus content (mg/kg) ranged from 2.15 to 2.50. The potassium content (mg/kg) ranged from 21.20 to 21.50. The iron content (mg/100g) ranged from 1.30 to 1.55. The zinc content (mg/100g) ranged from 0.51 to 0.52. The copper content (mg/100g) ranged from 0.10 to 0.11. The manganese content (mg/100g) ranged from 0.10 to 0.12.

The essential amino acids content (mg/100g) of mushroom samples were: arginine content ranged from 6.30 to 6.40. Histidine content ranged from 1.90 to 2.20. Lysine content ranged from 5.00 to 5.10. Tryptophan content ranged from 0.88 to 0.90. Phenylalanine content ranged from 2.00 to 2.10. Methionine content ranged from 1.00 to 1.15. Threonine content ranged from 4.05 to 4.10. Leucine content ranged from 3.90 to 4.10. Isoleucine content ranged from 5.56 to 5.70. Valine content ranged from 4.20 to 4.50.

All samples contain high levels of protein, minerals and essential amino acids. However the *Volvariella volvacea* sample is the best sample in terms of containing the protein so it can be entered as a dietary supplement in some processed foods that contain very small amounts of protein.

ملخص البحث

هذه الدراسة أجريت لتحديد المكونات الكيميائية والقيمة الغذائية لعدد ثمانية عينات من المشروم منها سبعة عينات برية صالحة للأكل تم جمعها من ولاية النيل الأزرق وعينة واحدة تجارية. حيث تم تصنيف جميع العينات بواسطة mushroom و mycokey software program و expert web site .

وأصناف المشروم التي تم العثور عليها هي:

Agaricus bisporus, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and commercial sample (*Agaricus bisporus*).

ايضا تم الكشف عن السمية كيميائياً باستخدام هيدروكسيد البوتاسيوم (4% KOH). اثناء جمعها من الحقل وكان اختبار السمية لجميع العينات سالبا.

ولقد أظهرت النتائج أن العينات تحتوي على محتوى رطوبة متفاوت بين العينات تراوح من 5.26% الى 11.11%. محتوى الدهن يتراوح من 0.94% الى 2.99%. محتوى البروتين يتراوح من 19.41% الى 34.14%. محتوى الرماد يتراوح من 0.22% الى 1.47%. محتوى الالياف يتراوح من 5.25% الى 23.86%. محتوى الكربوهيدرات يتراوح من 26.76% الى 77.91%. محتوى الكالسيوم (mg/kg) يتراوح من 5.80 الى 8.60. محتوى الماغنيسيوم (mg/kg) يتراوح من 3.07 الى 5.50. محتوى الفسفور (mg/kg) يتراوح من 2.15 الى 2.50. محتوى البوتاسيوم (mg/kg) يتراوح من 21.20 الى 21.50. محتوى الحديد (mg/100g) يتراوح من 1.30 الى 1.55. محتوى الزنك (mg/100g) يتراوح من 0.50 الى 0.52. محتوى النحاس (mg/100g) يتراوح من 0.10 الى 0.11. محتوى المانجنيز (mg/100g) يتراوح من 0.10 الى 0.12.

تحتوي هذه العينات على الأحماض الأمينية الأساسية (mg/100g) الأتية: محتوى الأرجينين يتراوح ما بين 6.30 الى 6.40 ، محتوى الهستيدين يتراوح ما بين 1.90 الى 2.20، محتوى اللايسين يتراوح ما بين 5.00 الى 5.10، محتوى التربتوفان يتراوح ما بين 0.88 الى 0.90، محتوى الفينيل الانين يتراوح ما بين 2.00 الى 2.10، محتوى الميثيونين يتراوح ما بين 1.00 الى 1.15، محتوى الثريونين يتراوح ما بين 4.05 الى 4.10، محتوى الليوسين يتراوح ما بين

3.90 الى 4.10، محتوى الايزوليوسين يتراوح ما بين 5.56 الى 5.70، محتوى الفالين يتراوح ما بين 4.20 الى 4.50.

كل العينات تحتوي على نسب عالية من البروتين والمعادن والأحماض الأمينية الأساسية بينما العينة *Volvariella volvacea* أفضل عينة من حيث إحتوائها على البروتين لذلك يمكن أن تدخل كمكمل غذائي في بعض الأغذية المصنعة التي تحتوي على نسب قليلة من البروتين.

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CHAPTER ONE

INTRODUCTION

Mushrooms are the fruiting bodies of macro-fungi. They include both edible/medicinal and poisonous species. However, originally, the word “mushroom” was used for the edible members of macro-fungi and “toadstools” for poisonous ones of the “gill” macro-fungi. Scientifically the term “toadstool” has no meaning at all and it has been proposed that the term is dropped altogether in order to avoid confusion and the terms edible, medicinal and poisonous mushrooms are used.

Mushroom nutraceuticals describe a new class of compounds extractable from either the mycelium or fruit body of mushrooms and embodies both their nutritional and medicinal features. They are consumed as a dietary supplement which has potential therapeutic applications (Chang and Miles, 1989).

Mushroom Nutraceuticals are enriched food materials which are used for Maintenance of healthy diet. These are part of a meal (Chang and Miles, 1989; Shiuan, 2004).

Infusion of mushrooms has been used to prevent beriberi. In addition, the decoction has been used for the treatment of abscesses and wounds (Yu *et al.*, 2009).

Edible mushrooms once called the “food of the gods” and still treated as a garnish or delicacy can be taken regularly as part of the human diet or be treated as healthy food or as functional food. The extractable products from medicinal mushrooms, designed to supplement the human diet not as regular food, but as the enhancement of health and fitness, can be classified into the category of dietary supplements/mushroom nutraceuticals (Chang and Buswell, 1996).

Dietary supplements are ingredients extracted from foods, herbs, mushrooms and other plants that are taken without further modification for their presumed health-enhancing benefits.

Identification study help to assure the safe use of mushroom as food. This study is considered a pioneer study aimed to shed some light on some of the wild species of mushrooms in Blue Nile State that are used as edible mushrooms by the local people and the intervention of food supplement in diets that are deficient of protein.

Many local people in Blue Nile State taking some types of wild edible mushrooms, collecting the mushroom during the begging of the rainfall season, consuming it fresh or drying it and using it in steaks of the dried meat (shormot) in their food, did not have a food culture that will enable them to know that these species contain toxin or not. This study was conducted in order to identify some of the species of wild edible mushrooms in Blue Nile state and the determination of nutritional value, minerals, and essential amino acids and learn about their toxicity. To ensure the safety of the food supplement them.

General objective:

To study some wild mushroom and their suitability for human consumption.

Specific objectives

1. To identify of wild mushrooms species in the Blue Nile State.
2. To study the nutritional value of edible mushroom.
3. To determine of chemicals composition of edible mushroom.
4. To determine of the toxicity in edible mushroom.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of mushroom

Mushrooms with other fungi are something special in the living world, being neither plants nor animals. They have been placed in a kingdom of their own called the kingdom of Myceteae. But what are mushrooms? The word mushroom may mean different things to different people and countries. It has emerged that specialized studies and the economic value of mushrooms and their products had reached a point where a clear definition of the term “mushroom” was warranted. In a broad sense “Mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogenous and large enough to be seen with naked eye and to be picked by hand” (Chang and Miles, 1992).

Wild mushrooms have been extensively consumed since the primers of human civilization, due to their unique and delicate flavor, being rich sources of minerals and having high amounts of water, protein, fiber, and carbohydrates. Lipids are present in low values, which make them excellent to be included in low-caloric diets (Heleno *et al.*, 2009; Kalac, 2012).

Beyond the nutritional characteristics, mushrooms have been also extensively studied for their medicinal properties, mainly due to their richness in bioactive compounds that presented antioxidant, anticancer and antimicrobial properties, among other bioactivities (Ferreira *et al.*, 2009; Ferreira *et al.*, 2010; Alves *et al.*, 2012).

According to Chang and Miles (2004) about 7000 species possess varying degrees of edibility and more than 3000 species may be

considered prime edible species, of which only 200 species have been experimentally grown, 100 economically cultivated, approximately 60 commercially cultivated, and about 10 species cultivated on an industrial scale. In addition, 2000 species have been suggested to possess medicinal properties.

Teng (2008) carried a comparison between mushroom, animal and plant: organisms that are classified in the fungi kingdom are unique as they possess both animal and plant qualities. Although the cell walls of fungi are rigid, similar to those of plant cells, they are made out of chitin which is a chemical compound found in the exoskeleton of insects. Some mushrooms store glycogen, an animal polysaccharide, while others form amoeboid cells and flagellated zoospores which are a characteristic of animals. Mushrooms are heterotrophic (lack chlorophyll) and are unable to produce food for themselves. Abdalla (2003) explain the similarities between mushroom and plant stating that mushrooms is similar to plant in respect to its inability to move and they have similarities in the intake of water, nutrients and substances into cell by uptake process.

2.2 Mushrooms structure

A mushroom or a fruit body has three main parts above the substratum, the cap, the gills and the stipe. The cap forms the upper protective layer of the mushroom while the stipe or the stem lifts the spore-producing region above the substratum to enable release of the spores. The gills radiate out on the underside of the cap. The gills are lined with reproductive cells called basidia from which spores are produced (Shu-Ting, 2011).

2.3 Nutritional value of mushrooms

The greatest difficulty in feeding man is to supply a sufficient quantity of the body-building material protein. The other three nutritional categories are: the source of energy food carbohydrates and fats; accessory food factors vitamins; and inorganic compounds which are indispensable to good health. Of course, water, too, is essential. In terms of the amount of crude protein, mushrooms rank below animal meats, but well above most other foods, including milk, which is an animal product (Chang and Miles, 1989).

Furthermore, mushroom protein contains all the nine essential amino acids required by man. The moisture content of fresh mushrooms varies within the range 70 - 95% depending upon the harvest time and environmental conditions, whereas it is about 10 - 13% in dried mushrooms. In addition to their good proteins, mushrooms are a relatively good source of the following individual nutrients: fat, phosphorus, iron, and vitamins including thiamine, riboflavin, ascorbic acid, ergosterine and niacin. They are low in calories, carbohydrates and calcium. Mushrooms also contain a high proportion of unsaturated fat. In recent years, there has been a trend toward discovering ways of treating mushrooms so as to give them added value. For example, Werner and Beelman (2002) have reported on growing mushrooms enriched in selenium. The desirability of a food product does not necessarily bear any correlation to its nutritional value. Instead, its appearance, taste, and aroma, sometimes can stimulate one's appetite (preference). In addition to nutritional value, mushrooms have some unique color, taste, aroma, and texture characteristics, which attract their consumption by humans.

2.4 Medicinal value of mushrooms

The major attribute of mushrooms, their medicinal properties, has also been drawn to our attention for study, e.g., hypotensive and renal effects (Tam *et al.*, 1986 and Yip *et al.*, 1987), immunomodulatory and antitumor activities of polysaccharide-protein complex (PSPC) from mycelia cultures, immunomodulatory and antitumor activities of lectins from edible mushrooms (Wang *et al.*, 1996), isolation and characterization of a Type I Ribosome-Inactivation protein from *V. volvacea* (Yao *et al.*, 1998), and medicinal effects of *Ganoderma lucidum* (Chang and Buswell, 1999; Chang and Miles, 2004).

2.5 Medicinal importance of mushrooms

Medical mycology is as old as traditional uses of mushrooms. They have been used in medicine since the Neolithic and Paleolithic eras (Samorini, 2001).

Mushrooms have been used in health care for treating simple and age old common diseases like skin diseases to present day complex and pandemic disease like AIDS. They are reputed to possess anti-allergic, anticholesterol, anti-tumor and anti-cancer properties (Jiskani, 2001).

The main components proved to be polysaccharides especially β -D- glucans. Chihara *et al.*, (1969) isolated from the shiitake fruiting bodies, an antitumor polysaccharide, which was named lentinan. Bahl (1983) reported that mushrooms cure epilepsy, wounds, skin diseases, heart ailments, rheumatoid arthritis, cholera besides intermittent fevers, diaphoretic, diarrhea, dysentery, cold, anesthesia, liver disease, gall bladder diseases and used as vermicides. *Lentinus edodus* has been used to enhance vigour, sexuality, energy and as an anti-aging agent (Gareth, 1990). Lentinansulphate obtained from *Lentinus* species inhibits HIV

(Gareth, 1990). Puffballs have been used in urinary infections (Buswell and Chang, 1993).

Mushrooms act as biological response modifiers by promoting the positive factors and eliminating the negative factors from the human body and thus regarded as the fourth principal form of the conventional cancer treatment (Yang *et al.*, 1993).

Auricularia species were used since times for treating hemorrhoids and various stomach ailments (Chang and Buswell, 1996). *Pleurotus tuber-regium* mushroom have been used for curing headache, high blood pressure, smallpox, asthma colds and stomach ailments (Oso, 1997; Fasidi and Olorumaiye, 1994). Puffballs (*Clavatia*, *Lycoperdon*) have been used for healing wounds (Delena, 1999). Pharmaceutical substances with potent and unique health enhancing properties have been isolated from mushrooms (Wasser and Weis, 1999). Fresh mushrooms are known to contain both soluble and insoluble fibers; the soluble fiber is mainly beta glucans polysaccharides and chitosan's which are components of the cell walls (Sadler, 2003). Soluble fiber present in mushrooms prevents and manages cardiovascular diseases (Chandalia *et al.*, 2000).

Cordyceps sinensis also treated as half caterpillar and half mushroom has been known and used for many centuries in traditional Chinese medicine. *Cordyceps* has been used to induce restful sleep, acts as anticancer, antiaging, and antiasthma agents besides proved effective for memory improvement and as sexual rejuvenator (Sharma, 2008).

2.6 Mushrooms as a source of food

Man has been hunting for the wild mushrooms since antiquity (Cooke, 1977). Thousands of years ago, fructifications of higher fungi have been used as a source of food (Mattila *et al.*, 2001) due to their

chemical composition which is attractive from the nutrition point of view. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors (Rai, 1994, 1997). Present use of mushrooms is totally different from traditional because, lot of research has been done on the chemical composition of mushrooms, which revealed that mushrooms can be used as a diet to combat diseases. The early history regarding the use of mushrooms in different countries has been reviewed by number of workers (Buller, 1915; Rolfe and Rolfe, 1925; Singer, 1961; Atkinson, 1961; Bano *et al.*, 1964; Jandaik and Kapoor, 1975; Bano and Rajarathnam, 1982; Abou-Heilah *et al.*, 1987 and Houghton, 1995).

The oriental use of mushrooms is older than the European (Lambert, 1938). Rolfe and Rolfe (1925) mentioned that the mushrooms like *Agaricus campestris*, *Morchella esculenta*, *Helvella crispa*, *Hydnum coralloides*, *Hypoxylon vernicosum* and *Polyporus mylittle* were used much earlier in India. Lintzel (1941, 1943) recommended that 100 to 200 g of mushrooms (dry weight) is required to maintain an optimal nutritional balance in a man weighing 70 kg. Bano *et al.*, (1963) determined the nutritive value of *Pleurotus flabellatus* as 0.974% ash, 1.084% crude fiber, 0.105% fat, 90.95% moisture, 0.14% non-protein nitrogen and 2.75% protein. Bano (1976) suggested that food value of mushrooms lies between meat and vegetables. Crisan and Sands (1978) observed that mushrooms in general contain 90% water and 10% dry matter. More so, the protein content varies between 27 and 48%. Carbohydrates are less than 60% and lipids are between 2 to 8%. Orgundana and Fagade (1981) indicated that an average mushroom is about 16.5% dry matter out of which 7.4% is crude fiber, 14.6% is crude protein and 4.48% is fat and oil. Gruen and Wong (1982) indicated that

edible mushrooms were highly nutritional and compared favourably with meat, egg and milk food sources. Of several thousand mushroom species known worldwide, only around 2000 are considered edible, of which about 20 are cultivated commercially with only 4 to 5 under industrial production (Chang, 1990). There is also a significant difference in the nutrient contents of pileus verses stalks (Latifah *et al.*, 1996; Zakia *et al.*, 1993).

Mushrooms have been a food supplement in various cultures and they are cultivated and eaten for their edibility and delicacy. They fall between the best vegetables and animal protein source. Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals (Jiskani, 2001). Mushrooms are good sources of vitamins like riboflavin, biotin and thiamine (Chang and Buswell, 1996).

