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KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

USING SOME INDUSTRIAL WASTES AS A SUBSTRATE FOR CULTIVATION OF OYSTER MUSHROOM (*Pleurotus ostreatus* spp.) AND CHEMICAL CHARACTERIZATION

LINA FARHAD HUSSEIN BAZYANI

MASTER'S THESIS

DEPARTMENT OF FOERST INDUSTRY ENGINEERING

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Thesis submitted in candidature for
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Prof. Dr. M. Hakkı ALMA (Supervisor) ……
Department of Forest Industry Engineering, Faculty of Forestry
Kahramanmaraş Sütçü İmam University

Assoc. Prof. Dr. Ekrem KIREÇCI (Member) ……
Department of Medical Microbiology, Faculty of Medicine.
Kahramanmaraş Sütçü İmam University

Assist. Prof. Dr. Bekir Cihad BAL (Member) …….
Department of Forest Industry Engineering, Faculty of Forestry
Kahramanmaraş Sütçü İmam University

I approve that the above signatures related to the members.

Prof. Dr. M. Hakkı ALMA ……
Director of Graduate School of Natural and Applied Scinces.
Kahramanmaraş Sütçü İmam University

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KAYIN MANTARI (*Pleurotus ostreatus* spp.) KÜLTÜRASYONUNDA YETİŞTİRME ORTAMI OLARAK BAZI ENDÜSTRİYEL ATIKLARIN KULLANIMI VE KİMYASAL KARAKTERİZASYONU

LINA FARHAD HUSSEIN BAZYANI

ÖZET

Bu çalışmada, değişik endüstriyel atıkların *Pleurotus ostreatus* mantarı yetiştiriciliğinde kullanım olanaklarının araştırılması yapılmış, verimlilik ve kimyasal bileşimi üzerine etkisi incelenmiştir. *Pleurotus ostreatus* mantarının tohumluk miselleri %100 kavak odunu talaşı, %100 zeytin posası, %100 ceviz odunu talaşı, yonca yaprağı ve bunların farklı kombinasyonlarından oluşan ortamlara aşılanmıştır. Ortamlar üzerine aşılı *Pleurotus ostreatus*'un misel gelişme süresi, mantar eldesi süresi, toplam verim, biyolojik etkinlik oranı, mantar kalite özellikleri, makro ve mikro besin elementi içeriği belirlenmiştir. Ortamların özelliklerinin belirlenmesi amacı ile karbon, azot, karbon/azot miktarları belirlenmiştir. Farklı ortamlarda yetiştirilen *Pleurotus ostreatus* mantarının, yağ asidi içeriği, mimoza kondanse tanen miktarı ve şeker miktarı belirlenmiştir.

Çalışmanın sonucuna göre, *Pleurotus ostreatus* aşılı ortamlar içerisinde en hızlı misel gelişimi % 70 PS+ % 20 W + %10 VSW ortamından, en kısa sürede mantar eldesi % 70 PS + % 20 W + %10 VSW ortamından elde edilmiştir. En yüksek toplam verim ve biyolojik etkinlik oranı, % 70 PS + % 20 W + %10 VSW ortamından elde edilmiştir. En yüksek mantar ağırlığı % 70 PS + % 20 W + %10 VSW ortamından 29.25 g, en yüksek şapka genişliği ve şapka kalınlığı % 70 PS + % 20 OW + %10 VSW ortamından 83.8 mm ve 8.23 mm, en yüksek sap uzunluğu %100 PS ortamından 11.93 mm olarak elde edilmiştir. Genel olarak kondanse tanen miktarı 3.66 ppm ile 6.18 ppm arasında olduğu belirlenmiştir. En yüksek sükroz içeriği %50 PS + %50 OW ortamından %88.04 ve en yüksek glikoz içeriği %100 PS ortamından %26.54 olarak tespit edilmiştir. En yüksek antioksidan özelliği % 75 PS + % 25 OW ortamından elde edilmiştir.

Anahtar Kelimeler: *Pleurotus ostreatus*, Verim, Antioksidan, Kimyasal Bileşim, Atık

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USING SOME INDUSTRIAL WASTES AS A SUBSTRATE FOR CULTIVATION OF OYSTER MUSHROOM (*Pleurotus ostreatus* spp.)AND CHEMICAL CHARACTERIZATION

(M.Sc. THESIS)

LINA FARHAD HUSSEIN BAZYANI

ABSTRACT

The aim of this study was to investigate the possibility of using different industrial wastes in cultivation of *Pleurotus ostreatus* and to determine its effects on chemical composition and yield. The spawns of *Pleurotus ostreatus* were inoculated on 100% poplar sawdust, 100% olive waste, 100% walnut wood waste, vicia sativa weed and different combination of this substrate. The mycelium growing duration of *Pleurotus ostreatus* which was inoculated on the medias, the mushrooms obtaining duration, total yield, biological efficiencies, morphological characteristics of the mushrooms and macro and micro nutrient contents were determined. Carbon, nitrogen, carbon/nitrogen were determined in order to find substrate contents. The content of fatty acid, the amount of condensed tannins and sugar were determined.

According to the results, the fastest mycelium growing duration was obtained from 70 % PS+ 20 % W + 10 % VSW, whereas the fastest mushroom obtaining duration was obtained from 70 % PS+ 20 % W + 10 % VSW inoculated with *Pleurotus ostreatus*. On the other hand, the highest total yield and biological efficiency were for 70 % PS+ 20 % W + 10 % VSW, and the highest weight of mushroom were obtained from 70 % PS+ 20 % W + 10 % VSW (29.25 g). In addition maximum diameter and thickness of cap were observed 70 % PS+ 20 % OW + 10 % VSW (83.8 mm and 8.23 mm) respectively. The highest diameter of stalk was determined PS %100 (11.93 mm). In general, the amount of condensed tannins was determined to be between 3.66 ppm and 6.18 ppm. The highest content of sucrose were obtained 50 % PS + 50 % OW (%88.04 g) and the highest content of glucose were obtained 100 % PS (%26.54 g). The maximum antioxidant were determined 75 % PS + 25 % OW substrates.

Key words: *Pleurotus ostreatus*, Yield, Antioxidant, Chemical Composition, Wastes

University of Kahramanmaras Sutcu Imam

Graduate School of Natural and Applied Sciences

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Lina Farhad Hussein

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LIST OF ABBREVIATIONS

DPPH:	Free radical scavenging activity
BHT:	Butylated hydroxytoluene
C:	Carbon
N:	Nitrogen
100% PS:	100% poplar sawdust
100% OW:	100% olive waste
50% PS + 50% OW:	50% poplar sawdust + 50% olive waste
45% PS + 45% OW+10% VSW:	45% poplar sawdust + 45% olive waste+ 10% vicia sativa weed
100% W:	100% walnut wood waste
50% PS + 50% W:	50% poplar sawdust + 50% walnut wood waste
45% PS + 45% W + 10% VSW:	45% poplar sawdust + 45% walnut wood waste+ 10% vicia sativa weed
75% PS + 25% OW:	75% poplar sawdust + 25% olive waste
70% PS + 20% OW + 10% VSW:	70% poplar sawdust + 20% olive waste + 10% vicia sativa weed
75% PS + 25% W:	75% poplar sawdust + 25% walnut wood waste
70% PS + 20% W + 10% VSW:	70% poplar sawdust + 20% walnut wood waste + 10% vicia sativa weed
40% PS + 40% OW + 20% VSW:	40% poplar sawdust + 40% olive waste + 20% vicia sativa weed
40% PS + 40% W + 20% VSW:	40% poplar sawdust + 40% walnut wood waste + 20% vicia sativa weed

1. INTRODUCTION

Mushroom is called "white vegetables" or "boneless vegetarian meat". It is the fruiting body of a fungus. Fungus (mycelium) can be compared to a root system. It takes nutrients from the environment, allowing the fungus to grow. Different types have their own nutrition preferences. Mushrooms are fungi that have been used for medicinal plants. It belongs to the Kingdom of Fungi. It has a distinct cellular structure like plants, but it does not have chlorophyll, therefore, it is unable to use the energy from the sun directly through chlorophyll. Thus fungus is heterotrophic and it depends on other organisms for grow up. They depend on the substrates on which they grow for all their nutritional requirements like carbon, water, nitrogen and minerals (Rajarathnam *et al*., 1997). As well as, edible mushrooms are the fungi which possess big fruiting bodies and it can be for culinary, medicinal or both. In China, there are 600 species of edible mushrooms, which are used for both. Since I960, Chinese scientists have researched into more than 40 types of edible mushrooms in more details, such as Armillariella mellea, Cordyceps sinensis, Coriolus versicolor, Ganoderma lucidum, Hericium erinaceus, *Lentinus edodes* (Chen, 2005). Culturing fungi on agricultural residues, wastes from human food in the conversion of the materials is important in economic terms. With this method, such residues have been used efficiently today's most effective method (Matthew *et al*., 1987).

Lignocellulosic material due to catching up on non-wood forest products industry within the scope of mushroom culture, when compared with other products, production per unit area is a branch of most products can be taken. Nature in the complex herbal organic matter of simple organic compounds and inorganic molecules, converting environmental waste removed from the medium and human nutrition have a role in the different fungal species food source and for medicinal purposes can be grown (Mathew *et al*., 1987).