2.6.1 Carbohydrates

The carbohydrate content of mushrooms represents the bulk of fruiting bodies accounting for 50 to 65% on dry weight basis. Free sugars amounts to about 11%. Florezak *et al.*, (2004) reported that *Coprinus atramentarius* (Bull.: Fr.) Fr. contain 24% of carbohydrate on dry weight basis. The mannitol, also called as mushroom sugar constitutes about 80% of the total free sugars, hence it is dominant (Tseng and Mau, 1999). Mc-Connell and Esselen (1947) reported that a fresh mushroom contains 0.9% mannitol, 0.28% reducing sugar, 0.59% glycogen and 0.91% hemicellulose. Carbohydrates of *Agaricus bisporus* were reported by Crisan and Sands (1978). Raffinose, sucrose, glucose, fructose and xylose are dominant in it (Singh and Singh, 2002). Water soluble polysaccharides of mushrooms are antitumor (Yoshioka *et al.*, 1975).

2.6.2 Proteins

Protein is an important constituent of dry matter of mushrooms (Chang and Buswell, 1996).

Protein content of mushrooms depends on the composition of the substratum, size of pileus, harvest time and species of mushrooms (Bano and Rajarathnam, 1982). Protein content of the mushrooms has also been reported to vary from flush to flush (Crisan and Sands, 1978). Haddad and Hayes (1978) indicated that protein in *A. bisporus* mycelium ranged from 32 to 42% on the dry weight basis.

Many mushroom varieties contain high level of proteins. On dry weight basis, protein content is reported to range 19 to 40 % (Binding, 1978). Proteins have been reported to be composed of albumins, globulins, glutelins, glutelin-like substances, prolamins and prolamin-like substances (Kalac, 2012).

Purkayastha and Chandra (1976) found 14 to 27% crude protein on dry weight basis in *A. bisporus*, *Lentinus subnudus*, *Calocybe indica* and *Volvariella volvacea*. On dry matter basis, the protein content of mushrooms varies from 19% to 39% (Weaver *et al.*, 1977; Breene, 1990). In terms of the amount of crude protein, mushrooms rank below animal meats but well above most other foods including milk (Chang, 1980). Mushrooms contains 19 to 35% proteins as compared to 7.3% in rice, 12.7% in wheat, 38.1% in soybean and 9.4% in corn (Crisan and Sands, 1978; Li and Chang, 1982; Bano and Rajarathnam, 1988). Verma *et al.*, (1987) reported that mushrooms are very useful for vegetarian because they contain some essential amino acids which are found in animal proteins. The digestibility of Pleurotus mushrooms proteins is as that of plants (90%) whereas that of meat is 99% (Bano and Rajarathnam, 1988).

Rai and Saxena (1989) observed decrease in the protein content of mushroom on storage. The protein conversion efficiency of edible mushrooms per unit of land and per unit time is far more superior compared to animal sources of protein (Bano and Rajarathnam, 1988). Mushrooms in general have higher protein content than most other vegetables (Bano and Rajarathnam, 1988) and most of the wild plants (Kallman, 1991). Mushrooms contain all the essential amino acids required by an adult (Hayes and Haddad, 1976).

Friedman (1996) reported that, the total nitrogen content of dry mushrooms is contributed by protein amino acids and also revealed that crude protein is 79% compared with 100% for an ideal protein.

Protein is the major component next to carbohydrates in mushrooms. Wide variations occur in the content of crude protein because not only the species of mushroom differ largely but also different converting factors are used based on the determination by Kjeldahl method. Although many researchers widely used the Nitrogen converting factor of 6.25 to calculate crude protein in mushrooms. Rajarathnam *et al.*, (2003) used a factor of 4.38 by considering the high proportion of non-protein nitrogen, mainly in chitin. To avoid overestimating the content of crude protein.

The proteins in mushrooms are composed of most of the essential amino acids. Nonetheless, some essential sulfur-containing and aromatic amino acids are scarce. The free amino acids account for nearly 20% of the total nitrogen. Even though their contents are low, they play an important role in the taste of mushrooms. Glutamic acid and alanine were found as the dominant free amino acids in *T. portentosum* and *T. terreum* (Rajarathnam *et al.*, 2003; Diez and Alvarez, 2001).

2.6.3 Fats

The fat content in different species of mushrooms ranges from 1.1 to 8.3% on a dry weight basis, with an average content of 4.0%. In general, the crude fat of mushrooms has representatives of all classes of lipid compounds including free fatty acids, monoglycerides, diglycerides, triglycerides, sterols, sterol esters, and phospholipids. (Huang *et al.*, 1989).

In mushrooms, the fat content is very low as compared to carbohydrates and proteins. The fats present in mushroom fruiting bodies are dominated by unsaturated fatty acids. Singer (1961) determined the fat content of some mushrooms as 2.04% in *Suillusgranulatus*, 3.66% in *Suillusluteus* and 2.32% in *A. campestris*. Hughes (1962) observed that mushrooms are rich in linolenic acid which is an essential fatty acid. Total fat content in *A. bisporus* was reported to be 1.66 to 2.2/100 g on dry weight basis (Maggioni *et al.*, 1968).

Orgundana and Fagade (1981) indicated that mushrooms have 4.481% fats on dry weight basis.

Mushrooms are considered good source of fats and minerals (Jiskani, 2001).

2.6.4 Vitamins

Mushrooms have been considered as a good source of vitamins because of the high levels of riboflavin (vitamin B₂), niacin, folic acid and traces of vitamin C, vitamin B₁, vitamin D, β-carotene (precursor of vitamin A), vitamin E and vitamin B₁₂ (Mattila *et al.*, 2001). Mushrooms are notable for their B-complex vitamins (niacin, thiamin, and B₁₂) and folic acid. Their ability to accumulate these vitamins eventually

substantiates their biosynthetic capacities even when they are grown on lignocellulosic wastes. The fact is that folate synthetase and B₁₂ synthetase enzyme systems have been proven in mushroom cells (Rajarathnam *et al.*, 2003). Compared to plants, mushrooms appear to have a limited occurrence of carotenoids including those which can act as precursors of retinol (Kalac, 2009).

Mushrooms are the only natural food source that can provide vitamin D to vegetarians since they are the only non-animal-based food containing vitamin D. There is a remarkable amount of vitamin D₂ (ergocalciferol) in numerous wild mushroom species, but is almost absent in cultivated species due to lacking exposure to sunshine (Mattila *et al.*, 2001). It has been well known that vitamin D₂ is originated by photo irradiation from its precursor ergosterol. When exposed to UV light, ergosterol undergoes photolysis to generate various photo irradiation products, mainly previtamin D₂, tachysterol and lumisterol. The previtamin D₂ then undergoes spontaneous thermal rearrangement to vitamin D₂ (Mattila *et al.*, 2002).

According to Mattila *et al.*, (1994), wild mushrooms contains higher amounts of vitamin D₂ than dark cultivated *A. bisporus*. Mushrooms also contain vitamin C in small amounts (Mattila *et al.*, 2001) which are poor in vitamins A, D, and E (Anderson and Fellers, 1942).

2.7 Mineral constituents

The fruiting bodies of mushrooms are characterized by a high level of well an assimilated mineral elements. Major mineral constituents in mushrooms are K, P, Na, Ca, Mg and elements like Cu, Zn, Fe, Mo, Cd form minor constituents (Bano and Rajarathanam, 1982; Chang, 1982).

K, P, Na and Mg constitute about 56 to 70% of the total ash content of the mushrooms (Li and Chang, 1982) while potassium alone forms 45% of the total ash. Abou-Heilah *et al.*, (1987) found that content of potassium and sodium in *A. bisporus* was 300 and 28.2 ppm. Respectively. *A. bisporus* ash analysis showed high amount of K, P, Cu and Fe (Anderson and Fellers, 1942). The mineral proportions vary according to the species, age and the diameter of the fruiting body. It also depends upon the type of the substratum (Demirbas, 2001). The mineral content of wild edible mushrooms has been found higher than cultivated ones (Aletor, 1995; Mattilla *et al.*, 2001; Rudawska and Leski, 2005).

A reasonable content of many mineral elements can be observed in mushrooms. Manzi *et al.*, (1999) reported that ash content of mushroom was around 6-10.5% of dry matter, this result was supported by Kalac (2009) who showed it to be about 5-12%. The principle constituents in the ash are potassium, phosphorus, magnesium, calcium, copper, iron, and zinc (Kalac, 2009; Guillamón *et al.*, 2010). In fruiting body, the distribution of potassium is not even. Its concentration indicates a decreasing trend in the order: cap > stipe > spore-forming part > spores (Kalac, 2009). Some species also hold germanium that has the ability to maintain vitality in humans (Rajarithnam *et al.*, 2003). Mushrooms possess a special feature to accumulate minerals that are available in their growth medium. This property can be ambivalent, for it is not only useful in providing desired minerals in good quantities but also is dangerous for consumption when toxic elements are accumulated (Rajarithnam *et al.*, 2003; Kalac, 2009). Mushrooms are able to accumulate potassium and phosphorus in their fruiting bodies. The concentrations of potassium and phosphorus are respectively 20-40 folds and 10-50 folds higher than those in the underlying substrates. On the other hand, mushrooms growing in

highly polluted areas or some accumulating species appear to contain considerably elevated contents of harmful elements, even one or two orders of magnitude higher than those in substrates. Great attention has been drawn regarding the accumulation of trace heavy metals in the mushroom, especially toxic elements such as cadmium, lead, mercury, chromium, arsenic, silver and tin (Kalac, 2009; Guillamon *et al.*, 2010).

2.8 Antioxidant activity

Antioxidants are chemical compounds that protect cells from the damage caused by unstable molecules known as free radicals. Free radicals are powerful oxidants and those chemical entities that contain unpaired electrons. They are capable of randomly damaging all components of the body, viz. lipids, proteins, DNA, sugars and are involved in mutations and cancers (Przybytniak *et al.*, 1999).

The nascent oxygen is trapped by enzymes like superoxide dismutase, catalase and glutathione peroxidase. Over production of free radicals creates oxidative stress. The antioxidants are an important defense of the body against free radicals and mushrooms are rich sources of these antioxidants (Puttaraju *et al.*, 2006; Oyetayo *et al.*, 2007).

Waxy cap mushroom extracts (*Hygrocybecoccinea*) are inhibitory to sarcoma (Ohtsuka and Asami, 1997). Schizophyllan from *Schizophyllum commune* is effective against head and neck cancer (Borchers *et al.*, 1999).

Antioxidant property of compounds is correlated with their phenolic compounds (Velioglu *et al.*, 1998).

Many species of mushrooms have been found to be highly potent immune enhancers, potentiating animal and human immunity against cancer (Borchers *et al.*, 1999). Tyrosine from *A. bisporus* is antioxidant (Shi *et al.*, 2002).

Phenolic acids are the major low molecular weight bioactive components usually found in mushroom species, responsible for their antioxidant properties (Ferreira *et al.*, 2009).

Our daily diet could be enriched with food rich in antioxidant such as fruits, vegetables and mushrooms in order to help the organism in the combat against oxidative stress, taking advantage of the additive and synergistic effects of all the antioxidant compounds present. Public health authorities consider prevention with nutraceuticals/functional foods as a powerful instrument in maintaining and promoting health, longevity and life quality (Ferreira *et al.*, 2009).

2.9 Categories of mushrooms

Mushrooms can be roughly divided into four categories: (1) those that are fleshy and edible fall into the edible mushroom category, e.g., *Agaricus bisporus* ; (2) mushrooms that are considered to have medicinal applications are referred to as medicinal mushrooms, e.g., *Ganoderma lucidum*; (3) those that are proved to be or suspected of being poisonous are named poisonous mushrooms, e. g., *Amanita phalloides* ; (4) those in a miscellaneous category, which includes a large number of mushrooms whose properties remain less well defined. These may tentatively be grouped together as “other mushrooms.” Certainly, this approach of classifying mushrooms is not absolute. Many kinds of mushrooms are not only edible, but also possess tonic and medicinal qualities. The above categories of mushrooms have been included in Ainsworth and Bisby’s Dictionary of the Fungi, 8th edition (Hawksworth *et al.*, 1996). This grouping certainly is not a perfect one, but it has been useful for estimating numbers of mushroom species. (Hawksworth, 2001). It should be noted that toadstool has been used frequently to refer to poisonous mushrooms. Scientifically, the term has no meaning at all. It is suggested

that the term toadstool be dropped altogether in order to avoid confusion and to use edible, medicinal, and poisonous mushrooms as described above.

2.10 Poisonous mushrooms

Because there is no known test by which to tell if a mushroom is edible or not, a mushroom should never be eaten unless it has been accurately identified and the edibility of the species is known. Even though poisonous mushrooms represent less than 1% of the world's known mushrooms, we cannot ignore the existence of the relatively few dangerous and sometimes fatal species. Mushrooms must be identified by a competent mycological authority. Therefore, if one is not absolutely sure whether a given mushroom is edible or otherwise, it should not be tasted, and the unidentified mushroom should be left alone. The toxins contained in various species are very different in chemical composition, and thus the effects of poisoning differ considerably according to the species involved. In any case, suspected mushroom poisoning should never be regarded lightly and medical assistance should be sought at once. The following summary of mushroom poisoning is taken from the account by Shepherd and Totterdell (1988).

2.10.1 Amanita -type poisoning

Unquestionably, the *Amanita phalloides* group causes the most dangerous type of mushroom poisoning. The toxins involved belong to the Phallotoxin and Amatoxin complexes. The Phallotoxin phalloides binds specifically to actin. While the Phallotoxin are not active following ingestion, although they are potent when injected intravenously, they have proved useful in experimental studies. In such studies phalloides, binding to actin, is coupled with fluorescent groups. By this means actin can be localized in the cells. It is the Amatoxin such as α - amatine that is

involved in amanita poisoning. α -Amatine is a specific inhibitor of RNA polymerase present in all eukaryotes. This blocking of the enzymes associated with the replication of RNA inhibits the formation of new cells. These toxins tend to accumulate in the liver and damage that organ severely. The RNA polymerase of the fungus is not affected. This group has caused the majority of recorded deaths from mushroom poisoning, especially in Europe. The general symptoms of this type of poisoning are severe abdominal pains, nausea, violent vomiting, diarrhea, cold sweats, and excessive thirst. These may last for 48 hours, with dehydration, cramps, and anuria (Miles and Chang 2004).

2.10.2 Muscarine-type poisoning

Two toxins, muscarine and ibotenic acid, are involved. They occur in *Amanita muscaria*, *A. pantherina*, and also in a number of *Inocybe* and *Clitocybe* species. Muscarine is known to be responsible for “pupil contraction, blurred vision, lachrymation, salivation, perspiration, reduced heart rate, lowering of blood pressure, and asthmatic-like breathing. (Alexopoulos *et al.*, 1996). Ibotenic acid is responsible for the insecticidal properties of *A. muscaria*, the fly agaric. Both muscarine and ibotenic acids are intoxicants, and there is a long history of different cultures using these compounds from *A. muscaria* for this purpose and in religious rites. The symptoms usually appear soon after eating the mushrooms, with vomiting, diarrhea, and salivation. The most characteristic symptoms are nervous excitement, difficulties in breathing, shivering, and a tendency to collapse (Miles and Chang 2004).

2.10.3 Psychotropic or hallucinogenic poisoning

Several different toxins are involved, including psilocin and psilocybin, which are found in species of *Psilocybe*, *Conocybe*, and *Stropharia*. These compounds are similar in their reaction to d-lysergic

acid diethylamide (LSD). They act on the central nervous system, producing distortions in vision and of tactile sensations as well as mixed emotional feelings of happiness or depression. Other symptoms are varied, including vomiting, increased rate of heartbeat, and hallucinations, which may last for various lengths of time (Miles and Chang 2004).

2.10.4 Coprinus poisoning

Several *Coprinus* species, such as *C. micaceous* And *C. atramentarius*, when consumed with an alcoholic drink, produce unpleasant but not dangerous symptoms. The symptoms include reddening of the face, increased rate of heartbeat and in some cases, vomiting and diarrhea. The mode of action of the chemical in *C. atramentarius* mushrooms is similar to Antabuse, which is a drug used to induce nausea and vomiting in individuals who are trying to overcome an addiction to alcohol (Miles and Chang 2004).

2.10.5 Poisoning from external sources

The poisoning is not caused by mushrooms themselves but by toxic substances that have accumulated in the mushrooms. The principal causes are (1) heavy metals due to polluting environmental conditions where the mushrooms are harvested that are far in excess of permissible levels, and (2) radioactive contaminants due to the pollution by contaminating radioactive materials in mushroom hunting areas and subsequent consumption of the collected mushrooms (Miles and Chang 2004).

2.11 Toxic components in poisonous mushrooms

Although there are thousands of mushroom species on earth, only 30-50 poisonous mushrooms species are found. However, even though no more than 10 are fatally poisonous, mushroom poisoning has caused

about 70% of natural poisoning and often results in death. Learning the physical features of each species of poisoning mushrooms is the best way to avoid risk instead of conducting the convenient tests of folklore claims. For instance, it has been proven that silver spoons will not be blackened when cooked with poison mushrooms (Rajarathnam *et al.*, 2003).

Amanita phalloides is known as the green death cap that is the most dangerous and poisonous mushroom responsible for 90-95% of fatal mushroom poisonings. Amanita poisonings are resulted from the toxic compounds such as the cyclic peptides, the Amatoxin, and the Phallotoxin (Table 1). *Amanita virosa* is as toxic as *A. phalloides* and is described as 'destroying angel', containing an Amatoxin, amaninamide. Amino acids that have the relevant structure to glutamic acid have been observed as mushroom toxins. Acromelic acids obtained from *Clitocybe acromelalga* are neurotoxins, exhibiting highly potent activity as glutamate agonists. *Amanita muscaria* presenting as a brilliant red cap flecked with white spots is the best known poisonous mushroom in the world. These mushrooms are cataloged under genus *Inocybe*, which contains abundant quantities of a toxic chemical Muscarine. In addition, some alkaloids, such as psilocybin and psilocin found in most *psilocybe* species, can act on the central nervous system since the structure and activity are similar to those of the hallucinogen, lysergic acid diethylamide (LSD) (Rajarathnam *et al.*, 2003).

Table 1: Mushroom poisoning

Mushroom species	Compounds	Nature of compound	Symptoms
Amanita phalloides	Phallotoxin	Cyclopeptide	Fatal
Amanita phalloides	Amatoxin	Cyclopeptide	Fatal
Amanita virosa	Virotoxin	Cyclopeptide	Fatal
Gyromitra esculenta	Gyromitrin	Cyclopeptide	Affects autonomic nervous system
Coprinus atramentarius	Coprine Clitidine	Amino acid Novel amino acids	Drunken sickness Neurotoxin
Clitocybe acromelalga	Acromelic acid Clithioneine	Novel amino acids Novel amino acids	Neurotoxin Neurotoxin
Amanita muscaria	Muscarine	Amino acid	Affects parasympathetic, cholinergic nervous system
Inocybe patouillardii	Muscarine	Amino acid	Affects parasympathetic, cholinergic nervous system
Clitocybe acromelalga	Muscarine	Amino acid	Affects parasympathetic, cholinergic nervous system
Amanita pantherina	Mycoatropine	Alkaloids	Psychotropic poisoning
Amanita muscaria	Mycoatropine	Alkaloids	Psychotropic poisoning
Amanita regalis	Mycoatropine	Alkaloids	Psychotropic poisoning
Psilocybe species	Psilocybin	Alkaloids	Psychotropic poisoning
Panaeolus species	Psilocybin	Alkaloids	Psychotropic poisoning
Panaeolina species	Psilocybin	Alkaloids	Psychotropic poisoning
Stropharia species	Psilocybin	Alkaloids	Psychotropic poisoning
Cortinarius orellanus	Orellanin	Polypeptide	Fatal
Ramaria formosa	Emodin		Laxative effects

Source: Rajarathnam *et al.*, (2003)

2.12 Mycological terms

The basic terminology of the fruiting body of a mushroom is represented in figure (1) the gathered edible mushrooms are commonly described as higher fungi or macro fungi. The fruiting body (carpophore, mycocarp) in higher fungi is found mostly above ground. A fruiting body grows from spacious underground mycelia (hyphae) by the process of fructification. The bulk of fruiting bodies have a short lifetime only about 10-14 days (Kalac, 2009).

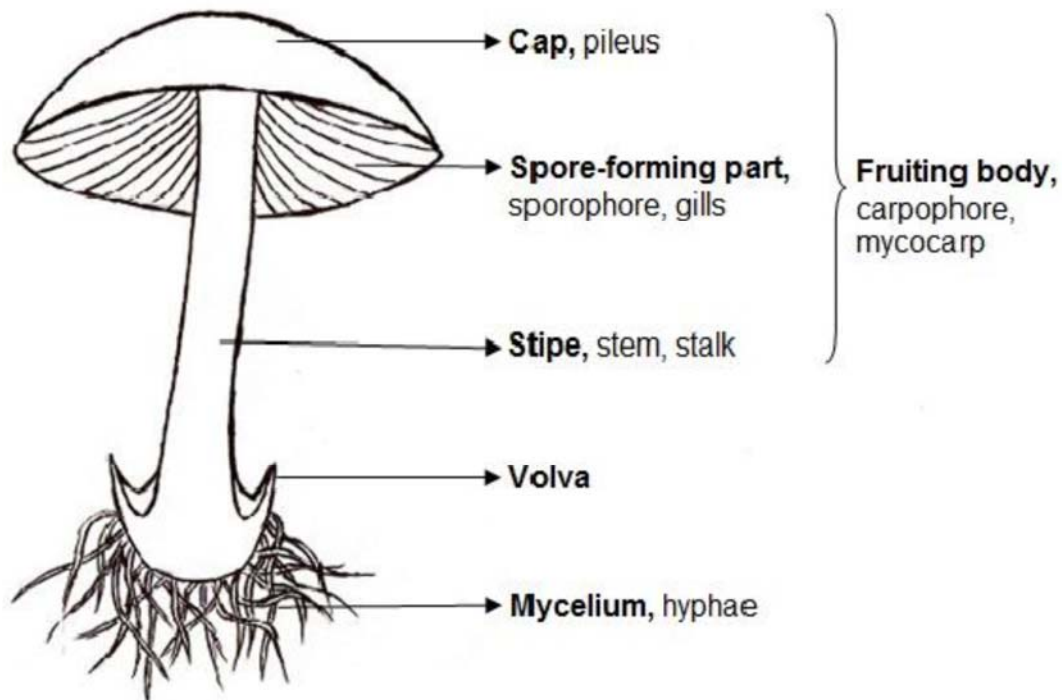


Figure 1: Schematic image of a mushroom and basic mycological terms.

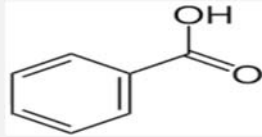
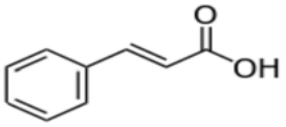
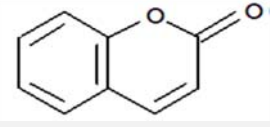
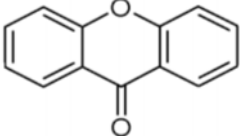
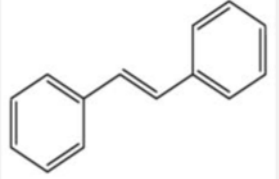
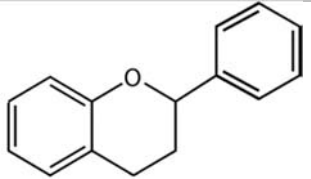
Most types of mushrooms are commonly found in the shape of umbrella with pileus (cap) and stipe (stem). Nonetheless, some species additionally possess an annulus (ring), or a volva (cup), or have both. The forms of some unusual mushrooms look like pliable cups, golf balls, or small clubs (Chang and Miles, 2004).

2.13 Phenolic acids

Phenolic compounds represent about 8000 different phenolic structures. They have one or more aromatic rings with one or more hydroxyl groups, including different subclasses such as flavonoids, phenolic acids, stilbenes, lignin's, tannins, and oxidized polyphenols (Crozier *et al.*, 2006; Barros *et al.*, 2009; Fraga. 2010; Carochoand Ferreira, 2013). Table 2 displays their structure as well as the number of carbons and representative compounds.

They are secondary metabolites that have an important role in health-promoting and nutraceutical potential of mushrooms in food (Chan *et al.*, 2014; Tulio *et al.*, 2014). The content of these compounds is highly correlated with the antioxidant activity of mushrooms and thus can be used as the important determinant in evaluating free radicals and scavenging potential of the mushroom extracts (Chan *et al.*, 2011; Bertalanic *et al.*, 2012; Skotti *et al.*, 2014).

Classification through number of carbons and basic structure of molecules within the phenolic compounds family

Number of carbons	Classification	Example	Basic structure
7	Hydroxybenzoic acids	Gallic acid	
9	Hydroxycinnamic acids	p-Coumaric acid	
9	Coumarins	Esculetin	
13	Xanthons	Mangiferin	
14	Stilbenes	Resveratrol	
15	Flavonoids	Naringenin	

Source: Carocho and Ferreira (2013).

2.14 Filed collection and identification

Chang and miles (2004) argued that to identify wild mushroom you need to use keys. Collectors should always remember when using keys that the mushroom in hand might not in book being consulted (or in any other book, for that matter). Once a name has been obtained by using a key, the detailed description provided for the mushroom must be read and compared with the one being identified. If the description does not fit the specimen, the key must be checked again, following a different route. Now days there are a lot of books, web site and electronic program that consider references for mushroom identification.

Chemical tests in mushroom identification are methods that aid in determining the variety of some fungi. The most useful tests are potassium hydroxide (KOH) 3% -5%.

2.15 Potassium hydroxide (KOH) test

The sample had chemical test for color change using potassium hydroxide (KOH) 4% by dropped KOH in the cap and dropped other drop in stalk and recorded the change of color.

According to mushroom expert web site (2008) KOH is used in the identification of the many mushrooms, including boletes, polypores, and gilled mushrooms. Note any color changes that take place. A change to yellow is sometimes found in species of *Agaricus* and *Amanita*; *magenta* or olive reaction can help identify species of *Russula* and *Lactarius*; deep red or black reactions can help sort out many gilled mushrooms; black reactions among polypores are crucial separators; and various colors are the produced with boletes. The negative reactions (no color change) many also be an informative character.

2.16 Identification of mushroom species

Different sources of keys are used, Mycokey software program, mushroom expert web site, Mykoweb, web site, Mycobank web site, Russell,2006; Huffman and Evenson, 2008; McKnight (1987). All these references starting by know the color of spores of mushrooms and the way of produced spores dose in gills, teeth, inside a leathery pouch (the puffball), inside of shallow cups (the cup fungi, including the morels) or simply on the surface of the mushroom (coral fungi and others).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Sample collection

Seven samples of wild edible mushrooms were collected from Blue Nile State – the city of Al-Damazin in addition to one sample was purchased from super-market in Khartoum North. During collection process from the field the toxicity test was conducted for all samples by potassium hydroxide with concentration of 4% using dropper on the caps and stem, during this test there was no positive toxicity in all samples.

3.2 Methods

3.2.1 Toxicity test

According to mushroom expert web site (2008) was used Chemical tests in mushroom identification are methods that aid in determining the variety of some fungi. The most useful tests are potassium hydroxide (KOH) 3% -5%. On to attend (KOH) weighing 4g, then dissolved in part of distilled water and then complete volume to 100 ml with distilled water. Then (KOH) solution was used for the detection of toxicity by dropping one or two drops of KOH solution into pieces of the caps and stem samples of the mushroom and then the colour change was observed (no colour change means negative reaction).

3.2.2 Identification of mushroom samples

In this study mushroom samples were identified by mycokey software program and mushroom expert web site.

3.2.3 Preparation of samples

The samples collected between April and September 2016 from the city of Al-Damazin nursery. After it was dried in the shade at room temperature and then was crushed by blender and saved in plastic bags, then the bags were packed in aluminum foil until chemical analysis.

3.3 Chemical analysis

3.3.1 Moisture content

The moisture content of the dried samples were determined according to standard methods of association of official analytical chemists (AOAC, 2003).

Principle

The moisture content is a weighed sample removed by heating the sample in an oven under atmospheric pressure at $105 \pm 1^\circ\text{C}$. Then the difference in weight before and after drying is calculated as a percentage from the initial weight.

Procedure

A sample of $2\text{g} \pm 1\text{mg}$ was weighed into a pre-dried and tarred dish. Then the sample was placed into an oven (NO.03-822, fn400, turkey) at $105 \pm 1^\circ\text{C}$ until a constant weight was obtained. After that the covered sample was transferred to desiccators and cooled to room temperature before reweighing. Triplicate results were obtained for each sample and the mean value was reported.

Calculation

$$\text{Moisture content \%} = \frac{(M2 - M3)}{(M2 - M1)} \times 100$$

Where:

M1= weighed dish+ cover

M2= weight of dish + cover +sample before drying

M3= weight of dish + cover+ sample after drying

The dry matter (DM) percentage was calculated by subtracting the percentage of moisture from 100%.

3.3.2 Fat content

The crude fat in the product was determined according to the standard methods of AOAC (2003).

Principle

The method determines the substances which are soluble in hexane (B.P, 40 – 60°C) and extractable under the specific conditions of Soxhlet extraction method. The dried hexane extract is weighed and reported as percentage of the dry matter as crude fat.

Procedure

A sample of 5g+1mg was weighed into an extraction thimbles (30-100 mm) and covered with cotton that previously extracted with hexane. Then, the sample and a pre-dried and weighed Erlenmeyer flask containing about 150 ml hexane (No1622,BDH,England) were attached to the extraction unit (Electro thermal ,England) and the temperature was adjusted to produce about 150 to 200 drops of the condensed solvent per minute for 16 hours . At the end of the distillation period, the flask with was disconnected from the unit and the solvent was redistilled. Later, the flask with the remaining crude hexane was put in an oven at 105°Cfor 3

hours, cooled to room temperature in a desiccators, reweighed and the dried extract was registered as crude fat (% DM) according to the following formula:

$$\text{Fat content (\%)} = \frac{(W_1 - W_2)}{W_3} \times 100$$

Where:

W_1 = weight of flask and ether extract

W_2 = weight of empty flask

W_3 = initial weight of sample

3.3.3 Crude protein

The crude protein was determined in all samples by micro – Kjeldahl technique following the method of AOAC (2005).

Principle

The method consists of sample oxidation and conversion of nitrogen to ammonia, which reacts with the excess amount of sulphuric acid forming ammonium sulphate. The solution is made alkaline and the ammonia is distilled into a standard solution of boric acid (2%) to form the ammonia –boric acid complex, which is titrated against a standard solution of HCL (0.1). Accordingly, the crude protein content is calculated by multiplying the total N% by 4.38 as a conversion factor for protein.

Procedure

0.2 gm of sample was accurately weighed into micro-Kjeldahl flask, 0.4gm of catalyst mixture (90% potassium sulphate and 10 % cupric sulphate) and 3.5 ml of concentrated sulphuric acid were added

into Kjeldahl digestion flask. After that, the flask was placed into a Kjeldahl unit (Tecator, Sweden) for about 3 hours, until a colorless digest was obtained. Following, the flask was left to cool to room temperature. The distillation apparatus; 20 ml of 40% NaOH sodium hydroxide were added. The ammonia evolved was received in 10 ml of 2% boric acid solution. The trapped ammonia is titrated against 0.02 M HCL using universal indicator. Finally, the distillate was titrated with standard solution of 0.02 HCL in the presence of 2-3 drops of indicator (Bromocreasol green and methyl red) until a brown reddish colour was observed.

Calculation

$$\text{Crude protein \%} = \frac{(\text{TV} \times \text{N} \times 14.00 \times \text{F}) \times 100\%}{1000 \times \text{sample weight (g)}}$$

Where:

TV= actual volume of HCL used for sample

N= normality of HCL

F= protein conversion factor = 4.38

3.3.4 Ash content

The standard analytical methods of AOAC (2003) were used for determination of ash content in the samples.

Principle

The inorganic materials which are varying in concentration and composition are customary determined as a residue after being ignited at a specified heat degree.

Procedure

A sample of 2gm \pm 1mg was weighed into a pre-heated, cooled weighed and tarred porcelain crucible and placed into a muffle furnace (Carbolite, Sheffield, England) at 50 to 600°C until a constant weight and a white gray ash was obtained. The crucible was transferred to a descanter then allowed to cool to room temperature and weighed. The ash content was calculated as a percentage based on the initial weight of sample.

Calculation

$$\text{Ash content \%} = \frac{(\text{Wt}_1 \text{ of crucible +ash}) - (\text{Wt}_2 \text{ of empty crucible})}{\text{Initial weight (S)} \times 100}$$

Where

Wt₁ = weight of the crucible with sample.

Wt₂ = weight of the empty crucible.

S = initial weight of sample.

3.3.5 Crude fiber content

The crude fiber was determined according to the official method of the AOAC (2003).

Principle:

The crude fiber is determined gravimetrically after the sample is being chemically digested in chemical solutions. The weight of the residue after ignition is then corrected for ash content and is considered as a crude fiber.