Lignocellulosic materials that are rich in the removal of the lignocellulosic residues and processing continuously remain problematic. Due to their chemical structure and dissociation complex, this procedure is difficult. Recently, lignocellulosic agricultural residues can be used as valuable nutrients in mushroom productions (Philippousis and Zervakis, 2000). Specifically, the mechanism involved in conversion of complex organic molecules into simple compounds is via enzymes for edible mushroom growth (Martinez *et al*., 1994). These biotechnological approaches in other areas of unused and potentially fouling agricultural residues can provide high protein and vitamin content of foods and medicinal properties (Rambell, 1983). In addition, the white cultivated mushrooms

(Agaricus) may open new job opportunities, social and economic developments also (Philippousis *et al*., 2000).

Oyster mushroom (*Pleurotus* spp.), popularly known as 'Dhingri', is edible mushroom having excellent flavour and taste. It belongs to class *Basidiomycetes*, subclass *Hollobasidiomycetidae*, Family *Agaricaceae* and order *gricals*. Presently there are three mushrooms namely *Pleurotus* species (Oyster Mushroom), *Volvariella volvaceae* (Straw Mushroom) and *Auricularia* spp (Ear Mushroom). Oyster mushroom can grow at moderate temperature; therefore, it is suitable for most places as it grows wild in the forests of hilly areas, and it is cultivated in temperate and subtropical regions of the world. It may also grow on decaying organic matter (Randive, 2012).

Most cultivated mushrooms belong to the Basidiomycetes produce spores called basidiospores. It has been estimated that our planet harbour about 1.5 million different species of mushrooms. Only 64000 species have been described so far (Oei, 1996). Cultivation of oyster mushroom (*Pleurotus ostreatus*) has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and utilizing various agro-based residues. *Pleurotus* species are efficient lignin degraders, which can grow on different agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandiak and Goyal, 1995).

Mushrooms produce huge amount of carbon dioxide that slows the growth and twister, distorts and stunts the fruiting body (Nagaya and Kapoor, 1990). During cultivation, it is important to release the carbon dioxide from the surroundings of mushroom spawn packets. Light is an important factor for the development of the pinhead of the Oyster mushroom. It requires about 80 to 210 lux light intensity for maximum number of fruiting body per unit area. Mushroom emits heat during growing period and requires high relative humidity. These factors influenced by the line-to-line and packet-to-packet spacing of Oyster mushroom (Alicbusan and Santiago, 1975).

The technology of artificial cultivation of mushroom is recent innovation. Incorporation of non-conventional crops in existing agricultural system can help for improving the social as well as economic status of small farmer's. Several agricultural wastes, such as compost or wheat compost, paddy straw, banana leaves, sugarcane leaves, wheat barn, rice husk, and sawdust can be used as a substrate for growing mushrooms (Gupta, 1986). Sawdust and sugarcane were the best substrates for growing of oyster mushroom than other agro-based substrates (Ahmed, 1998). Iqbal *et al*., (1988) studied the

effects of various composts, such as blow gutter, weaving sweeping cotton waste and straw on spawn age. They concluded that when the fresh spawn was used to inoculate these composts, as well as blow gutter cotton gave higher yield followed by paddy straw and weaving cotton waste. The fresh spawn gave comparatively higher yield with blow gutter cotton waste than the older spawn (Iqbal *et al*., 1988).

In the study of Saghir (1998), the fresh weight of mushroom from each bed was recorded up to the third flush and the biological efficiency was calculated. Paddy straw supported the highest yield (450 g/kg dry straw, 45% BE). The BE values for water hyacinth, paddy straw + rice husk, banana leaves, sugarcane bagasse and areca nut husk were 35.2, 27.8, 22.6, 16.7 and 12.9% respectively. It was concluded that water hyacinth could be used as substitute for paddy straw in areas where it is scarce.

In general, mushrooms contain 90% water and 10% dry mater. Their nutritional value can be compared to those of eggs, milk and meat (Oei, 2003). The economic importance of the mushroom lies primarily in its use as food for human consumption. It is rich in Vitamin C and B complex and the protein content varies between 1.6 to 2.5%. The mineral salts required for the human body is also present in mushroom. Besides its nutritive value, its medicinal value for diabetics and cancer therapy has been emphasized (Sivrikaya *et al*., 2002).

Pleurotus species contain many types of minerals, such as high potassium to sodium ratio. This makes mushrooms an ideal food for patients suffering from hypertension and heart diseases. It is the source of extra ordinary power used in the preparation of many continental dishes and have medicinal properties like anti- cancerous, anti-cholesterol, anti-tumors. It is also useful against diabetes, ulcer and lungs diseases. It demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and economic way (Quimio, 1976).

The search has necessitated for alternative source of protein because the production of pulses has not kept pace with our requirement due to high population growth. Edible mushrooms are recommended by the FAO as food, contributing to the protein nutrition of developing countries dependent largely on cereals. Cultivation of oyster mushroom is the way that helps to increase source of protein. Culturing fungi on agricultural residues and

wastes from human food in the conversion of the materials are important economic terms (Mathew *et al*., 1987).

Many edible and inedible mushrooms in southern and eastern Asia are used for medicinal purposes for a long time. Most of them are used in medical practice today, especially the ganoderma species. Recently, 270 types of fungi are known to have beneficial properties (Ying *et al*., 1987). New techniques using sawdust mixed with various ingredients as a substrate for growing mushrooms under controlled environmental conditions have been developed (Yamunaka, 1995).

The objective of the current study was to determine the production of oyster mushroom by using laboratory techniques for cultivation. The growth performance was researched with locally available substrates, such as sawdust, olive waste, walnut wood waste (*Juglonus* spp.), vicia sativa weed, and their mixtures in different percentages. In addition, some chemical properties of mushrooms, such as carbon, nitrogen, carbon/nitrogen, protein, and type of carbohydrate were also determined using high performance liquid chromatography (HPLC). Fatty acid composition, antioxidant activity, total tannin concentration and inorganic contents were also tested with gas chromatography flame ionization detection (GC-FID), UV- spectrophotometer, X- Ray fluorescence, respectively.

2. LITRETURE REVIEW

2.1. *Pleurotus Ostreatus*

2.1.1. History and distribution

Pleurotus ostreatus is a common mushroom prized for edibility. It was widely distributed throughout the Northern Hemisphere, such as Europe, North Africa, Asia and North America (Singer, 1986). First cultivation was in Germany and it was grown commercially around the world for food. Then it was cultivated in many countries in sub-tropical and temperate regions. The oyster mushroom is widespread in many temperate and subtropical forests throughout the world, although it is absent from the Pacific Northwest of North America, being replaced by *P. pulmonarius* and *P. populinus* (Steve and Joe, 2009).

The standard oyster mushroom can grow in many places, but some other related species, such as the branched oyster mushroom, grow only on trees. While this mushroom is often seen growing on dying hardwood trees, it only appears to be acting parasitically. As the tree dies of other causes, *P. ostreatus* grows on the rapidly increasing mass of dead and dying wood. They actually benefit the forest by decomposing the dead wood, returning vital elements and minerals to the ecosystem in a form usable to other plants and organisms (Stamets and Paul, 2000).

2.1.2. Classification and morphological characteristics

Fungi comprise one of five kingdoms that make up the eukaryotic domain in the scientific classification system of living organisms (Carlile *et al*., 2001). *P. ostreatus* is well-defined group of Basidiomyceteous fungi. It belongs to Domain: *Eukaryotic,* kingdoms*; fungi,* Phylum; *Basidiomycota*, Class*; Agaricomycetes*, Order; *Agaricales* family *leurotaceae*, which are belong to the genus *Pleurotus* (Vilgalys and Sun, 1994). Although, 70 species of *Pleurotus* have been recorded, and new species are discovered more or less frequently. However, some of these are considered identical to previously recognized species (Inamulhaq *et al*., 2010).

Several closely related species of mushroom were commonly known as oyster mushrooms: *Pleurotus ostreatus, Pleurotus pulmonaris* and *Pleurotus populinus*. These three types were similar in many respects but were different enough to classify as different

species. Mating experiments and DNA analysis demonstrate these differences in the laboratory, while morphological and ecological differences were apparent in the field (Oecd, 2005).

Morphological description, species identification within the genus *Pleurotus* is difficult because of the morphological similarities and possible environmental effects. Mating compatibility studies have demonstrated the existence of eleven discrete inter-sterility groups in *Pleurotus* to distinguish one species from the others. *P. columbinus, P. florida, P. salignus,* and *P. spodoleucus* are the synonyms or subspecies taxa for the species of *P. ostreatus*. Fruit body color is an extremely variable characteristic in *Pleurotus* spp and depending on cultivation conditions. Fruit bodies of various colors, such as white, cream, brown, gray and blue gray, are produced (Eger-Hummel 1980; Li, 1980). *P. ostreatus*, Cap; 40-250 mm broad, oyster-shape, spatulate to lingulate when young, convex then later was becoming conchate to flabellate, surface smooth. Gills: crowded, whitish to cream or pale greyish, edge smooth, later somewhat undulating, lamellulae 1- or 3-tiers. Stipe: 10-20×10-25 mm, rudimentary, usually lateral, several co crescents, surface longitudinally striate (Oecd, 2005).

2.1.3. Life cycle of *Pleurotus ostreatus*

The life cycle of most mushrooms is the same or very similar but macroscopic and microscopic features are different, such morphological variations enable us to identify individual mushroom species.

The general life cycle of mushrooms start from a mature fruit body or basdiospore (Abate, 1998). Four basidiospores form at the end of each basidum on the gill of a fruit body. Each spore has one nucleus, spores germinate to become primary mycelia, and then form secondary mycelia by plasmogamy, and chances are 25% that a primary mycelium will meet with a compatible one, and the secondary mycelia of oyster mushroom can be distinguished by the clamp connections and each cell has two nuclei. Only secondary mycelia can produce fruit bodies under the proper conditions. In the basidia of a mature fruit body, the two nuclei fuse into one, then pass through meiosis, and produce four haploid nuclei. The four haploid nuclei are then made into four new basidiospores as shown in Fig. 2.1.