Procedure:

About 2gm \pm 1 mg of a defatted sample was placed into a conical flask containing 200 ml of H₂SO₄ (0.26 N). The flask was then, fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digest was filtered (under vacuum) through a porcelain filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200 ml NaOH (0.23 N) solution for 30 minutes under reflux condenser and the precipitate was filtered, rinsed with hot distilled water, 20ml ethyl alcohol (96%) and 20 ml diethyl ether.

Finally, the crucible was dried at 105 °C (overnight) to a constant weight, cooled , weighed, ashed in a Muffle furnace (No.20. 301870, Carbolite, England) at 550-600 °C until a constant weight was obtained and the difference in weight was considered as crude fiber.

Calculation:

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2}{S}$$

Where

W₁ = weight of sample before ignition (gm).

W₂ = weight of sample after ignition (gm).

S = weight of sample (gm).

3.3.6 Carbohydrates content

Total carbohydrates were calculated by difference according to the following equation:

Total carbohydrates = 100% - (Moisture + Protein + Fat + Ash).

3.3.7 Mineral content

The mineral content was determined in all samples by Elmer

(1996). Using atomic absorption spectrophotometer model 210 VGP USA (AAS 2005).

Principle:

Atomic absorption spectrophotometer model 210 VGP USA (AAS 2005). Quantitatively measured the concentrations of elements present in a liquid sample. It utilized the principle that elements in the gas phase absorb light at very specific wavelengths which gives the technique excellent specificity and detection limits. The sample may be an aqueous or organic extract, indeed it may even be solid provided it can be dissolved successfully. The liquid was drawn in to a flame where it is ionized in the gas phase. Light of a specific wavelength appropriate to the element being analyzed was shone through the flame, the absorption was proportional to the concentration of the element. Quantification was achieved by preparing standards curve for all elements.

Procedure:

A sample of $2\text{gm} \pm 1\text{gm}$ was weighed into a porcelain crucible and ashed at 550°C for three hours; then the ash was cooled at room temperature, after that the sample was digested with 50 ml of 0.1N HCL and solution was filtered with $20\mu\text{m}$ filter papers. The filtrate was passed through the AAS system using different lamps and calibrated with standard minerals.

3.3.8 Amino acids content

The amino acid was determined according to the official method of the AOAC (2006). Performed by using High Performance Liquid Chromatography (HPLC).

Procedure:

A sample of $2\text{g} \pm 1\text{mg}$ was weighed into a pre-dried and tarred dish. Then the sample was placed into an oven (NO.03-822, fn400, turkey) at $60 \pm 1^\circ\text{C}$ for four hours. After that 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 . After that 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.

3.3.9 Statistical analysis

The results were subjected to statistical analysis (SAS). One-way by using completely randomized design (CRD). The mean values were also tested and separated by using Duncan's Multiple Range Test (DMRT) as described by Montgomery and Douglas, (2001).

CHAPTER FOUR

RESULTS AND DISCUSSION

4. Results and discussion

4.1 Identification of mushroom species

All of the following plates from 1 to 8 were the field pictures of the studied mushroom samples collected from Blue Nile State and the commercial one. They identified by using Mycokey software program and mushroom expert web site. The results were named as shown below each plate.



Plate 1: Edible mushrooms *Agaricus bisporus* (identification by Mycokey installed version 4.1 and mushroom expert web site)



Plate 2: Edible mushrooms *Chlorophyllum rhacodes* (identification by Mycokey installed version 4.1 and mushroom expert web site)



Plate 3: Edible mushrooms *Agaricus lutosus* (identification by Mycokey installed version 4.1 and mushroom expert web site)



Plate 4: Edible mushrooms *Volvariella Volvacea* (identification by Mycokey installed version 4.1 and mushroom expert web site)



Plate 5: Edible mushrooms *Agaricus impudicus* (identification by Mycokey installed version 4.1 and mushroom expert web site)



Plate 6: Edible mushrooms *Agaricus arvensis* (identification by Mycokey installed version 4.1 and mushroom expert web site)



Plate 7: Edible mushrooms *Agaricus silvicola* (identification by Mycokey installed version 4.1 and mushroom expert web site)



Plate 8: Edible mushrooms commercial sample *Agaricus bisporus* (identification by Mycokey installed version 4.1 and mushroom expert web site)

4.2 Moisture content

Figure (2) shows the moisture contents of wild edible mushrooms *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola*, (*Agaricus bisporus* commercial sample). The highest moisture content 11.11% was in *Volvariella volvacea*, *Agaricus arvensis* and *Agaricus silvicola*, whereas the lowest 5.26% was in *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Agaricus impudicus* and the commercial sample (*Agaricus bisporus*), respectively. There was no significant differences ($P \leq 0.05$) between the varieties *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Agaricus impudicus* and the commercial sample (*Agaricus bisporus*), regarding moisture content, while between the varieties *Volvariella volvacea*, *Agaricus arvensis*, *Agaricus silvicola* there was no significant differences ($P \leq 0.05$) in moisture content. Abdalla (2015) reported that the moisture content of some wild edible mushrooms ranged between 6.97% and 14.07% also Saiqa *et al.*, (2008) reported that the moisture content of wild edible mushrooms was in *Agaricus bisporus* was 5.9% and moisture content 9% for the variety *Volvariella volvacea* was reported by Mshandete and Cuff (2007).

4.3 Fat content

Figure (3) shows the fat contents of wild edible mushrooms *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*). The highest value of fat content was in *Agaricus lutosus* 2.99%, whereas the lowest value of fat content was in *Agaricus impudicus* 0.94%. The values within all samples showed significantly difference ($P \leq 0.05$). Abdalla (2015) reported that

the fat content of some wild edible mushrooms in (*Agaricus bisporus*) was 3.50% and Mshandete and Cuff (2007) reported the fat content in *Volvariella volvacea* was 3.3% and Barros *et al.*,(2007) reported the fat content in *Agaricus arvensis* was 2.7%.

4. 4 Protein content

Figure (4) shows the protein contents of wild edible mushrooms *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*). The highest value of protein content was in *Volvariella volvacea* 34.14%, whereas the lowest value of protein content was in *Agaricus bisporus* 19.41%. The values within the *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus* and the commercial sample (*Agaricus bisporus*) showed significantly difference ($P \leq 0.05$). While the samples *Agaricus arvensis*, *Agaricus silvicola* are similar in values and have no significant difference between them. Abdalla (2015) reported that the protein content of *Agaricus bisporus* was 21.35%, other study Saiqa *et al.*, (2008) reported that the protein content in *Agaricus bisporus* was 16.4% and Mshandete and Cuff (2007) reported the protein content in *Volvariella volvacea* was 28.0% and Barros *et al.*, (2007) reported the protein content in *Agaricus arvensis* was 56.3%.

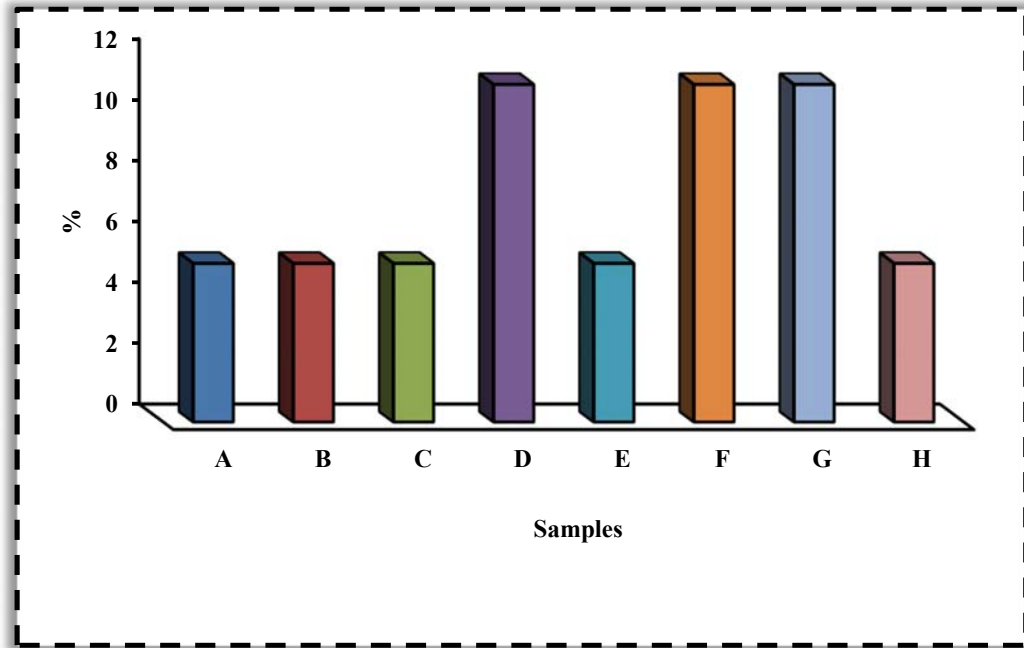


Figure 2: Moisture content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

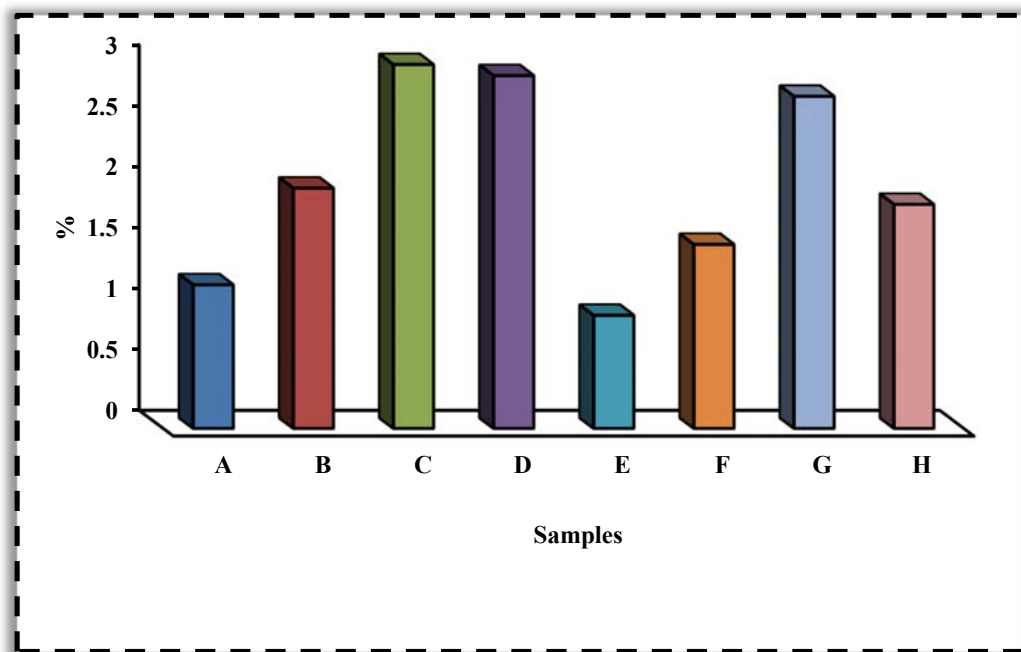


Figure 3: Fat content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

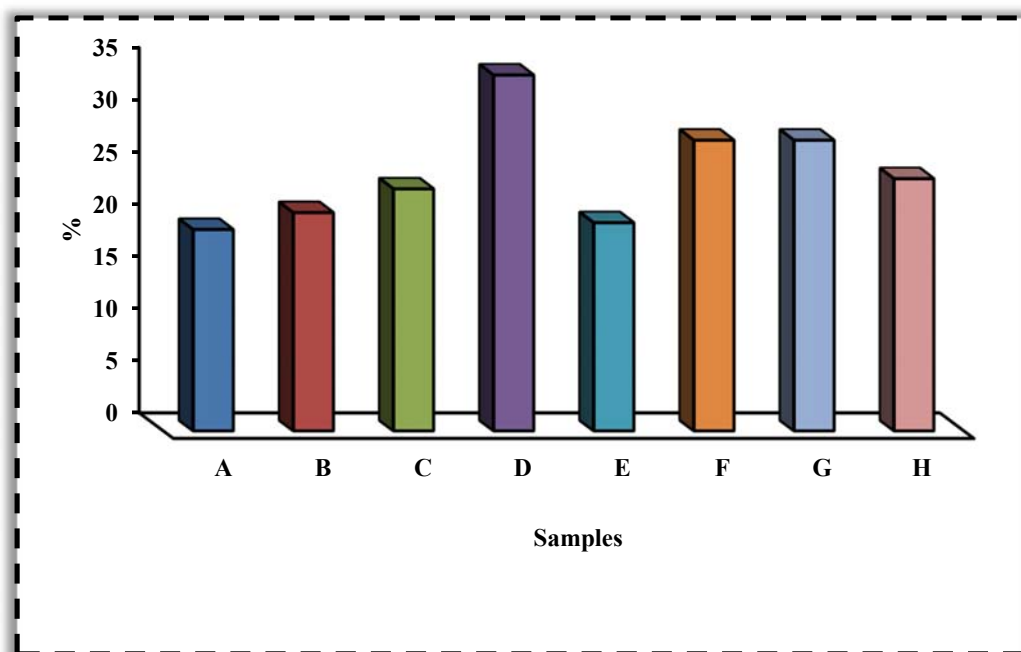


Figure 4: Crude protein of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

4. 5 Ash content

Figure (5) shows the ash contents of wild edible mushrooms *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*). The highest value of the ash content were in *Agaricus bisporus* 1.47%, whereas the lowest value of ash content were in *Agaricus impudicus* 0.22%. The values within all samples showed significantly difference ($P \leq 0.05$). Abdalla (2015) reported that the ash content of *Agaricus bisporus* was 26.00% and Saiqa *et al.*, (2008) founded the ash content in *Agaricus bisporus* as 11.01% and Mshandete and Cuff (2007) reported the ash content in *Volvariella volvacea* was 10.0% and Barros *et al.*, (2007) reported the ash content in *Agaricus arvensis* was 3.5%.

4. 6 Crude fiber content

Figure (6) shows the crude fiber of wild edible mushrooms *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*). The highest value of crude fiber was in *Agaricus impudicus* was 23.86%, whereas the lowest value of crude fiber was in *Agaricus silvicola* 5.25%. The values within the *Agaricus bisporus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis* and *Agaricus silvicola* showed significantly difference ($P \leq 0.05$). While the *Chlorophyllum rhacodes*, *Agaricus lutosus* and the commercial sample (*Agaricus bisporus*) are similarity in values and have no significant difference. Abdalla (2015) reported that the crude fiber content of *Agaricus bisporus* was 14.33% and Mshandete and Cuff (2007) reported the fiber content in *Volvariella volvacea* was 9.8%.

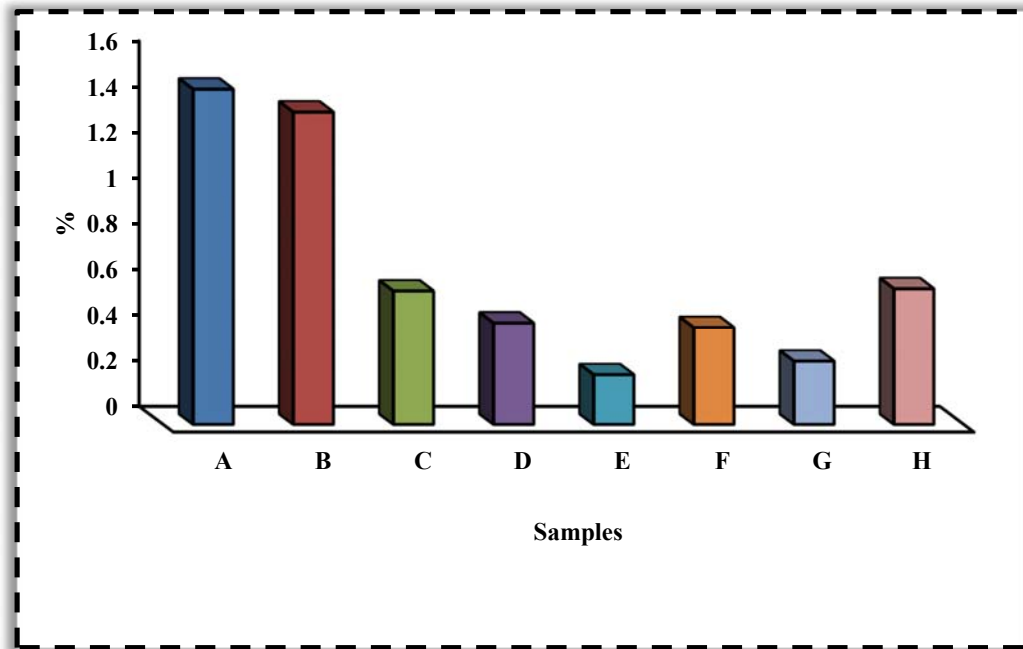


Figure 5: Ash content of edible Mushroom samples

Key:

- | | |
|-------------------------------|--|
| A ≡ <i>Agaricus bisporus</i> | B ≡ <i>Chlorophyllum rhacodes</i> |
| C ≡ <i>Agaricus lutosus</i> | D ≡ <i>Volvariella volvacea</i> |
| E ≡ <i>Agaricus impudicus</i> | F ≡ <i>Agaricus arvensis</i> |
| G ≡ <i>Agaricus silvicola</i> | H ≡ <i>Agaricus bisporus</i> (commercial sample) |

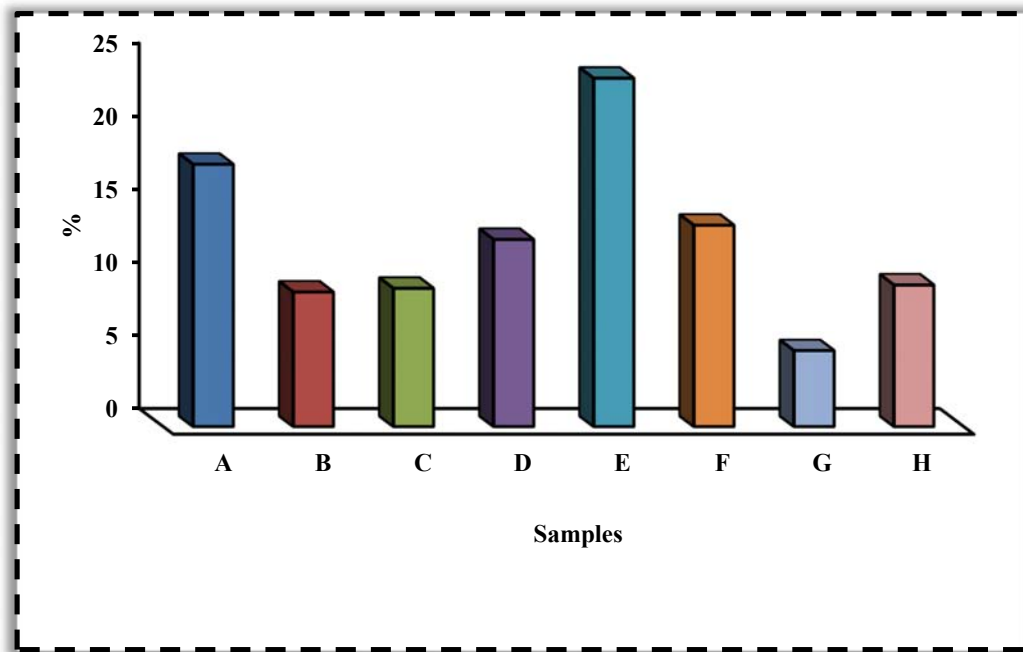


Figure 6: Crude fiber of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

4. 7 Carbohydrate content

Figure (7) shows the carbohydrate contents of wild edible mushrooms *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*). The highest value were in *Agaricus bisporus* was 77.91%, followed by *Agaricus lutosus* 54.01%, whereas the lowest value of carbohydrate content were in *Agaricus impudicus* was 26.76%. The values within all samples showed significantly difference ($P \leq 0.05$). Abdalla (2015) reported that the carbohydrate content of some wild edible mushrooms in *Agaricus bisporus* was 20.75% and Saiqa *et al.*, (2008) reported that in *Agaricus bisporus* was 56.47% and Mshandete and Cuff (2007) reported the carbohydrate content in *Volvariella volvacea* was 50.0% and Barros *et al.*, (2007) reported the carbohydrate content in *Agaricus arvensis* was 37.5%.

4. 8 Mineral content

Figure (8) shows the calcium contents of wild edible mushrooms *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*). The highest levels of calcium were in *Agaricus lutosus* was 8.60 mg/kg, whereas the lowest levels of calcium were in *Volvariella volvacea* was 5.80 mg/kg. The values within the *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*) showed significantly difference ($P > 0.05$). While the *Agaricus lutosus*, *Agaricus arvensis* and the commercial sample (*Agaricus bisporus*) are similar in values. The reported calcium values for *Agaricus bisporus* are 6.7 mg/kg, (Abdalla, 2015).

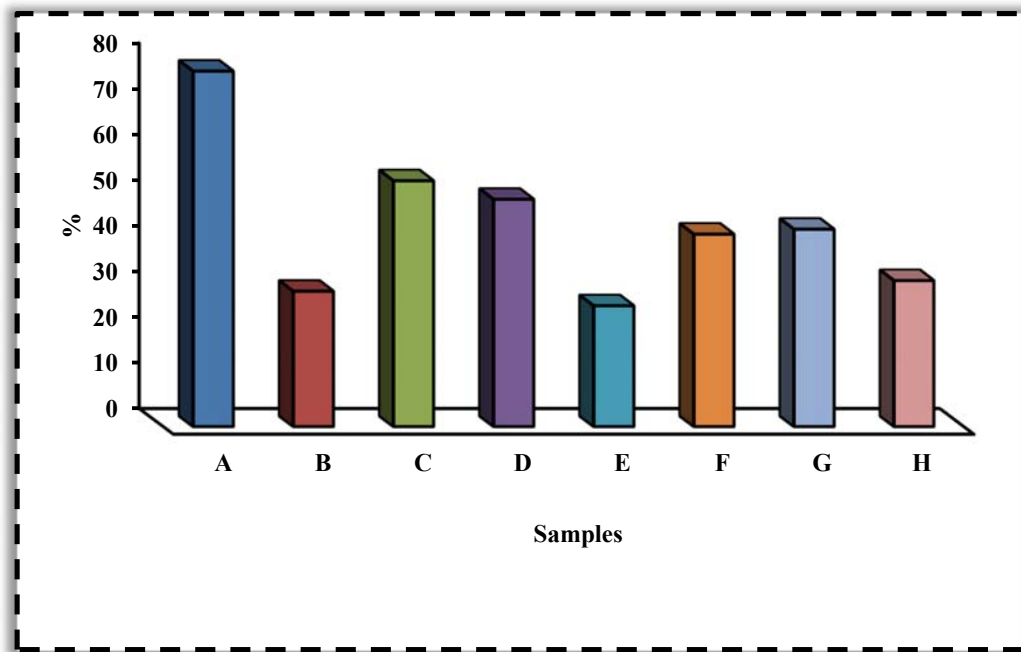


Figure 7: Total carbohydrates of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

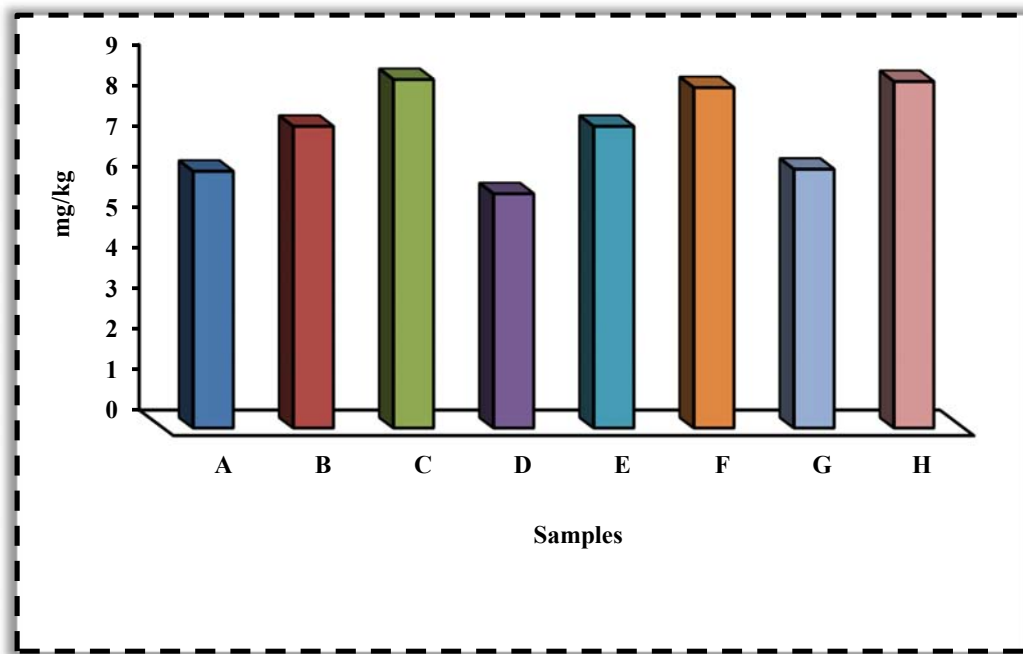


Figure 8: Calcium content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

Figure (9) shows the magnesium content of wild edible mushrooms. The highest levels of magnesium was in *Agaricus lutosus* was 5.50 mg/kg, whereas the lowest levels of magnesium was in *Volvariella volvacea* was 3.07 mg/kg.

Figure (10) shows the phosphorus content of wild edible mushrooms. The highest levels of phosphorus was in *Volvariella volvacea* was 2.50 mg/kg, whereas the lowest levels of phosphorus was in *Agaricus silvicola* was 2.15 mg/kg.

Figure (11) shows the potassium content of wild edible mushrooms. The highest levels of potassium was in *Agaricus arvensis* was 21.50 mg/kg, whereas the lowest levels of potassium was in *Agaricus silvicola* was 21.20 mg/kg.

Figure (12) shows the iron content of wild edible mushrooms. The highest levels of iron was in *Agaricus bisporus* was 1.55 mg/100g, whereas the lowest levels of iron was in *Volvariella volvacea* was 1.30 mg/100g.

Figure (13) shows the zinc content of wild edible mushrooms. The highest levels of zinc were in *Volvariella volvacea* 0.52 mg/100g, whereas the lowest levels of zinc was in commercial sample (*Agaricus bisporus*) was 0.51 mg/100g.

Figure (14) shows the copper content of wild edible mushrooms in this study the highest levels of copper was in *Agaricus bisporus* was 0.11 mg/100g, whereas the lowest levels of copper was in commercial sample (*Agaricus bisporus*) was 0.10 mg/100g

Figure (15) shows the manganese content of wild edible mushrooms in this study the highest levels of manganese was in *Chlorophyllum rhacodes* was 0.12 mg/100g, whereas the lowest levels of manganese was in *Agaricus silvicola* was 0.10 mg/100g.

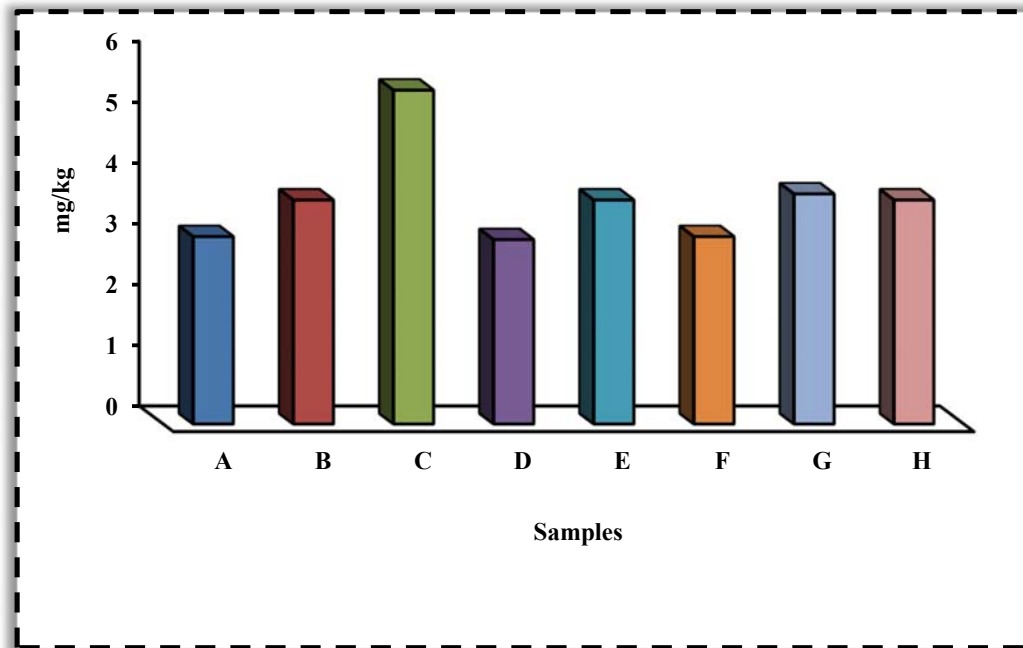


Figure 9: Magnesium content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

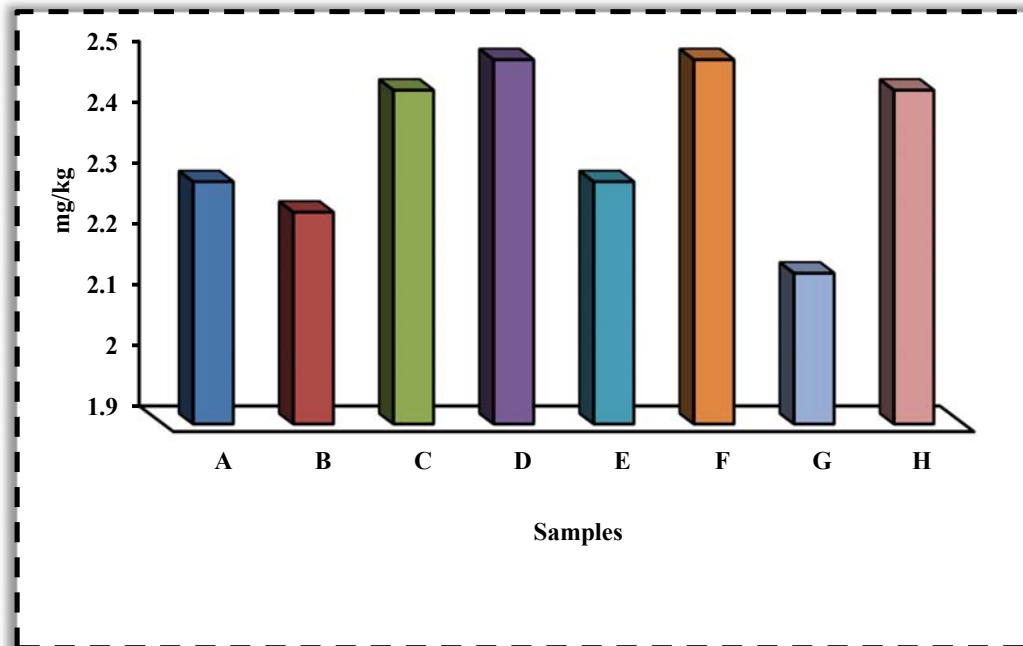


Figure 10: Phosphorus content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

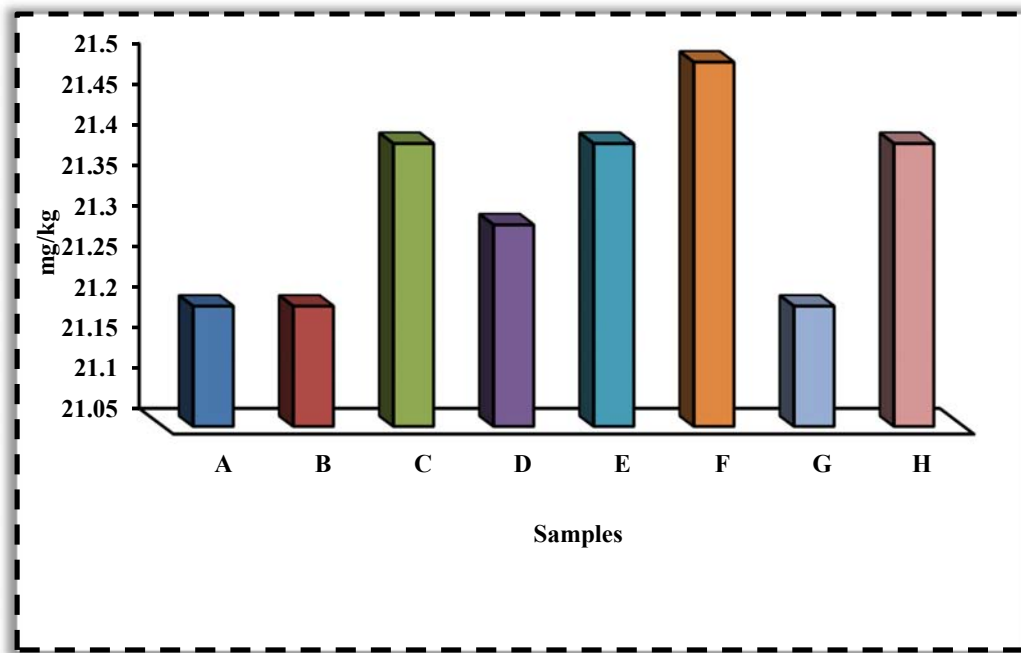


Figure 11: Potassium content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