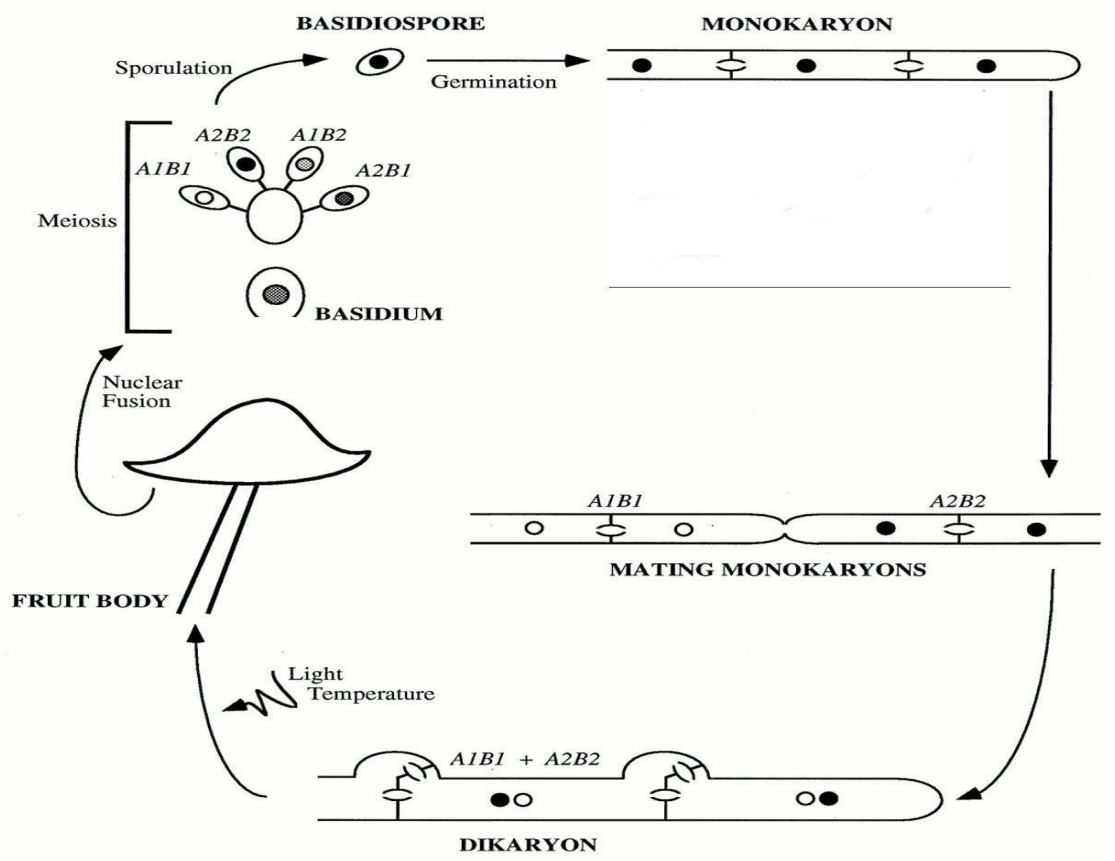

Figure 2.1: Life cycle of *Pleurotus ostreatus* (Casselton, 1995).

2.1.4. Growth requirements and conditions

2.1.4.1. Mycelia growth

The carbon sources suitable for mycelia growth are starch, glucose, fructose, maltose, mannose, sucrose, pectin, cellulose and lignin. Ethanol is also a source of carbon for mycelia growth; however, citrate, oxalate and other organic acids are not beneficial to the growth of the mycelium. The nitrogenous sources utilized by *Pleurotus* spp. are peptone, corn steep liquor, soybean cake powder, yeast powder, ammonium sulfate, asparagine, serine, alanine and glycine (Nwokoye *et al*., 2010).

The optimal temperatures for growth of the mycelium are around 25-28 °C and the range of pH is about 5.5 to 6.5. The tolerance of mycelia for carbon dioxide is rather strong. The mycelia of *Pleurotus* spp can still grow flourishingly at the carbon dioxide concentration of 15 to 20%. When the concentration of carbon dioxide is raise to 30%, the growth of mycelia rapidly decreases (Chang and Miles, 1989). The optimum pH value of

substrate for the cultivation of *Pleurotus ostreatus* is 6.5. The pH below 5 and above 7 hindered the mycelial growth (Kalita *et al*., 1977).

2.1.4.2. Fruiting body production

For fruiting body formation, CO_2, light and temperature are key environmental factors. When the CO_2 concentration in the mushroom house or growing bags is higher than 600 ppm (0.06%), the stipe elongates and the growth of the caps will be prevented. The requirements for light are different for the various stages of growth. The growth of mycelium does not need any light and cultivation of the oyster mushroom in a dark place is better than in a bright place (Oecd, 2005).

The formation of primordial and the growth of fruiting bodies require light. The former requires light of 200 lux intensity for over 12 hrs. The growth of the fruiting body requires light of 50 to 500 lux intensity. The color of the caps is closely related to the intensity of light, and if it is low then the color will be pale. The optimal temperature for the development of fruiting bodies was ranged from 10 to 18 °C; growers can choose a suitable strain for their own natural environment. Each *Pleurotus* species need different environmental conditions for fruit body development (Chang and Miles, 1989).

Like the cultivation of *Pleurotus* spp. and all white-rot fungi, several factors are critical for successful fruiting. Certain environmental conditions are required to cue the organism into the reproductive phase. Moisture, temperature, gas exchange, and light are involved in mushroom initiation and development. When choosing or creating a site for mushroom production, consideration of environmental conditions is important (Tisdale, 2004).

2.1.4.3. Moisture and temperature

Maximum humidity of 90 to 100% is recommended for optimal primordial formation. Once primordia have formed, humidity should be lowered to 85 to 90%. Ideally, humidity levels should be managed so that mushrooms are regularly certain contaminates. Inadequate moisture can prevent primordia formation and stunt fruit body growth (Kang, 2004).

Oyster mushrooms are able to cultivate and thrive in a wide range of temperature environments. Recommended temperatures are between 10 and 21 °C for development of

oyster mushrooms. Achieved successful fruiting of *P. ostreatus* with daily temperatures was ranging between 8 °C - 33 °C (Stamets, 2000).

For cultivation of *Pleurotus* species, cotton waste substrate was found to be superior to rice straw and banana leaf substrate, that temperature between 20 °C - 28 °C and relative humidity between 70-80% was ideal for its cultivation (Bano *et al*., 1979). Moreover, supplementation of substrate with cottonseed powder enhance yield up to 85%. Khan *et al*. (1980) indicated that the oyster mushroom (*Pleurotus ostreatus*) had six different temperature requirements.

2.1.4.4. Light

For forest-dwell mushroom, indirect natural light has been considered ideal for the formation of *Pleurotus* spp. fruit bodies. Although the mycelium of the oyster mushroom does not require light, proper fruit body formation requires moderate light. Too little or too much light can lead to discolored, malformed fruit bodies or the inability to fruit. Stamets (2000) found that oyster mushroom yield was maximized using light levels of 60 to 86 µmol/m^2/sec (300 to 430 lux) for twelve-hour, which the recommended levels are around 200 to 300 µmol/m^2/sec (1,000 to 1,500 lux) for commercial production.

2.2. General information about Oyster Mushroom (*Pleurotus ostreatus)*

2.2.1. Cellular properties of lignocellulosic materials

Lignin polymers are formed by precursor alcohols, which give rise to three main types of polymer units. All three related polymer types are referred to as lignin, but individually they are termed as guaiacyl units. Syringyl units are more resistant to degradation than the syringyl units, these units vary by wood type. In most gymnosperms, lignin is primarily composed of guaiacyl units, while lignin in angiosperms is approximately half guaiacyl and half syringyl units. Both wood types contain only small quantities of p-hydroxyphenyl units (Kirk and Farrell, 1987).

Only a small group of organisms, including the fungi, has developed the ability to degrade lignin. It is an aerobic process involving many specific enzymes. The decay rate of particular plant debris is set to be proportional to its lignin content. A mathematical formula, developed in 1980 calculates the bioavailability of a substrate based on its lignin

content (Chandler *et al*., 1980). An adaptation of this equation accommodating high lignin substrates was presented several years later and involves computing biodegradable carbon (C) nitrogen (N) ratio. In addition to the composition of the substrate itself, conditions that favor the decomposer (adequate N, temperature and moisture) are significant factors in lignin decomposition (Richard, 1996).

2.2.2. Degradation of wood by white rot fungi

White-rot fungi possess the ability to break down all major components wood. For the few organisms with this capacity, they are the most efficient and therefore the primary agents of lignin degradation in natural ecosystems (Buswell and Odier, 1987). The enzymatic breakdown of lignin allows access to the carbon and energy rich celluloses and hemicelluloses. The lignin destroying properties of *Phanerochaete chrysosporium* have been studied extensively for practical application in the paper industry. It serves as a model organism for the physiological, biochemical, and genetic factors related to lignin biodegradation. Using *P. chrysosporium*, optimal culture conditions for lignin degradation have been described and the highest degradation rates have been reported. When grown using wood pulp, *P. chrysosporium's* degradation rate was approximately 200 mg lignin per gram of mycelium per day (Yang *et al*., 1980).