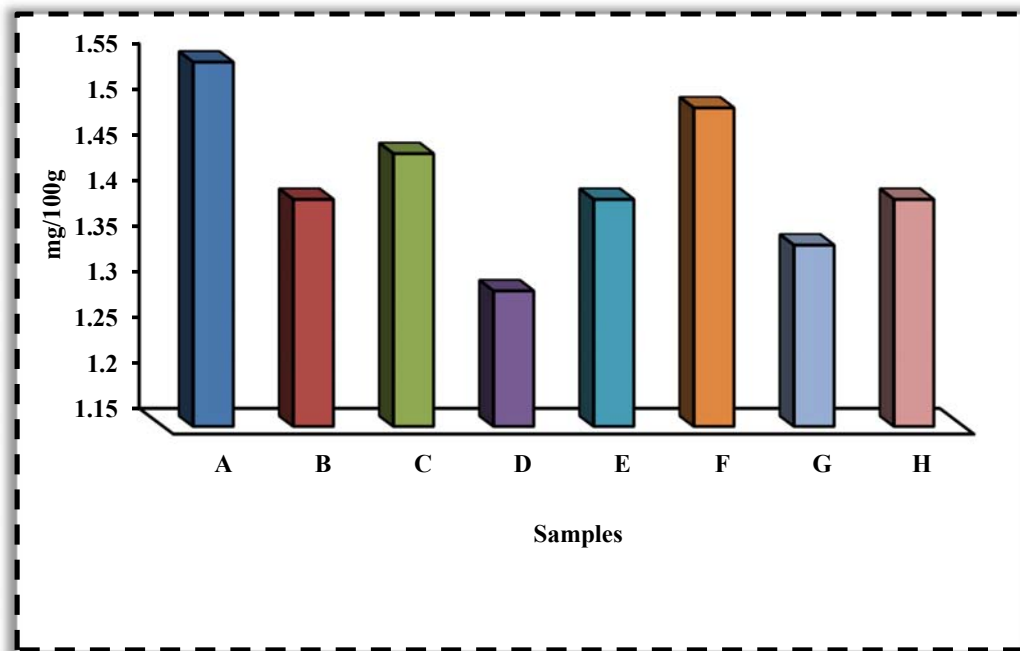


Figure 12: Iron content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

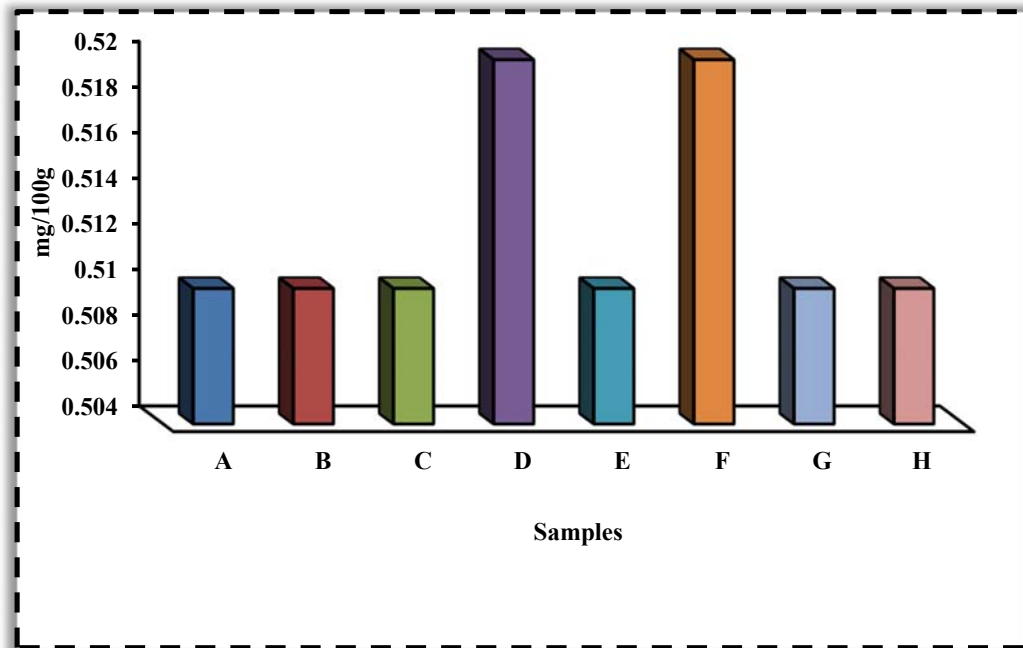


Figure 13: Zinc content of edible Mushroom samples

Key:

- | | |
|-------------------------------|--|
| A ≡ <i>Agaricus bisporus</i> | B ≡ <i>Chlorophyllum rhacodes</i> |
| C ≡ <i>Agaricus lutosus</i> | D ≡ <i>Volvariella volvacea</i> |
| E ≡ <i>Agaricus impudicus</i> | F ≡ <i>Agaricus arvensis</i> |
| G ≡ <i>Agaricus silvicola</i> | H ≡ <i>Agaricus bisporus</i> (commercial sample) |

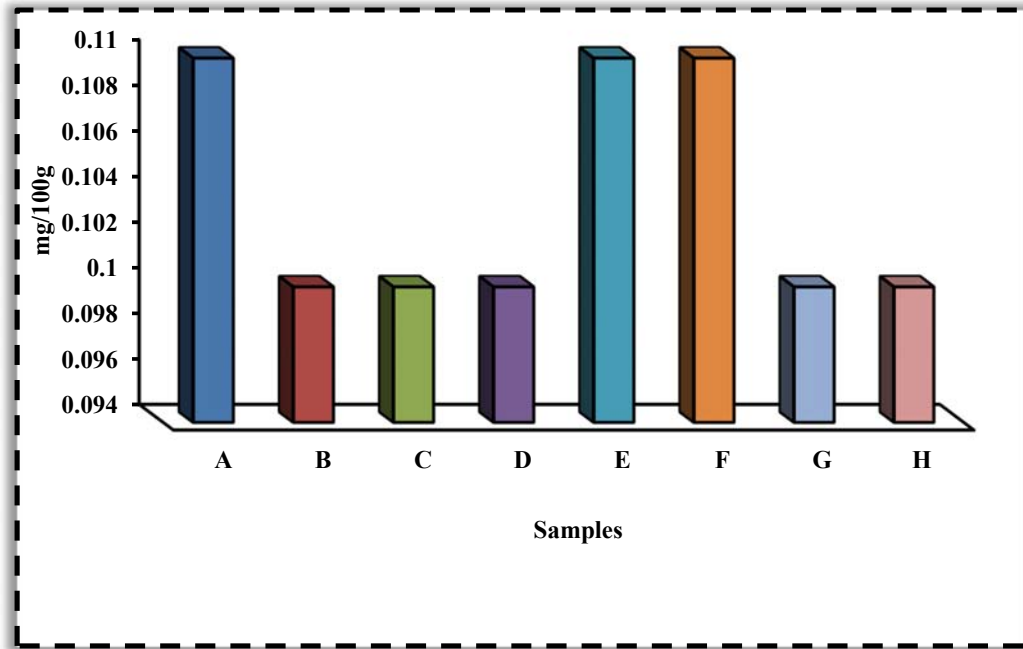


Figure 14: Copper content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

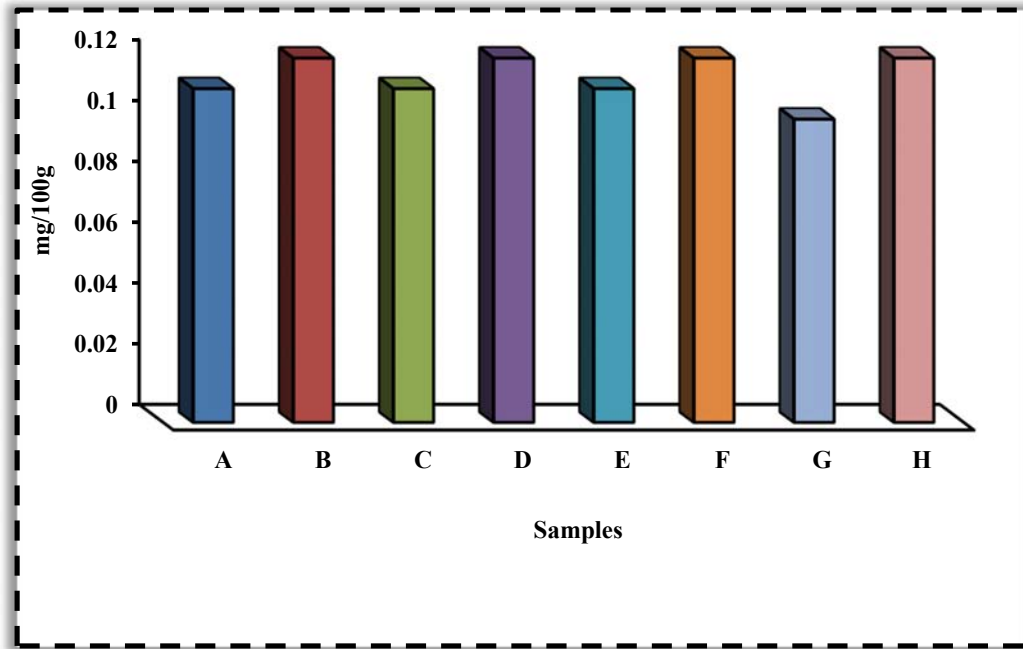


Figure 15: Manganese content of edible Mushroom samples

Key:

- | | |
|-------------------------------|--|
| A ≡ <i>Agaricus bisporus</i> | B ≡ <i>Chlorophyllum rhacodes</i> |
| C ≡ <i>Agaricus lutosus</i> | D ≡ <i>Volvariella volvacea</i> |
| E ≡ <i>Agaricus impudicus</i> | F ≡ <i>Agaricus arvensis</i> |
| G ≡ <i>Agaricus silvicola</i> | H ≡ <i>Agaricus bisporus</i> (commercial sample) |

4. 9 Amino acids content

Figure (16) shows the arginine contents of wild edible mushrooms *Agaricus bisporus*, *Chlorophyllum rhacodes*, *Agaricus lutosus*, *Volvariella volvacea*, *Agaricus impudicus*, *Agaricus arvensis*, *Agaricus silvicola* and the commercial sample (*Agaricus bisporus*). The highest values of arginine was in *Chlorophyllum rhacodes* (6.40 mg/100g) whereas the lowest values of arginine was in *Agaricus arvensis* was (6.30 mg/100g). Tseng and Mau (1999) reported that the arginine content of some wild edible mushrooms was in *Agaricus bisporus* (3.83 mg/100g).

Figure (17) shows the histidine content of wild edible mushrooms. The highest values of histidine was in *Chlorophyllum rhacodes* (2.20 mg/100g), whereas the lowest values of histidine was in *Agaricus impudicus* (1.90 mg/100g). Crisan and sands (1978) reported that the histidine content of some wild edible mushrooms was in *Agaricus bisporus* (2.7 mg/100g) and *Volvariella volvacea* (3.8 mg/100g).

Figure (18) shows the lysine content wild edible mushrooms. The highest values of lysine was in *Chlorophyllum rhacodes* (5.10 mg/100g), whereas the lowest values of lysine was in *Agaricus bisporus* (5.00 mg/100g). Crisan and sands (1978) reported that the lysine content of some wild edible mushrooms was in *Agaricus bisporus* (9.1 mg/100g) and *Volvariella volvacea* (7.1 mg/100g).

Figure (19) shows the tryptophan content of wild edible mushrooms. The highest values of tryptophan was in *Agaricus bisporus* (0.90 mg/100g), whereas the lowest values of tryptophan was in *Chlorophyllum rhacodes* (0.88 mg/100g). Crisan and sands (1978) reported that the tryptophan content of some wild edible mushrooms was in *Agaricus bisporus* (2.0 mg/100g) and *Volvariella volvacea* (1.5 mg/100g).

Figure (20) shows the phenylalanine content of wild edible mushrooms. The highest values of phenylalanine was in *Agaricus arvensis* (2.10 mg/100g), whereas the lowest values of phenylalanine was in *Agaricus silvicola* (2.00 mg/100g). Crisan and sands (1978) reported that the phenylalanine content of some wild edible mushrooms was in *Agaricus bisporus* (4.2 mg/100g) and *Volvariella volvacea* (2.6 mg/100g).

Figure (21) shows the methionine of wild edible mushrooms. The highest values of methionine was in *Agaricus bisporus* (1.15 mg/100g), whereas the lowest values of methionine was in *Agaricus silvicola* (1.00 mg/100g). Crisan and sands (1978) reported that the methionine content of some wild edible mushrooms was in *Agaricus bisporus* (0.9 mg/100g) and *Volvariella volvacea* (1.1 mg/100g).

Figure (22) shows the threonine content of wild edible mushrooms. The highest values of threonine was in *Agaricus bisporus* (4.10 mg/100g), whereas the lowest values of threonine was in *Volvariella volvacea* (4.05 mg/100g), the values within all samples showed no significantly difference ($P \leq 0.05$). Crisan and sands (1978) reported that the threonine content of some wild edible mushrooms was in *Agaricus bisporus* (5.5 mg/100g) and *Volvariella volvacea* (3.5 mg/100g).

Figure (23) shows the leucine content of wild edible mushrooms. The highest values of leucine was in *Chlorophyllum rhacodes* (4.10 mg/100g), whereas the lowest values of leucine was in commercial sample (*Agaricus bisporus*) was (3.90 mg/100g). The values within all samples showed significantly difference ($P \leq 0.05$). Crisan and sands (1978) reported that the leucine content of some wild edible mushrooms was in *Agaricus bisporus* (7.5 mg/100g) and *Volvariella volvacea* (4.5 mg/100g).

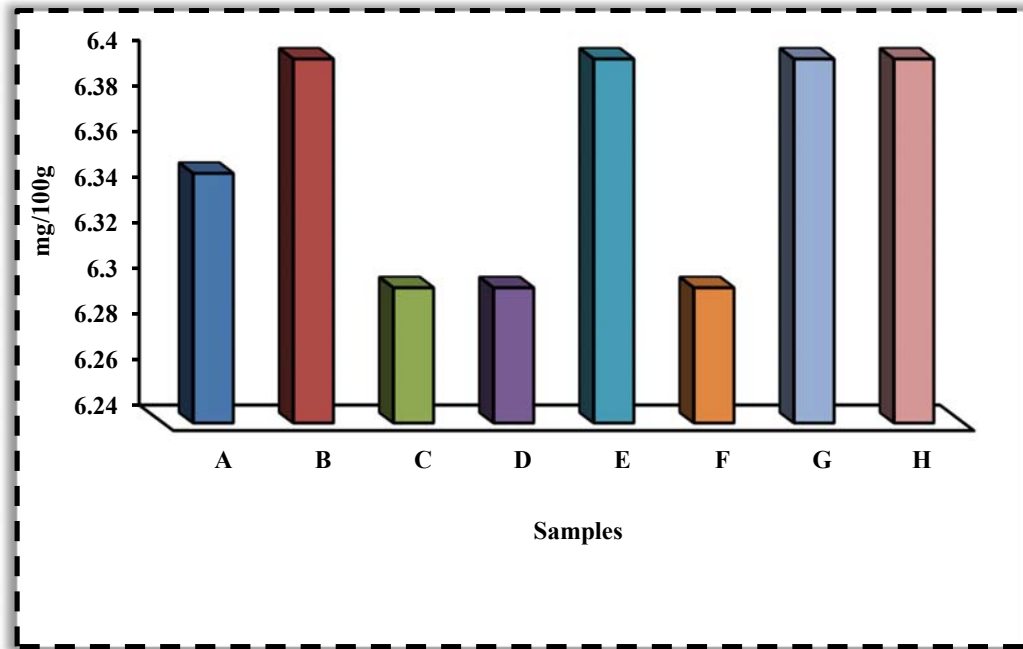


Figure 16: Arginine content of edible Mushroom samples

Key:

- A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

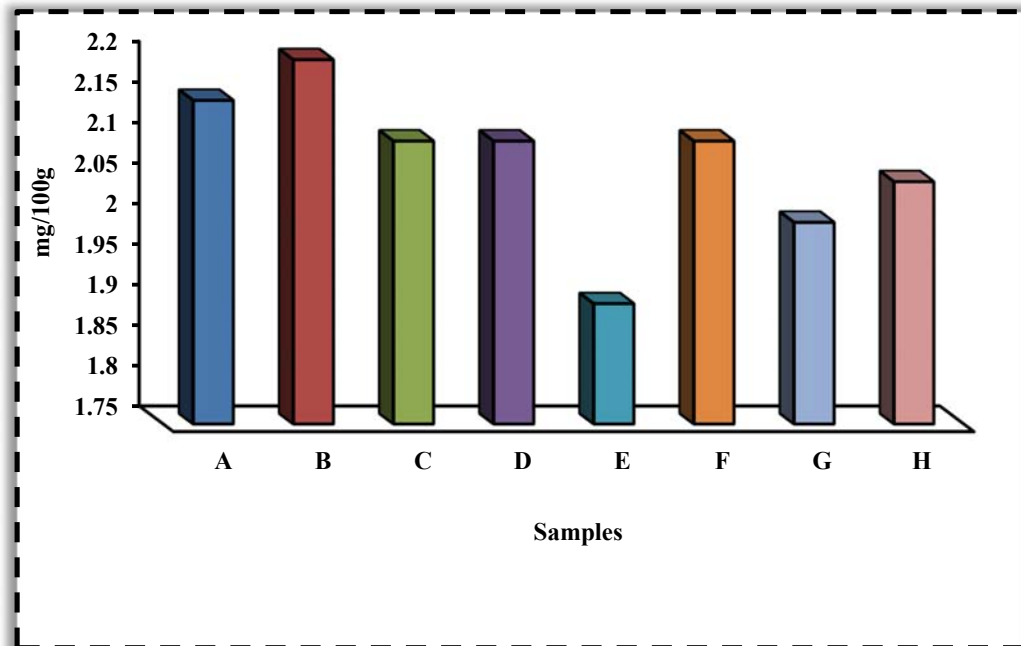


Figure 17: Histidine content of edible Mushroom samples

Key:

- | | |
|-------------------------------|--|
| A ≡ <i>Agaricus bisporus</i> | B ≡ <i>Chlorophyllum rhacodes</i> |
| C ≡ <i>Agaricus lutosus</i> | D ≡ <i>Volvariella volvacea</i> |
| E ≡ <i>Agaricus impudicus</i> | F ≡ <i>Agaricus arvensis</i> |
| G ≡ <i>Agaricus silvicola</i> | H ≡ <i>Agaricus bisporus</i> (commercial sample) |

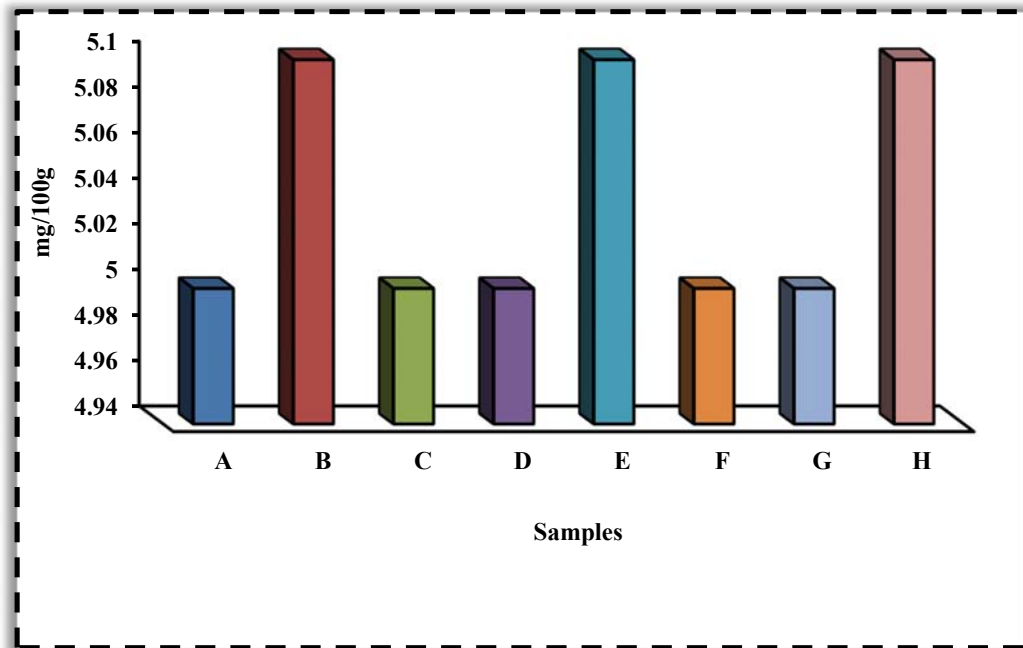


Figure 18: Lysine content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

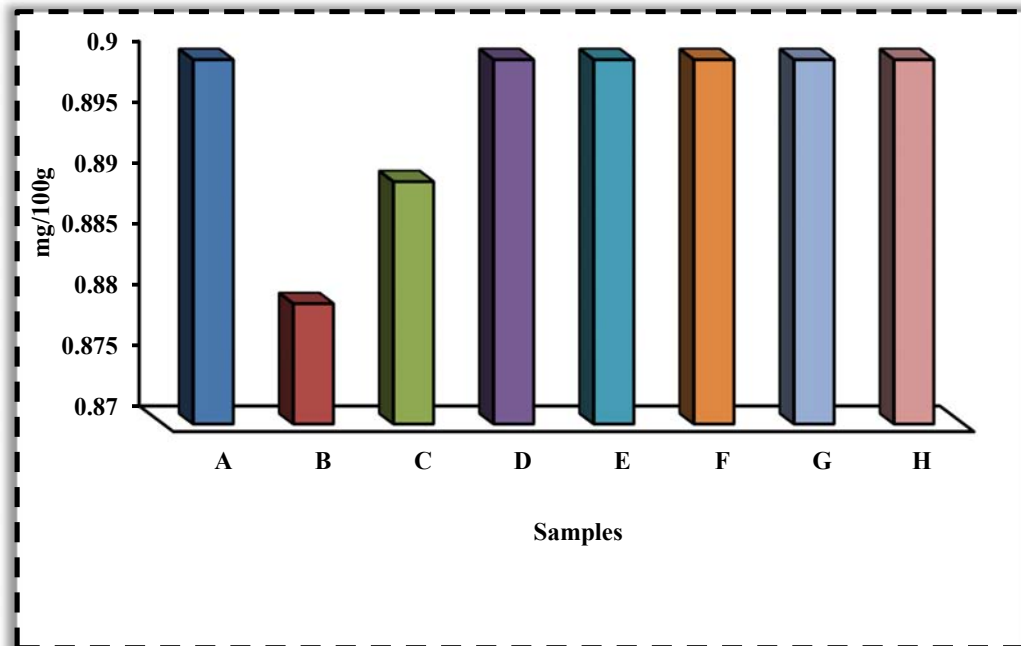


Figure 19: Tryptophan content of edible Mushroom samples

Key:

- | | |
|-------------------------------|--|
| A ≡ <i>Agaricus bisporus</i> | B ≡ <i>Chlorophyllum rhacodes</i> |
| C ≡ <i>Agaricus lutosus</i> | D ≡ <i>Volvariella volvacea</i> |
| E ≡ <i>Agaricus impudicus</i> | F ≡ <i>Agaricus arvensis</i> |
| G ≡ <i>Agaricus silvicola</i> | H ≡ <i>Agaricus bisporus</i> (commercial sample) |

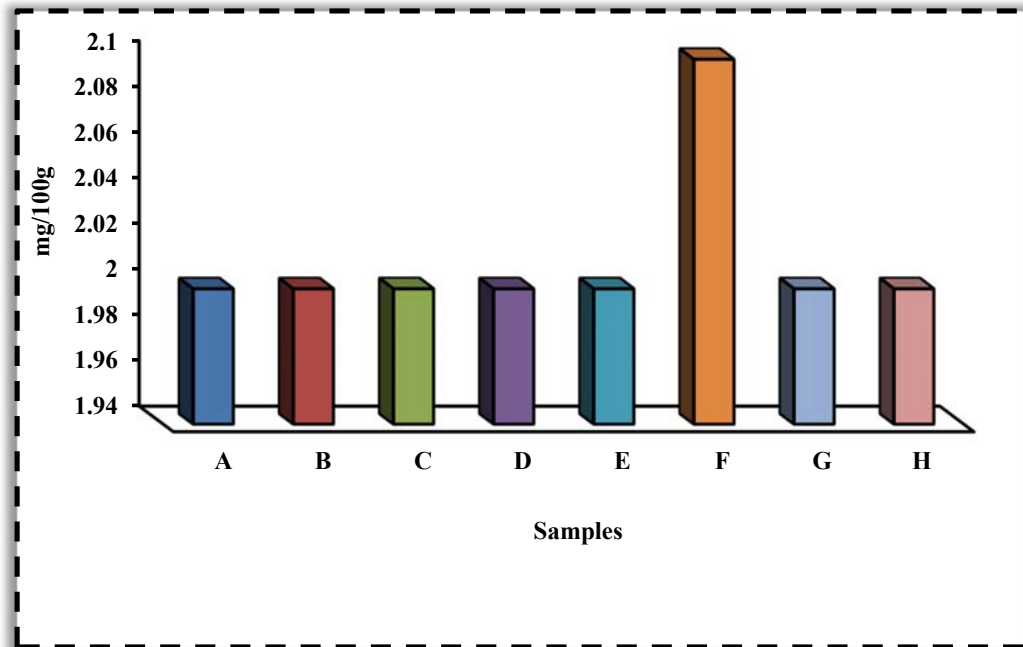


Figure 20: Phenylalanine content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

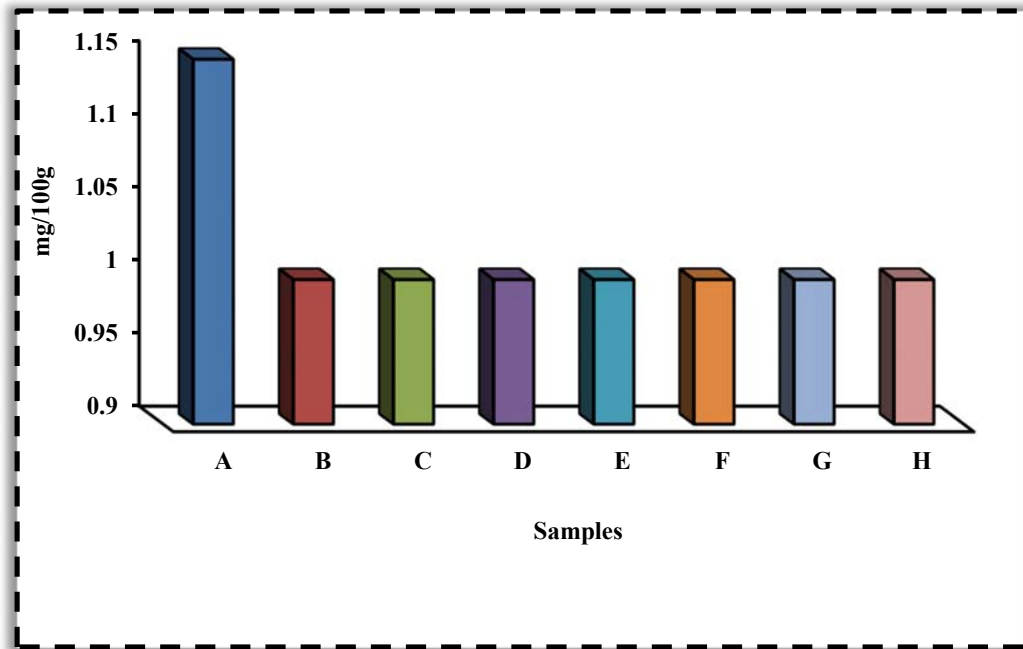


Figure 21: Methionine content of edible Mushroom samples

Key:

- | | |
|-------------------------------|--|
| A ≡ <i>Agaricus bisporus</i> | B ≡ <i>Chlorophyllum rhacodes</i> |
| C ≡ <i>Agaricus lutosus</i> | D ≡ <i>Volvariella volvacea</i> |
| E ≡ <i>Agaricus impudicus</i> | F ≡ <i>Agaricus arvensis</i> |
| G ≡ <i>Agaricus silvicola</i> | H ≡ <i>Agaricus bisporus</i> (commercial sample) |

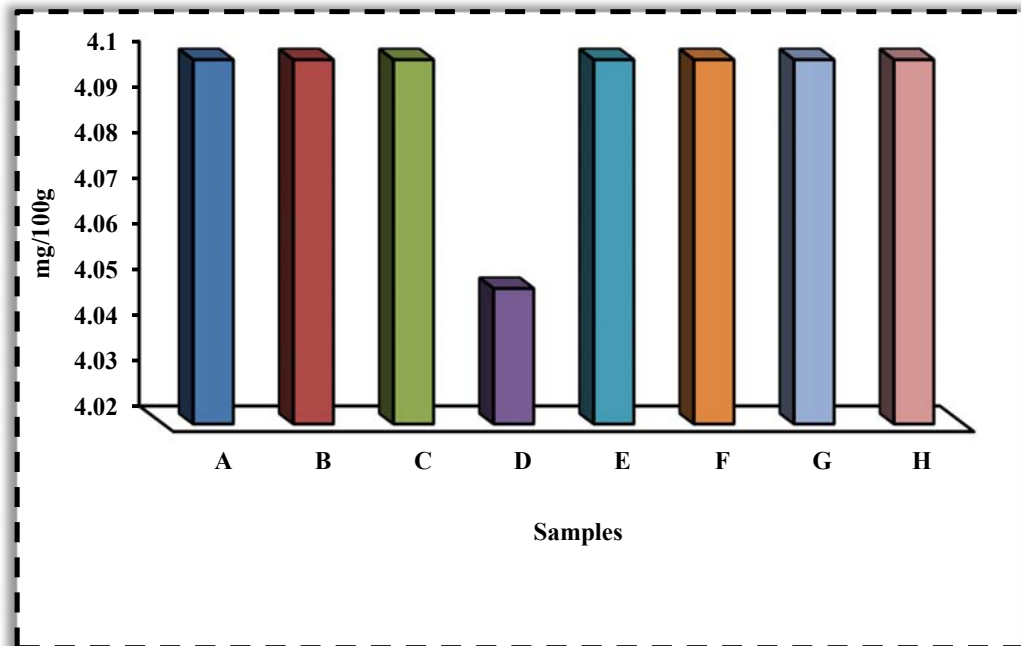


Figure 22: Threonine content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

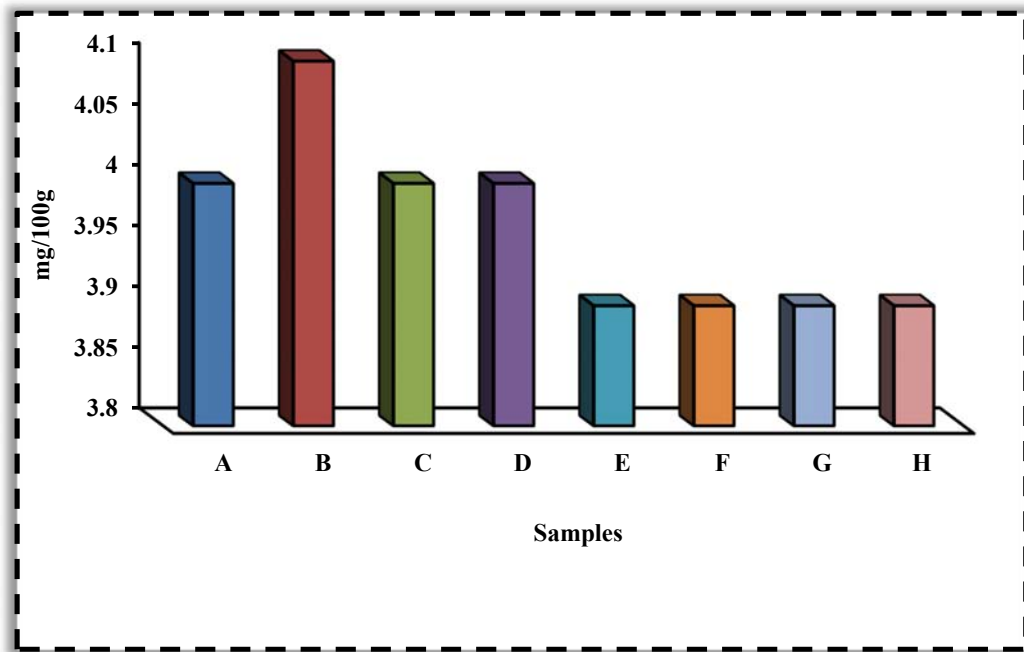


Figure 23: Leucine content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

Figure (24) shows the isoleucine content of wild edible mushrooms. The highest values of isoleucine was in *Chlorophyllum rhacodes* (5.70 mg/100g), whereas the lowest values of isoleucine was in *Agaricus bisporus* (5.56 mg/100g). The values within all samples are not significantly ($P \leq 0.05$) different. Crisan and sands (1978) reported that the isoleucine content of some wild edible mushrooms was in *Agaricus bisporus* (4.5 mg/100g) and *Volvariella volvacea* (3.4 mg/100g).