Lignin degradation appears to be associated with the vegetative phase of fungal growth, while cellulose degradation is associated with fruit-body formation. Tan and Wahab (1997) found activity of lignin degrading enzymes increased in *P. pulmonarius* mycelium up until the formation of fruiting bodies, at which point activity of cellulose-degrading enzymes increased. The same pattern in enzyme production has been observed in *P. ostreatus* (Velazquez-Cedeno *et al*., 2002). Some reports suggest that substrates with high cellulose are favored by the white rot fungi *P. pulmonarius*, over those with high lignin contents (Sivaprakasam and Kandaswamy, 1981).

White rot fungi can degrade the syringyl units of lignin more effectively than the guaiacyl units. As mentioned earlier, the lignin of gymnosperms have considerably higher levels of guaiacyl units than that of angiosperms. The resistance of guaiacyl to degradation may explain why white rot fungi are found more commonly on angiosperm wood than on gymnosperms (Hatakka, 2004).

2.2.3. Physiology

Nutritional and cultural parameters do influence the physiology of lignin degradation by white rot fungi. The following parameters have been noted to affect lignin decomposition: appropriate substrate, oxygen availability, correct level of certain minerals and trace elements, and N level. Recent studies have revealed that many white rot fungi metabolize lignin in conjunction with cellulose and hemicellulose, and will only degrade lignin when the other energy sources are present. Molecular oxygen is essential for lignin degradation and increased O_2 levels resulted in heightened ligninase reactions. Several inorganic nutrients are closely associated with lignin degradation processes. Correct levels of Mn, Cu, Ca, Zn and Fe showed beneficial effects in lignin degradation. N-limitation was found to stimulate lignin degradation of *P. chrysosporium* (Kirk and Farrell, 1987). However, *P. ostreatus* and *L. edodes* degrade lignin at higher rates when grown in N-rich versus N-limited media (Kaal *et al*., 1995).

2.2.4. Wild and cultivation of *Pleurotus ostreatus*

Mushrooms growing in the wild have been found to be nutritious and important for medicinal purposes. During the rainy season, different species of both edible and non-edible species usually grow on various natural substrates, such as garden soil, decaying wood, termite nest, palm wastes, leaf litters, cocoa, tea, coffee and rubber plantations. It had been considered as rich food because they contain protein, sugars, glycogen, lipids, vitamins, amino acids and crude fibers. They also contain important minerals, which are required for normal body functioning (Gbolagade *et al*., 2006).

In most rural African village communities, indigenous edible mushrooms are highly treasured. Since they start growing soon after the first rains and become very handy vegetables long before the agricultural crops planted are ready for harvesting (Masamba and Mwale, 2010).

In the study of Meulen *et al*., (2004), different ratios of wheat bran, sugarcane and wheat, straw was used for cultivation of mushroom (*Pleurotus ostreatus*). Fungal growth on wheat stalk was better than the sugarcane. In addition, lignin degradation was faster on wheat straw grown with *Pleurotus ostreatus* mushroom. The addition of wheat bran to media has also increased the mushroom yield.

Kalita *et al*., (1997) studied the effects of some agro wastes as substrates for oyster mushroom (*Pleurotus sajor- caju*) cultivation in Assam. *Pleurotus sajor- caju* was grown in polyethylene bags with the following substrates: rice straw, rice straw + rice husk, water hyacinth, chopped banana leaves, betel nut and sugarcane bagasse. Spawn running was completed within 12-14 days on paddy straw + rice husk and areca nut husk, but it took longer on other substrates.

Jadhav *et al*., (1996) also studied the effects of different substrates on the yield of oyster mushroom. These substrates were wheat straw, paddy straw, stalks and leaves of maize and cotton, jowar, soybean straw, and groundnut creepers plus wheat straw. Cotton stalks and leaves were resulted the best yields as compared to the other substrates.

Baysal *et al*., (2003) studied the effects of waste paper supplemented with peat, chicken manure and husk rice (90+ l0; 80 +20 wzw) on spawn running, pin head, fruit body formation, and yield of oyster mushroom (*Pleurotus ostrearus*). The fastest spawn running (mycelia development) was observed at 15.8 days. While pin head formation (21.4 days), fruit body formation (25.6 days), and the highest yield (350.2 g) were observed with the substrate composed of 20% rice husk in weight. Increasing the proportion of rice husk within the substrate accelerated the spawn running, pin head and fruit body formation. So that, this outcome resulted an increment in mushroom yield. However, more peat and chicken manure had a negative effect on growing.

Ragunathan *et al*., (1996) researched the cultivation of three species of *Pleurotus* (*P. sajor-caju*, *P. platypus*and, *and P. citrinopileatus*) with mixtures of agro-residues, such as paddy straw, maize stover and sugarcane bagasse. Primordium initiation was observed on day 22 to 27 after spawning. Maximum yield was obtained in *P. sajor-caju* cultivated on paddy straw. Maximum yields of *P. platypus* and *P. citrinopileatus* were observed on coir pith and sugarcane bagasse, respectively.

In the study of Muhammad and Khan (1993), four strains oyster mushroom (*Pleurotus sp*.) was cultivated on cotton waste. Spawn running was the fastest in blue-gray strain of *P. ostreatus*. This strain gave the highest yield in the first and third flush. The highest yield for yellow strain of *sajor-caju*gave was in the second flush.

The biological efficiency, i.e. kg of fresh mushroom harvested from 100 kg of dry substrate, was 121.3% and 116.2% for *P. ostreatus* and *P. florida*, respectively. Lower mushroom yields were obtained from the two species when substrates composed of either

lower or higher percentages of legume wastes. Both dry matter and protein contents of the two mushroom species continued to increase with higher percentages of legume waste. However, *P. ostreatus* was more responsive than *P. florida* when rice straw substrate was enriched with legume waste. The highest mushroom yield was obtained with substrates composed of equal amounts of rice straw and legume waste with 5% gypsum; these yields were however lower than those obtained without gypsum addition. Clearly, the gypsum addition adversely inhibited the favorable effect observed with legume waste on both mushroom yield and quality (El-Kartan and Salama, 1996).

Bugarski *et al*., (2007) examined the chemical composition of oyster mushrooms grown on seven different substrates (wheat straw, soybean straw, corn stalks, sunflower stems, wheat straw 50% + soybean straw 50%, wheat straw 50% + corn stalks 50%, wheat straw 50% + sunflower stems 50%). Ash, protein, cellulose, fats, sodium, potassium, magnesium, iron, manganese, copper and zinc concentrations were examined. They concluded all the contents besides magnesium were affected by the type of substrate.

2.2.4.1. Importance of mushroom cultivation

The habitual use of mushrooms is well documented in several cultures and religions. They began to be used as food and medicine in 600 A.D. in Asia. At first, they were harvested in forests only, and sometime later began to be cultivated by man. Cultivation of edible mushrooms combines both skill and scientific technology in which agricultural wastes are recycled to produce a protein rich but cheap human food (Chang, 2008).

Interestingly, among commercially cultivated mushrooms, the oyster mushroom is the third most important mushroom in production in the world. The genus *Pleurotus* has gained much popularity world over and presently believed to be potential rival of commonly cultivated button mushroom (*Agaricus bisporus)*. In this genus, more number of species has been reported as cultivated than in any other edible fungus. *Pleurotus ostreatus* is the most cultivated species among the oyster mushroom. This species was first cultivated in the USA in 1900 and several other species of the mushroom such as *Pleurotus sajor-caju* were initially cultivated in India after the late of 1940s. Oyster mushroom is grown worldwide, and in China was the major producer. It has been regarded as one of the most profitable cash crops in Korea, accounting for 65% of total domestic mushroom production (Oecd, 2005).

Cultivated mushroom are generally saprophytes, utilizing substrate as primary or secondary decomposers. *Pleurotus oyster* is one of the four important wood inhabiting cultivated mushrooms in Japan, China together with *Lentinus edodes, Flammulina velutipes*, and *Pholiota nameko* (Chang and Hayes, 1978; Cohen *et al*., 2002).

The Chinese were pioneers in the development of fungi culture techniques, being shiitake them first mushroom produced, by using tree logs (Kues and Liu, 2000). Later, the culture spread to several countries of North America and Europe. Now a day the world production of mushrooms was around 3.4×10^6 tons in 2008, the largest producers being China, with 1.5×10^6 tons and the USA with 0.38×10^6 tons. Although there are more than 2000 species of edible mushrooms, nowadays only approximately 22 species are intensively cultivated (Manzi *et al.*, 2001). Among these only the champignon (*Agaricus bisporus*), the giant mushrooms (*Pleurotus ostreatus and Pleurotus ostreatoroseus*) and shiitake (*Lentinula edodes*) are the most cultivated ones and well appreciated (Carvalho *et al*., 2010).

2.2.5. Importance of *Pleurotus ostreatus* and other mushrooms

Oyster mushroom is the only one among mushrooms, which is called as "meat of the forest" due to its meat like taste and texture. It is consumed by local population and meets the requirements concerning the flavor and taste. Only a few species of this mushroom have been cultivated commercially. Mushrooms are liked all over the world due to their taste, flavor and health giving properties as a balanced food. Oyster mushrooms also contain considerable amount of phosphorus, potassium, copper and iron but low level of calcium.

The oyster mushrooms can also be used industrially for myco-remediation purposes. Both the Latin and common names refer to the shape of the fruiting body. The Latin *pleurotus* (sideways) refers to the sideways growth of the stem with respect to the cap, while the Latin *ostreatus* (and the English common name, oyster) refers to the shape of the cap which resembles the bivalve of the same name. Many also believe that the name is fitting due to a flavor resemblance to oysters. The name Oyster mushroom is also applied to other *Pleurotus* species, so *P. ostreatus* is sometimes referred to as the (Tree Oyster Mushrooms), (Stamets and Paul, 2000).