Figure (25) shows the valine content of wild edible mushrooms. The highest values of valine was in *Agaricus bisporus* (4.50 mg/100g), whereas the lowest values of valine was in *Agaricus arvensis* (4.20 mg/100g). The values within all samples are highly significantly ($P \leq 0.05$) different. Crisan and sands (1978) reported that the valine content of some wild edible mushrooms was in *Agaricus bisporus* (2.5 mg/100g) and *Volvariella volvacea* (5.4 mg/100g).

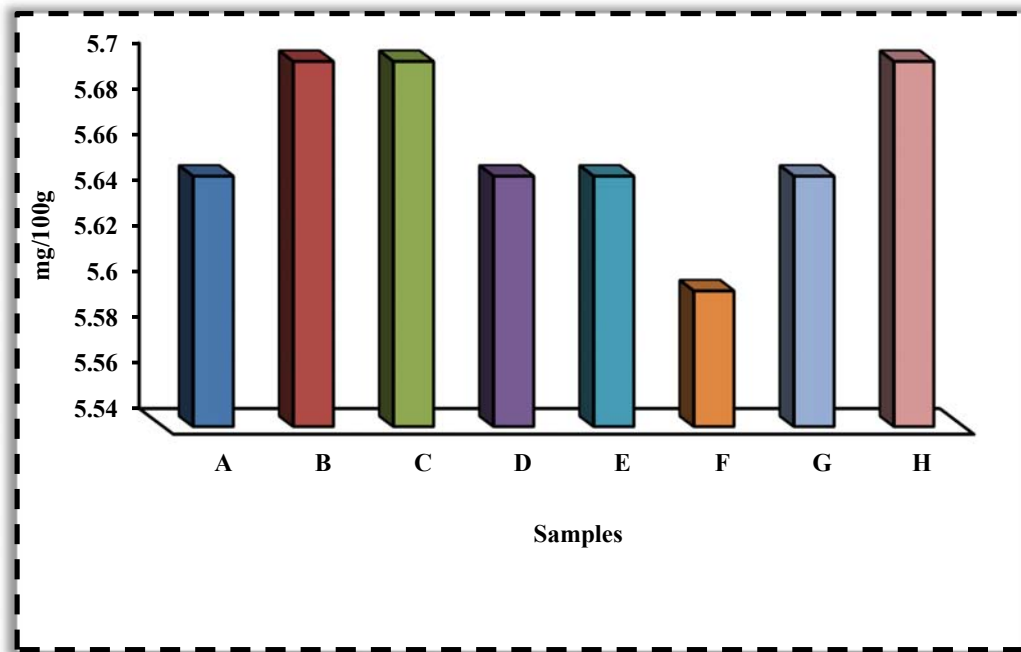


Figure 24: Isoleucine content of edible Mushroom samples

Key:

A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

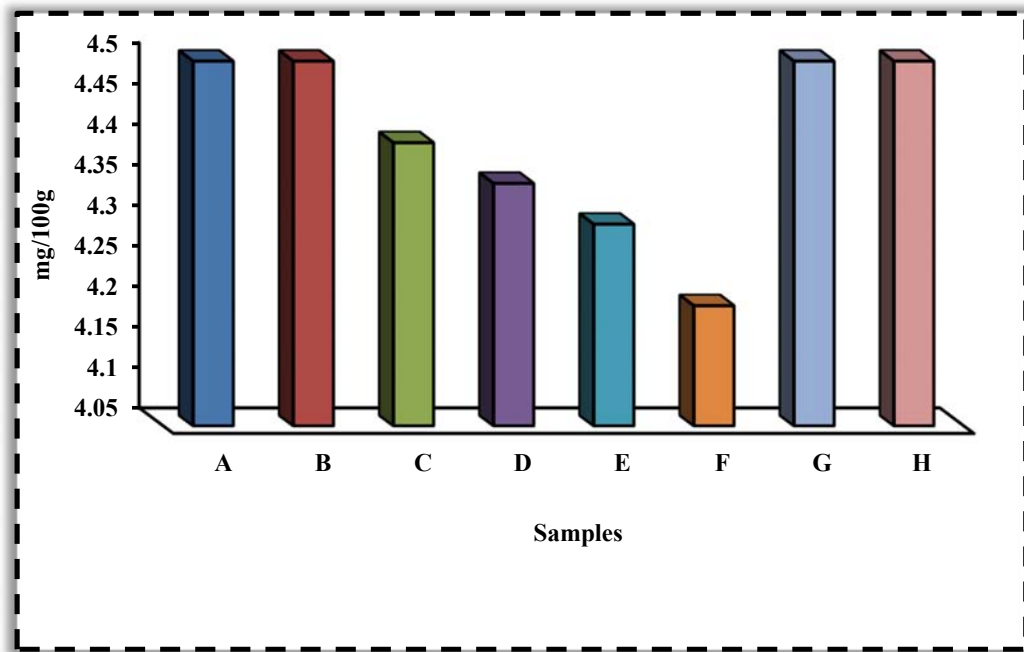


Figure 25: Valine content of edible Mushroom samples

Key:

- A ≡ *Agaricus bisporus* B ≡ *Chlorophyllum rhacodes*
 C ≡ *Agaricus lutosus* D ≡ *Volvariella volvacea*
 E ≡ *Agaricus impudicus* F ≡ *Agaricus arvensis*
 G ≡ *Agaricus silvicola* H ≡ *Agaricus bisporus* (commercial sample)

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Based on the results obtained in this study it can be concluded that the studied edible mushroom samples (seven of them are wild varieties collected from Blue Nile State) are nontoxic.
2. These mushrooms are good sources of protein, minerals and essential amino acids.
3. Also these varieties could be enter as dietary supplement in the local people food to increase the nutritional value and thus a cheap protein and minerals source will be available to all people during the rainfall season.
4. The study revealed that the proximate composition, mineral and essential amino acids are of edible mushrooms sample ware considerable amount.

5.2 Recommendations

1. The edible mushroom is recommended as a dietary supplement in some foods that contain small amounts of protein, essential amino acids and minerals.
2. The public awareness of the people regarding mushrooms nutritional values, their uses and the toxic and nontoxic varieties should be practiced.
3. The edible mushrooms should be cultivated in a commercial level to be available to consumers.
4. Further studies are needed on wild edible mushrooms to highlight on their nutritional value and suitability for human consumption.

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Appendices

Appendix 1: Chemical composition of edible mushroom (g/100 g dry weight basis)

Sample	Moisture (%)	Fat (%)	Crude fiber (%)	Total CHO (%)	Crude protein %(Nx4.38)	Ash (%)
A	5.26 ^b ±0.00	1.19 ^g ±0.04	18.00 ^b ±1.00	77.91 ^a ±1.00	19.41 ^g ±0.01	1.47 ^a ±0.01
B	5.26 ^b ±0.00	1.98 ^d ±0.05	9.30 ^c ±0.10	29.93 ^g ±1.00	21.02 ^c ±0.02	1.37 ^b ±0.01
C	5.26 ^b ±0.00	2.99 ^a ±0.00	9.55 ^c ±0.00	54.01 ^b ±0.01	23.29 ^d ±0.01	0.59 ^d ±0.00
D	11.11 ^a ±0.00	2.90 ^b ±0.00	12.88 ^d ±0.10	49.98 ^c ±0.01	34.14 ^a ±0.12	0.45 ^c ±0.00
E	5.26 ^b ±0.00	0.94 ^h ±0.02	23.86 ^a ±0.01	26.76 ^h ±0.01	20.06 ^f ±0.01	0.22 ^h ±0.00
F	11.11 ^a ±0.00	1.52 ^f ±0.01	13.85 ^c ±0.01	42.37 ^c ±0.01	27.93 ^b ±0.01	0.43 ^f ±0.00
G	11.11 ^a ±0.00	2.73 ^c ±0.00	5.25 ^f ±0.01	43.44 ^d ±0.01	27.93 ^b ±0.01	0.28 ^g ±0.00
H	5.26 ^b ±0.00	1.85 ^e ±0.01	9.78 ^c ±0.01	32.26 ^f ±0.01	24.26 ^c ±0.01	0.60 ^c ±0.00
P-value	0.003 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}
Lsd_{0.05}	0.0007292	0.0005474	0.6193	0.8654	0.6193	0.0005474
SE±	0.0002236	0.0001826	0.2066	0.2887	0.2066	0.0001826

Mean±SD value(s) having different superscript(s) letters in a column are significantly different (P≤0.05) according to DMRT.

Key:

A ≡ *Agaricus bisporus*

C ≡ *Agaricus lutosus*

E ≡ *Agaricus impudicus*

G ≡ *Agaricus silvicola*

B ≡ *Chlorophyllum rhacodes*

D ≡ *Volvariella volvacea*

F ≡ *Agaricus arvensis*

H ≡ *Agaricus bisporus* (commercial sample)

* ≡ significantly different

** ≡ highly significantly different

NS ≡ not significantly different

Appendix 2: Minerals content of edible mushroom (dry weight)

Sample	Ca (mg/kg)	Mg (mg/kg)	P (mg/kg)	K (mg/kg)	Fe (mg/100g)	Zn (mg/100g)	Cu (mg/100g)	Mn (mg/100g)
A	6.35 ^c ±0.07	3.10 ^c ±0.00	2.30 ^{ab} ±0.00	21.20 ^a ±0.00	1.55 ^a ±0.07	0.51 ^b ±0.00	0.11 ^a ±0.01	0.11 ^b ±0.01
B	7.45 ^b ±0.07	3.70 ^b ±0.28	2.25 ^{ab} ±0.07	21.20 ^a ±0.00	1.40 ^{bcd} ±0.00	0.51 ^b ±0.00	0.10 ^b ±0.00	0.12 ^a ±0.01
C	8.60 ^a ±0.00	5.50 ^a ±0.14	2.45 ^{ab} ±0.21	21.40 ^a ±0.28	1.45 ^{abc} ±0.07	0.51 ^b ±0.00	0.10 ^b ±0.00	0.11 ^b ±0.01
D	5.80 ^d ±0.14	3.07 ^c ±0.07	2.50 ^a ±0.14	21.30 ^a ±0.00	1.30 ^d ±0.00	0.52 ^a ±0.01	0.10 ^b ±0.00	0.12 ^a ±0.01
E	7.45 ^b ±0.07	3.70 ^b ±0.14	2.30 ^{ab} ±0.00	21.40 ^a ±0.14	1.40 ^{bcd} ±0.00	0.51 ^b ±0.00	0.11 ^a ±0.01	0.11 ^b ±0.01
F	8.40 ^a ±0.00	3.10 ^c ±0.00	2.50 ^a ±0.14	21.50 ^a ±0.14	1.50 ^{ab} ±0.00	0.52 ^a ±0.01	0.11 ^a ±0.01	0.12 ^a ±0.01
G	6.40 ^c ±0.14	3.80 ^b ±0.14	2.15 ^b ±0.07	21.20 ^a ±0.00	1.35 ^{cd} ±0.07	0.51 ^b ±0.00	0.10 ^b ±0.00	0.10 ^c ±0.00
H	8.55 ^a ±0.07	3.70 ^b ±0.28	2.45 ^{ab} ±0.21	21.40 ^a ±0.28	1.40 ^{bcd} ±0.00	0.51 ^b ±0.01	0.10 ^b ±0.00	0.12 ^a ±0.01
P-value	0.0002 ^{**}	0.005 ^{**}	0.04867 [*]	0.4501 ^{NS}	0.0074 ^{**}	0.0456 [*]	0.050 [*]	0.0491 [*]
Lsd_{0.05}	0.2063	0.3859	0.3007	0.3646	0.1031	0.0007292	0.0007292	0.0007292
SE±	0.06325	0.1183	0.0922	0.1118	0.03162	0.0002236	0.0002236	0.0002236

Mean±SD value(s) having different superscript(s) letters in a column are significantly different (P≤0.05) according to DMRT.

Key:

A ≡ *Agaricus bisporus*

C ≡ *Agaricus lutosus*

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B ≡ *Chlorophyllum rhacodes*

D ≡ *Volvariella volvacea*

F ≡ *Agaricus arvensis*

H ≡ *Agaricus bisporus* (commercial sample)

* ≡ significantly different

** ≡ highly significantly different

NS ≡ not significantly different

Appendix 3: Amino acids content of edible mushroom (mg/100 g dry weight)

Sample	Arginine	Histidine	Lysine	Tryptophan	Phenylalanine	Methionine	Threonine	Leucine	Isoleucine	Valine
A	6.35 ^{ab} ±0.07	2.15 ^{ab} ±0.07	5.00 ^b ±0.00	0.90 ^a ±0.00	2.00 ^b ±0.00	1.15 ^a ±0.07	4.10 ^a ±0.00	4.00 ^b ±0.00	5.56 ^a ±0.07	4.50 ^a ±0.07
B	6.40 ^a ±0.00	2.20 ^a ±0.00	5.10 ^a ±0.00	0.88 ^c ±0.0	2.00 ^b ±0.00	1.00 ^b ±0.00	4.10 ^a ±0.00	4.10 ^a ±0.00	5.70 ^a ±0.00	4.50 ^a ±0.00
C	6.30 ^b ±0.00	2.10 ^{bc} ±0.00	5.00 ^b ±0.00	0.89 ^b ±0.00	2.00 ^b ±0.00	1.00 ^b ±0.00	4.10 ^a ±0.00	4.10 ^a ±0.00	5.70 ^a ±0.00	4.40 ^{ab} ±0.00
D	6.30 ^b ±0.00	2.10 ^{bc} ±0.00	5.00 ^b ±0.00	0.90 ^a ±0.00	2.00 ^b ±0.00	1.00 ^b ±0.00	4.05 ^a ±0.00	4.00 ^b ±0.00	5.65 ^a ±0.07	4.35 ^{abc} ±0.07
E	6.40 ^a ±0.00	1.90 ^c ±0.00	5.10 ^a ±0.00	0.90 ^a ±0.00	2.00 ^b ±0.00	1.00 ^b ±0.00	4.10 ^a ±0.00	3.90 ^c ±0.00	5.65 ^a ±0.07	4.30 ^{bc} ±0.00
F	6.30 ^b ±0.00	2.10 ^{bc} ±0.00	5.00 ^b ±0.00	0.90 ^a ±0.00	2.10 ^a ±0.00	1.00 ^b ±0.00	4.10 ^a ±0.00	3.90 ^c ±0.00	5.60 ^a ±0.00	4.20 ^c ±0.14
G	6.40 ^a ±0.00	2.00 ^d ±0.00	5.00 ^b ±0.00	0.90 ^a ±0.00	2.00 ^b ±0.00	1.00 ^b ±0.00	4.10 ^a ±0.00	3.90 ^c ±0.00	5.65 ^a ±0.07	4.50 ^a ±0.00
H	6.40 ^a ±0.0	2.05 ^{cd} ±0.07	5.10 ^a ±0.00	0.90 ^a ±0.00	2.00 ^b ±0.00	1.00 ^b ±0.00	4.10 ^a ±0.00	3.90 ^c ±0.00	5.70 ^a ±0.00	4.50 ^a ±0.00
P-value	0.0047 ^{**}	0.0007 ^{**}	0.00 ^{**}	0.0494 [*]	0.00 ^{**}	0.003 ^{**}	0.4934 ^{NS}	0.0001 ^{**}	0.4934 ^{NS}	0.0086 ^{**}
Lsd_{0.05}	0.07292	0.07292	0.0007292	0.0007292	0.0007292	0.07292	0.07292	0.0007292	0.1031	0.1458
SE±	0.02236	0.2236	0.0002236	0.0002236	0.002236	0.02236	0.02236	0.0002236	0.03162	0.04472

Mean±SD value(s) having different superscript(s) letters in a column are significantly different (P≤0.05) according to DMRT.

Key:

A ≡ *Agaricus bisporus*

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