2.2.5.1. Nutritional value

Mushrooms have been used as food and medicine in many parts of the world since time immemorial. Although mushrooms are often grouped with vegetables and fruits, they are actually fungi. They are macro-fungi that belong either to Basidiomycetes or to ascomycetes and they are very distinct from plants, animals and bacteria (Mushigeni and Chang, 2001). It is evident that the growing interest in the cultivation of mushrooms can help in solving many problems of global importance such as protein shortage as well as improving the health and well-being of people. Mushrooms are valuable health foods that are low in calories and provide essential minerals (Masamba and Mwale, 2010). Mushrooms are highly nutritious and are important features of human diet worldwide, and it can be compared with eggs, milk and meat. The content of essential amino acids in mushroom is high and close to the need of the human body (Belewu, 2005; Oei, 2003).

Mushrooms are considered as food with delicious taste and high nutritional value because their contents (g/100 g) of protein (23.22), carbohydrate (63.17), phosphorus (104.13) and fiber (34.0) are high, and the amount of lipids (4.71) is low. High protein content of as much as 50% to 84% dry matter has been detected in the fruit bodies and mycelia of *P. ostreatus*. Their mycelia also contain amino acids like glycine, valine, threonine, serine, leucine, proline, methionine, asparagine, glutamine, lysine, arginine, histidine, cysteine and alanine (Nwokoye *et al*., 2010).

The fresh fruiting bodies of oyster mushroom, *Pleurotus ostreatus* includes a large quantity of moisture (90.8%), whereas fresh as well as dry oyster mushrooms are rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), fiber (8.7%), and ash (9.8%). While vitamins, such as thiamin (4.8 mg), riboflavin (4.7 mg) and niacin (108.7 mg), minerals like calcium (98 mg), phosphorus (476 mg), ferrous (8.5 mg) and sodium (61 mg) on 100 g dry weight basis are also appeared (Bhatti *et al*., 2007).

2.2.5.2. Medicinal properties

The fungus is widely used in Asian as a medicine to treat all types of diseases. Some mushrooms contain compounds, which can contribute to the general health of man. As mushrooms are widely distributed all over the world, some of them have been used in traditional medicine as anti-inflammatory, analgesics, homeostatic, diuretic, nourishment, antibiotic and anti-tumor agents (Chang, 1999; Shittu *et al*., 2005).

Most of the medicinal extracts from mushrooms are different forms of polysaccharides and all of them are strengtheners of the immune system with little or no side effects. For example, a sizofiran, antitumour polysaccharide extracted from the culture broth of *Schizophyllum commune* is an effective immuno-therapeutic agent for cervical carcinoma. It stimulates a rapid recovery of the immunological status impaired by radiotherapy (Oei, 1991). Some mushrooms are used for the treatment of gastric ulcer, duodenal ulcer and chronic gastritis. A good example is *Hericium erinacius*. Some mushrooms, such as *Tremella fulciformis* are used for curing leukaemia, coughing, phlegm and asthma of patients suffering from chronic bronchitis (Oei, 1996).

Recent studies on various *Pleurotus* spp possess a number of beneficial medicinal properties, such as anti-tumour, immunomodulatory, anti-genotoxic, anti-oxidant, anti-inflammatory,anti-allergic, hypo-cholesterolaemic, anti-hypertensive, anti-hyperglycaemic, antimicrobial and antiviral activities. These activities have been reported for various extracts and isolated compounds, such as polysaccharides, polysaccharide-protein complexes, proteoglycans, proteins and DNA from oyster mushroom fermentation broth, mycelia or fruiting bodies. In particular, polysaccharides appear to be potent antitumor and immuno-modulating substances, besides possessing other beneficial activities (Gregori *et al*., 2007; Khan *et al*., 2011).

Various bioactive compounds isolated from culture extracts of Ethiopian higher fungi showed other biological properties, such as anti-protozoal, anthelmintic, phytotoxic and brine shrimp lethality activities (Dange and Abate, 1995). *P. ostreatus* has been used for disease management of patients with HIV and AIDS (Anonymous, 2007). The folic acid present in oyster mushrooms helps to cure anemia. It is suitable for people with hypertension, obesity and diabetes due to its low sodium: potassium ratio, starch, fat and calorific value. Alkaline ash and high fiber content make them suitable for consumption for those having hyperacidity and constipation and cholesterol inhibitors mushrooms (Sonali and Randive, 2012).

In the last 30 years, many scientific studies instead of old applications have been introduced new application forms in Japan, Korea and China (Wasser and Weis, 1999). In particular, these fungi caused to increase the factors for immunology, anti-cancer properties, anti-oxidant, anti-hypertensive, liver protection activity, anti-fibrotic, anti-diabetic, antiviral, antimicrobial properties and cholesterol-lowering efficacy (Jong and Donovic, 1989).

Pleurotus species are rich sources of proteins and an abundance of essential amino acids, minerals (Ca, P, K, Fe, Na) and contain vitamins C, B-complex – thiamine, riboflavin, niacin and folic acid (Kalač and Svoboda, 2000; Isiloglu *et al*., 2001; Sánchez, 2004; Çağlarırmak, 2007; Regula and Siwulski, 2007). Oyster mushroom (*Pleurotus ostreatus)*, is recommended in numerous countries as an addition to the daily diet due to its documented probiotic properties and relatively high nutritive value (Wang *et al*., 2000; Smith *et al*., 2002; Florczak *et al*., 2004; Bernaś *et al*., 2006).

Several reports showed that coffee has a great potential for cultivation of different types of mushrooms. It has been established that treated coffee husk is suitable for the growth of *L.edodes* (Fan *et al*., 2000).

Soccol *et al*., (2006) demonstrated that the strain of *Pluerotus ostreatus* LPB 09 was capable of producing the fruiting body on coffee husks. Even in the presence of high concentrations of caffeine and tannin in the medium, it was shown that toxic compounds decrease and the protein concentration increase after cultivation of *Pluerotus ostreatus* (Fan *et al*., 2003).

The feasibility of using the treated coffee husk suitable for the cultivation of *L. edodes* LPB 02 in solid state cultivation was demonstrated that the strain of *Pluerotus ostreatus* LPB 09 was capable of producing the fruiting body on coffee husks, even with high concentrations of caffeine and tannin in the medium (Fan *et al*., 2000).

Pleurotus species contain high ratio of potassium to sodium, which makes mushrooms an ideal food for patients suffering from hypertension and heart diseases. The practice of mushroom cultivation not only produces medicinal and nutritious food but also improves the straw quality. This takes place by reducing lignin, cellulose, hemicelluloses, tannin and crude fiber content of straw. This is ideal for animal feed, industrial sawdust and wood waste (Ortega *et al*., 1992).

2.2.5.3. Waste recycling and using as a substrate

Most of the fungi have strong enzymes and are capable of utilizing complex organic compounds, which occur as agricultural wastes and industrial by products. Mushroom fungi also belong to this group. Thus, agricultural wastes can also be used as a bedding material for mushroom cultivation (Baysal and Peker, 2001).

The oyster mushroom, *Pleurotus* spp. is cultivated in many countries both in sub-tropical and temperate regions of the world. Like other edible mushrooms, *Pleurotus* species can be grown on various agricultural waste products without addition of enrichment materials (Rajapakse, 2007). An attractive feature of this mushroom is due to the capacity of secreting spectrum of enzyme that can utilize a large variety of agricultural waste products containing lignin, cellulose, starch, sugars and fermented proteins. In addition, they can transform the lignocellulosic biomass into quality of food and flavor (Baysal and Peker, 2001).

The importance of edible mushrooms has increased due to the advances in cultivation technology, which makes the use of agricultural and industrial residues possible by recycling them as substrates (Pandey *et al*., 2000). Moreover, they represent an excellent alternative for discarding several residues, helping in reducing pollution caused by the presence of these materials in the environment (Carvalho, 2010).

Large volumes of lignocellulose agricultural residues (fish waste, vegetable materials) are generated annually through agricultural and food processing industries. These are disposed of either by burning or dumping in landfills, thus posing hazard to the environment and human health. However, these residues would be used for cultivation of edible and medicinal mushrooms (Atipko *et al*., 2008).

Mushroom spent can be used as an organic fertilizer. The spent is made of degraded and non-degraded plant polymers. Spent from already composted substrates, such as *Agaricus bisporus* could be applied directly to the gardens and horticulture plants (Abate, 1998).

Spent after mushroom cultivation on different substrates, such as cottonseed waste, cereal straw and sugarcane can also be served as animal feed supplements (Abate, 1998). The lignocellulosic substrate can be used for mushroom production, which left after harvesting of the mushrooms. It should be noted that this compost besides being rich in nitrogenous material contains partly degraded lignocelluloses components. When it is combined with animal dung or human excreta in a biogas digest, it would yield not only biogas but also a good quality organic nitrogenous fertilizer in the form of sludge. The sludge from the biogas plant as a nitrogenous fertilizer is far more beneficial than the compost from where it was derived. Part of the biogas that is produced near the mushroom house can also be conveniently used for pasteurization of the mushroom bed material (Chang, 2004; Chang, 2007).

2.3. Chemical Composition

2.3.1. Source of carbon and nitrogen

The nutrient requirements of *Pleurotus ostreatus* mushroom fruit bodies contain carbon, nitrogen and inorganic compounds. The main nutrients are carbon sources, such as cellulose, hemicellulose and lignin. Oyster mushrooms (*Pleurotus ostreatus*) require less nitrogen and more carbon source. Thus, most organic matters containing cellulose, hemicellulose and lignin can be used as mushroom substrate. Sawdust and industrial wastes however demanded each nutritional source differs for mushroom species and substrates used. Sawdust and industrial wastes are the principal substrate for oyster mushroom cultivation. Kumari and Achal (2008) cultivated *P. ostreatus* on different substrates and reported the highest yield on wheat straw, followed by the combination of paddy and wheat straw.

In addition, Yildiz *et al*., (2002) found that when weed plants were mixed with rice straw in 1:1 ratio, there was increase in yield than when it was used individually. However, our results are not consistent with the results of Das and Mukherjee (2007). Sawdust and sugarcane bagasse also contain high amount of lignin. Low degradation of lignocellulosic substances of sawdust by *P. ostreatus* might be another factor affecting the overall low yield values from sugarcane bagasse and sawdust.

2.3.2. High performance liquid chromatography (HPLC)

It is also known that High Pressure Liquid Chromatography is a form of Column Chromatography (CC) used in analytical chemistry as well as in biochemistry to separate, identify and quantify compounds. Chemical characteristics have been increasingly employed in taxonomy during the last two decades, and it is confirmed the distinctiveness of the chemistry of different type of hydrocarbohydrate (Wink and Waterman, 1999). Sugarcane bagasse contains cellulose and sucrose, which are easily degraded by oyster mushroom. Even though it is rich in cellulose, sucrose and nitrogen, the yield is found to be low in our experiment in comparison to other substrates. Furthermore, Taurachand (2004) reported that the natural substrates (woods on which *Pleurotus* species grow) were very poor in nitrogen content. Mushroom in paddy straw was easy due to getting sugars from the cellulosic substances (Wink and Waterman, 1999).

2.3.3. Gas chromatography flame ionization detection (GC-FID)

Fat and fatty acids especially polyunsaturated ones contribute to important aspects of fish, meat, and plant products' quality and are critical for their nutritional and sensory value. Therefore, it is required to improve methods for the separation of total lipids into their neutral and polar fractions and for the analysis of their fatty acids (FA). Neutral lipid FA composition of animal and marine fat is strongly associated with their diet, whereas polar lipids regulate the function of membrane cells. Iatroscan is an instrument that combines thin-layer chromatography (TLC) resolution efficacy with the capacity of quantification by flame ionization detection (FID) (Shantha, 1992; Estévez and Helleur, 2005)

Lipid oxidation leads to loss of quality in food and is one of the most important problems. This reaction can be found in foodstuffs and oxygen by unsaturated lipid reaction. Lipid oxidation process is thermodynamically favored, although the direct reaction between oxygen and polyunsaturated lipids are blocked. Therefore, activation for initiating the free radical reactions is energy required (Irwin and Hedges, 2004).

Lipid oxidation occurs in three stages: the beginning, development and the end. This reaction begins with light, temperature, heavy metal ions and oxygen exposition. The final step includes the release of hydrogen from fatty acids (RH). Thus, free radicals occur. After the formation of active radicals, binding of molecular oxygen to form active radicals and active peroxy radicals are developed. The first oxidation product is hydroperoxide (Irwin and Hedges, 2004).

There have been sufficient data to make generalization of essential fatty acids and their distribution. A peroxy radical that initiates lipid peroxidation root is not very long lasting. Bio-membranes and intracellular organelles (mitochondria, endoplasmic reticulum, etc.) of unsaturated fatty acids in membrane phospholipids are susceptible to oxidative attack due to the presence. Reactive oxygen species and cell lipid damage bringing free radicals and lipid peroxides are also formed (Irwin and Hedges, 2004).

2.3.4. Antioxidant composition

Antioxidant reactions called "free radical reaction or auto oxidation" are named and triggered to initiate the chain reaction (initiator initiator) factors. In the last step of the reaction, which is unstable compounds hydroperoxides, aldehydes, ketones, acids,

hydrocarbons and acids, such as epoxy oxidation products are broken. These cleavage products are specific to the rancid smell of oil and flavor (Pokorny *et al*., 2001; Kayak, 2003; Old, 2004).

Compared to other methods, DPPH free radical binding method covers the antioxidant activity as soon as it is determined to ensure relativity. Scavenging antioxidants also connect the effects of free radicals (Wang *et al*., 1998).

2.3.4.1. Phenolic compounds

Phenolic compounds are widely distributed in the plant Kingdom and are important in fruits because they are responsible for the color and flavor of the fresh fruit and processed products (Lee and Jaworski, 1987). They are particularly important products in ecology and contribute to the organoleptic nature of wines (flavors, astringency, and hardness). They are also important in chemotaxonomy and plant pathology (Boudet, 2007). Thirty eight phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. In excess of 8000 phenolic structures have been reported and they are widely dispersed throughout the plant kingdom and many can occur in food (Crozier *et al*., 2009). Phenolics range from simple, low molecular weight, single aromatic ring compounds to the large and complex tannins and derived polyphenols. They can be classified by the number and arrangement of their carbon atoms and are commonly found conjugated to sugars and organic acids. Phenolics occurring naturally in healthy plant tissue can be classified into two groups: the flavonoids and the non-flavonoids. Traditionally processed foods and beverages, such as black tea, matured red wine, coffee and cocoa may contain phenolic transformation products that are best described as ‘derived polyphenols (Dai and Mumper, 2010).

3. MATERIALS AND METHODS

3.1. Material

The experiment was conducted in the laboratory of Kahramanmaraş Sütçü İmam University Forest Industrial Engineering Laboratory. The substrates, which used for cultivation, are poplar sawdust sourced from Wood Company in Kahramanmaraş, olive wastes collected from Remzibey Company, walnut wood wastes collected from Kahramanmaraş strong box product, and vicia sativa weed supplied by a farm.

3.2. Methods

3.2.1. Preparation of pure culture and growing media

Suitable culture containing 20 g Agar-Agar and 16 g Malt-Extract agar was prepared to grow mycelia, and then was sterilized malt-agar medium in autoclave at 121°C for 1 hr. Then, the antibiotic was applied to growing pure plate cultures. It was then kept for three weeks in an incubator under 25°C for sufficient growth.

Spawn was formed from wheat grain seeds, which were cleaned and submerged by using water for 6 hr. Then, water was removed and wheat grains were subsequently packed in to polyethylene bags and sterilized in an autoclave at 121^{o}C for 20 min. After sterilized, the bags were inoculated with actively growing mycelium of *Pleurotus ostreatus* from petri dishes and incubated under 25^{o}C for 15 days, until the mycelium fully covered the grains (Fan *et al*., 2000)

For cultivation of *Pleurotus ostreatus* mushroom, poplar sawdust was used as a control sample. Based on Stamets (1993) recommendation, olive wastes, walnut wood wastes and vicia sativa were prepared by the ratio of 3:1, 1:3 and 1:1 respectively. All samples were mixed, and mixture was putted 1 kg in the plastic bags. Each treatment contained 10 plastic bags.

3.2.2. Inoculation and incubation

Most of the substrates require sterilization or pasteurization before using them for cultivation. Currently this is not recommended since it kills the beneficial microorganisms, and breaks down organic substances into the forms that are more favorable for the growth of contaminants (Quimio *et al*., 1990; Oei, 1996).

For destroying harmful organisms in all of the samples, the autoclave was used at 121 °C for 90 min. Then samples were put into a sterile cabinet. Fifty g of wheat grain was added and then stirred with compost for helping the immunization procedure in the cabinet hood. This step was carried out in the incubator, which placed in room temperature (25 °C) with 70-80% moisture content.

During spawn, running temperature in growth room was at 25 ºC. Edible mushrooms grow in 80-90% moisture, which is critical during the fruiting stage. After spawn, the room temperature should be between 16-18 ºC with the same humidity (Royse, 2002).

3.2.3. Substrate analysis

After the spawn and harvesting period, the pH and moisture (%) were determined. The substrates were dried in an oven at 65 ºC, and then ground by milling machine. The substrate was sieved by 1 mm diameter sieve. Sieve extraction was analyzed for carbon, nitrogen, carbon: nitrogen and protein ratios.

3.2.3.1. Determination of pH

Ten grams of each substrate was sampled and mixed with 100 mL of distilled water for 1.5 hr and then the pH was recorded.

3.2.3.2. Determination of moisture

The total moisture was determined by drying the both substrate and mushroom to a constant weight at 105 °C (Madan *et al*., 1987).

$$R\ (\%) = \frac{R2\text{-}R1}{R2} \times 100 \qquad (3.1)$$

where,

R is moisture percentage,

$R1$ is dry weight (g),

$R2$ is wet weight (g).

3.2.3.3. Determination of total nitrogen

Total N was analyzed using the Kjeldahl digestion (with 0.1*N* H_2SO_4), distillation and titration method as described by Bremner (1996).

3.2.3.4. Determination of Carbon

The carbon content of the substrates was determined by ash method (AOAC, 1990).

3.2.3.5. Determination of C/N ratio

C/N ratio was determined according to Yildiz and Karakaplan (2003).

3.2.4. Determination of spawn and harvesting time

The spawn data were collected daily if appearance of cultivation in substrate has 100% growth mycelia. In addition, harvesting time from spawn to maturation of fruiting bodies was recorded in all the substrates.

3.2.5. Total yield

The total mushroom yield was done every day in the experiment by weighing the products separately (g/bag).

3.2.6. Biological efficiency

The total weights of all fruiting bodies harvested from all three pickings were measured as total mushroom yield. The biological efficiency (BE – accumulated fresh weight of mushrooms expressed on the basis of dry weight of initial substrate) was calculated by the formula given by Mintesnol (2014).

$$BE\ (\%) = \frac{M.A}{S.A} \times 100 \qquad (3.2)$$

Where,

BE is percentage of biological efficiency,

M.A is mushroom weight (g),

S.A is dry weight of substrate (g).

3.2.7. Morphological measurements

3.2.7.1. Weight of mushrooms

Each mushroom body (stalk + cap) was weighed using digital balance and expressed as gram.

3.2.7.2. Cap length, Stalk length and Stalk diameter

Digital calipers (150 mm) were used to measure wide and narrow part of the cap, mushroom's stem width, and distance between the cap handle and surface.

3.2.7.3. Dry matter

Fresh mushrooms were weighed and placed in to the oven at 105 °C to get the constant weight. After that, the measurement was repeated to get the average between them.

3.2.7.4. Protein analysis

The nitrogen value was multiplied by the standard factor of 6.25.

3.2.8. Chemical analyses of *Pleurotus osteratus* mushroom

3.2.8.1. Extraction and determination of total tannin *Pleurotus osteratus*

Extraction solution was prepared by mixing 0.05 g of Fe_2SO_4, 95 ml *N*-butanol and 5 mL HCl 35% .Then, weighed 0.01 g form both mushroom sample and mimosa tannin, put in small plastic tube, and added 10 mL of the extraction solution and placed in water bath for heated during 1 hr. Finally, 1 mL from extraction solution was taken and placed into a spectrophotometer quartz cuvette and read at 541 nm wavelength (Makkar *et al*., 1995).

3.2.8.2. Sugar analysis by high performance liquid chromatography (HPLC)

Liquid chromatography of the extracts was carried out on a Shimadzu HPLC system with LC-20AD pumping system and a RID detector. The column used was Benson BP-100Pb^{++} (300 x 7.8 mm). The mobile phase was performed with de-ionize water. The flow rate was 0.4 mL/min. The chromatographic column was washed with the initial

conditions to stabilize for 10 minute. The concentrations of each compound were reported on mg/kg and percent amount based on total oven-dry *Pleurotus ostreatus* mushroom. In Table 3.1. HPLC operation condition is presented.

Table 3.1. Operating system of HPLC condition.

Properties	Conditions
Fluent	DI H_2O
Flow Rate	0.4 ml/min
Pressure	340 psi
Detector	RID
Temperature	80 °C
Sample Volume	20 µl
Column Type	Benson BP-100Pb^{++} (300 x 7.8 mm)

3.2.8.3. DPPH radical scavenging activity

This assay was performed by following the standard method of Blois (1958) with several modifications. The antioxidant activity of the mushroom extracts and the was assessed based on the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH)-free radical activities by Blois method. The diluted working solution of the test extracts was prepared in methanol. (BHT)-butylated hydroxytoluene was used prepared in methanol and 1 mL of this solution was mixed with 1 mL of sample solution and standard solution separately (Alma *et al*., 2003). These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm by using UV-Spectrophotometer. Methanol (1 mL) with DPPH solution (0.008%, 1 mL) was used as blank. The optical density was recorded and percentage inhibition was calculated using the formula given below (Bors *et al*., 1992)

$$\text{Inhibition of DPPH activity \%} = \frac{A\text{-}B}{A} \times 100 \qquad (3.3)$$

Where,

A is optical density of the DPPH,

B is optical density of the sample, and BHT.

3.2.8.4. Fatty acid analysis by gas chromatogram (GC-FID)

The lipid analysis was performed based on the fatty acid extracts. The Shimadzu GC 2025 was used as an instrument after the fatty acids were converted to methyl esters. For this analysis, 60 m long, 0.20 mm inside width with a film with thickness of 25 microns to (teknokro) dishes column (TRCN100) was used. Column temperature during the analysis from 80 to 240 °C, The temperature injection was 240 °C and the temperature of detector was kept in 250 °C. The column temperature program was 140 °C up to 80 °C was adjusted to 3 °C/min. 1 min to 240 °C as expected 140 °C we get it finally. After waiting 5 min we get the results. With 3 °C/min I hr 61 min., Helium gas used as a transferring gas in the method. During the analysis of examples of the fatty acid connections, methyl esters prior to analysis, the mixtures according to the standard fatty acid methyl esters by injecting each of a fatty acid retention times were determined. After this operation by making the necessary programming of examples of mixtures of fatty acid methyl ester analysis was performed. Results in the total fatty acids amount of each fatty acid was determined as a percentage, calculations were performed using GC Solution 2.3 program.

Table 3.2. GC-FID operating system conditions for lipid analysis

Properties	Conditions
Fluent	Helium gas (He)
Flow rate	3 °C/min
Length	60 m
Width	0.2 mm
Thickness	25 mic
Temperature	80-240 °C
Temperature of injection	240 °C
Colum	TRCN 100

3.2.8.5. Inorganic Content

Mineral content or macro organic compound of the samples was determined by X – Ray fluorescence wavelength dispersive equipment.

3.2.9. Statistical analyses

Statistical analyses were performed for all data by using statistical package (SPSS) program (SPSS, 1999). The differences between means were analyzed by Duncan comparison test (1955). Duncan mean separation test was also performed for homogeneity with ANOVA. Mean differences were classified as significant ($P< 0.05$) or non-significant ($P> 0.05$).

4. RESULTS AND DISCUSSION

4.1. pH changes of *Pleurotus ostreatus* at different stages

Table 4.1 illustrates the effect of various substrates on pH and moisture contents of cultivated *Pleurotus ostreatus*. The initial pH of all substrates before inoculation of the fungi was between neutral and acidic.

Table 4.1. pH and moisture contents of *Pleurotus ostreatus* cultivated on various substrates

Substrates	pH			Moisture (%)
	Cultivation	After Spawn	Harvesting	
100% PS	7.22	4.12	4.84	71.10
100% OW	6.69	5.38	5.15	44.01
50% PS+50% OW	6.78	3.98	4.46	61.14
45% PS+45% OW+10% VSW	5.97	4.30	4.98	59.11
100% W	6.51	4.92	4.74	64.49
50% PS+50% W	6.88	4.22	4.47	67.83
45% PS+45% W+10% VSW	5.87	5.15	5.20	63.57
75% PS+25% OW	6.75	4.47	4.71	65.83
70% PS+20% OW+10% VW	6.26	4.18	4.92	62.11
75% PS+25% W	6.67	4.21	4.66	67.84
70% PS+20% W+10% VSW	7.27	3.72	4.63	64.09
40% PS+40% OW+20% VSW	6.06	5.38	6.73	53.32
40% PS+40% W +20% VSW	6.22	5.20	5.26	62.67

The pH changed at different stages. The highest pH was observed on 70% PS + 20% W + 10% VSW substrate (7.27), while the lowest pH was 5.87 on 45% PS + 45% W + 10% VSW substrate. After spawning, the maximum pH was 5.38 on 40% PS + 40% OW + 20% VSW and 100% OW substrates. At this stage, the lowest pH was observed for 70% PS + 20% W + 10% VSW substrate. At harvesting stage, the highest pH was 6.73 on 40% PS + 40% OW + 20% VSW. The maximum and the minimum moisture contents were observed on 100% PS and 100% OW substrates (71.10 vs. 44.01%) (Table 4.1.).

In the present study, pH was higher for poplar sawdust substrate, which also showed high degradation. These pH changes were different in harvesting mushrooms, this may be due to dissimilar substrates (40% PS + 40% OW + 20% VSW, 40% PS + 40% W +

20% VSW). In another study (Kalita *et al*., 1977), the optimum pH of substrate for cultivation *Pleurotus ostreatus* was 6.5. The pH below 5 and above 7 hindered the mycelial growth (Kalita *et al*., 1977). The moisture contents were lower than 80%. Ideally, moisture content should be between 60 and 80% for good yield besides the other factors. These results show that the agro wastes have different absorptive properties and this may affect the mycelia produced in each substrate. Ahmad (2010) also showed pH between 6.0 and 6.6 when they used 100% cotton.

4.2. Nutritive value of *Pleurotus ostreatus* cultivated on various substrates

Table 4.2 illustrates the nutritive value of *Pleurotus ostreatus* cultivated on various substrates. The maximum nitrogen and carbon contents were observed on 100% OW substrate (2.90 and 43.99%). However, the minimum nitrogen and carbon contents were 0.81 and 12.86% on 100% W substrate. The highest carbon / nitrogen ratio was 22.24 on 100% PS substrate, while the minimum carbon / nitrogen ratio was 15.17 on 100% OW substrate (Table 4.2.).

Table 4.2. Nutritive value of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Nitrogen (%)	Carbon (%)	Carbon/Nitrogen	Protein (%)
100% PS	0.94	20.91	22.24	5.88
100% OW	2.90	43.99	15.17	18.13
50% PS+50% OW	1.92	32.45	16.9	12.00
45% PS+45% OW+10% VSW	1.97	31.98	16.23	12.31
100% W	0.81	12.86	15.88	5.06
50% PS+50% W	0.88	16.89	19.30	5.47
45% PS+45% W+10% VSW	1.03	17.97	17.45	6.44
75% PS+25% OW	1.43	26.68	18.66	8.94
70% PS+20% OW+10% VSW	1.48	26.21	17.71	9.25
75% PS+25% W	0.91	18.90	20.77	5.69
70% PS+20% W+10% VSW	1.06	19.99	18.86	6.63
40% PS+40 % OW+20% VSW	2.02	31.51	15.60	12.63
40% PS+40% W+20% VSW	1.18	19.06	16.15	7.38

In our study, carbon/nitrogen ratio increased until the end of mycelia growth, then decreased at the end of the harvesting time. However, carbon / nitrogen ratio increased maybe due to carbon and nitrogen contents of substrates. The reduction in nitrogen was

higher than the carbon. Carbon / nitrogen ratio has led to increase mycelia growth at the end of this stage. Moreover, the amount of protein content depends on substrate. For this reason, 100% olive wastes, 40% PS + 40% W + 20% VSW and 40% PS + 40% OW + 20% VSW substrates did not result in any product.

Ashraf *et al*., (2013) studied the effects of various substrates on mycelia growth and found that the growth rate was remained relatively low. The maximum nitrogen was 0.76% on 100% cotton waste, while the minimum nitrogen was 0.37% on 75% cotton waste + 25% maize leaves. In addition, the maximum protein percentage was 27.23 when cotton wastes and wheat straw were used as a substrate. Additionally, carbon/nitrogen ratio was lower than 23% and the mycelia growth rate varied between 23 and 45% (Vane *et al*., 2006).

4.3. *Pleurotus ostreatus* mushroom spawn time

Figure 4.1, depicts spawn time of *Pleurotus ostreatus* cultivated on various substrates. The maximum spawn time was 31 days on 45% PS + 45% W + 10% VSW and 70% PS + 20% OW + 10% VSW substrates. However, the minimum spawning running time was 18 days for 45% PS + 45% OW + 10% VSW substrate. In other study (Khan *et al*., 2010), the required minimum spawning time was 17.50 days when substrates contained 50% sawdust + 50% wheat straw. In addition, the maximum spawning time was recorded when 50% rice husk + 50% wheat straw was used (23.75 days). These results are somewhat agree with our study (Tan, 1981).

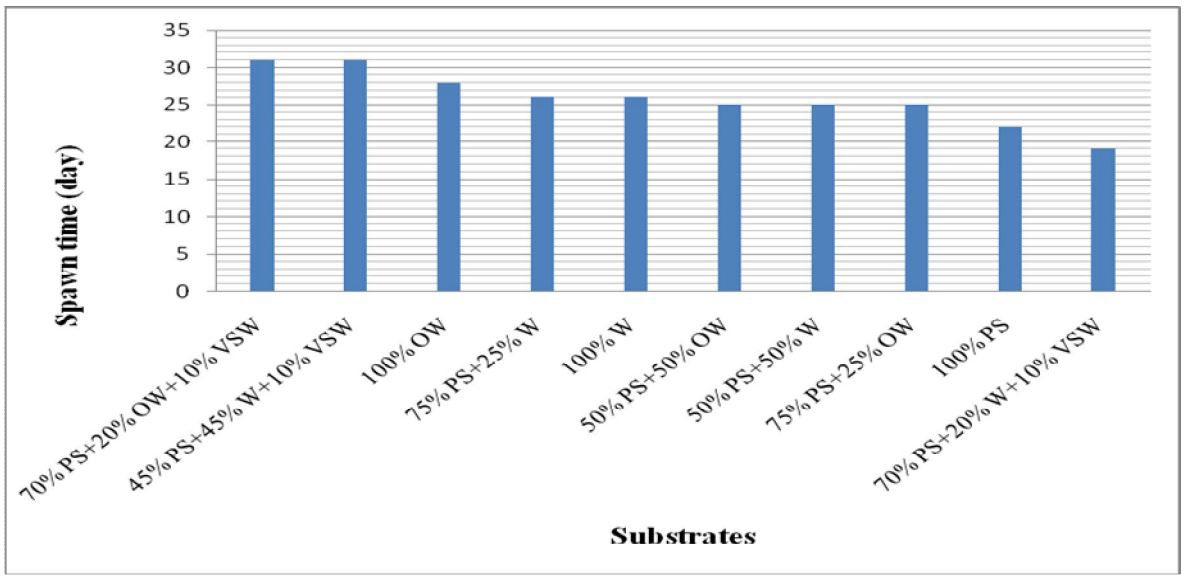

Figure 4.1. Spawn time of *Pleurotus ostreatus* cultivated on various substrates.

4.4. Harvesting time of *Pleurotus ostreatus* mushroom

The harvesting time of *Pleurotus ostreatus* grown on various substrates are presented in Table 4.3. There were statistically significant differences between the harvesting times of all substrates ($P \leq 0.05$).

Table 4.3. Harvesting time of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Harvesting Time (day)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	20	45.25 abc *	25.09	16	99
50% PS+50% OW	31	39.23 abcd	19.05	21	81
45% PS+45% OW+10% VSW	29	33.34 cd	12.87	23	57
100% W	22	33.73 bcd	23.97	21	96
50% PS+ 50% W	25	49.20 ab	31.47	21	95
45% PS+45% W+ 10% VSW	13	42.54 abcd	11.57	29	74
75% PS+25% OW	28	50.11 a	25.19	26	115
70% PS+20% OW+10% VSW	22	45.96 abc	15.56	20	67
75% PS+ 25% W	18	46.61 abc	22.12	22	76
70% PS+20% W+10% VSW	11	28.73 d	7.64	21	42

* Same letter shows no significant difference between groups

The maximum mean harvesting time was 50.11 days on 75% PS + 25% OW substrate. While the minimum mean harvesting time was 28.73 days on 70% PS + 20% W + 10% VSW substrate. Moreover, the 100% PS, 70% PS + 20% OW + 10% VSW and 75% PS + 25% W substrates had the same harvesting time.

Earnshaw *et al*., (2012) found that the maximum and minimum mean harvesting times varied when 100 % wheat bran was used as a substrate (85 to 60 days). These results are agreed with our study.

4.5. Total time during spawning and harvesting period

The total time of *Pleurotus ostreatus* grown on various substrates are given in Table 4.4. There were statistically significant differences between the total times of all the substrates ($P \leq 0.05$).

The minimum mean total time for spawning and harvesting stages were 47.73 days for 70% PS + 20% W + 10% VSW substrate. While the maximum mean total time was 76.95 days for 70% PS + 20% OW + 10% VSW substrate. Moreover, the 50% PS + 50% W 45% PS + 45% W + 10% VSW, 75% PS + 25% OW and 75% PS + 25% W substrates had the same total time for spawning and harvesting stages.

Table 4.4. Total time for spawning and harvesting stages of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Total Time (day)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	20	67.25 ab*	25.09	38	121
50% PS+50% OW	31	64.23 abc	19.06	46	106
45% PS+45% OW+10% VSW	29	51.35 cd	12.87	41	75
100% W	22	59.73 bcd	23.97	47	122
50% PS+50% W	25	74.2 ab	31.47	46	120
45% PS+45% W+10% VSW	13	73.54 ab	11.57	60	105
75% PS+25% OW	28	75.11 ab	25.19	51	140
70% PS+20% OW+10% VSW	22	76.95 a	15.56	51	98
75% PS+25% W	18	72.61 ab	22.11	48	102
70% PS+20% W+10% VSW	11	47.73 d	7.64	40	61

*Same letter shows no significant difference between groups

4.6. Total yield of *Pleurotus ostreatus*

The total yields of *Pleurotus ostreatus* grown on various substrates are presented in Table 4.5. There were statistically significant differences between the total yields of all substrate ($P \leq 0.05$).

Table 4.5. Effect of various substrates on total yield of *Pleurotus ostreatus* cultivation.

Substrates	Total Yield (g)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	7	26.02 d*	19.39	4.32	64.04
50% PS+50% OW	8	43.00 bcd	27.71	8.90	74.30
70% PS+20% OW+10% VSW	6	83.99 ab	46.76	20.44	164.89
100% W	7	38.24 bcd	26.88	7.71	70.66
50% PS+50% W	8	38.01 bcd	33.05	6.23	90.98
70% PS+20% W+10% VSW	3	107.49 a	69.73	31.00	167.52
75% PS+25% OW	8	36.25 cd	22.06	11.43	70.53
75% PS+20% OW+10% VSW	5	103.04 a	53.53	40.98	147.53
75% PS+25% W	8	23.33 d	15.60	3.86	56.54
70% PS+20% W+10% VSW	4	80.44 abc	29.06	43.72	108.39

*Same letter shows no significant difference between groups

The maximum mean yield was observed on 70% PS + 20% W + 10% VSW substrate (107.49 g), while the minimum mean yield was 26.02 g on 75% PS + 25% W substrate. Moreover, the 50% PS + 50% OW W, 100% W, 50% PS+50% W, 70% PS + 20% W + 10% VSW and 75% PS + 20% OW + 10% VSW substrates had the same total yield.

In a study of Sharma *et al*., (2013), the maximum yield was observed on 100% rice straw as a control (381.81 g). While the minimum yield was 247.87 g on 100% sawdust.

4.7. Biological efficiency of *Pleurotus ostreatus*

The biological efficiency of *Pleurotus ostreatus* grown on various substrates is presented in Table 4.6. There were significant differences between the biological efficiencies of all substrates ($P \leq 0.05$).

Table 4.6. Biological efficiency of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Biological Efficiency (%)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	7	9.00 cd*	6.71	1.49	22.16
50% PS+50% OW	8	11.06 bcd	7.13	2.29	19.12
70% PS+20% OW+10% VSW	6	20.54 abc	11.43	5.00	40.32
100% W	7	10.77 bcd	7.57	2.17	19.90
50% PS+50% W	8	11.82 bcd	10.27	1.94	28.28
70% PS+20% W+10% VSW	3	29.50 a	19.14	8.51	45.98
75% PS+25% OW	8	10.61 bcd	6.46	3.34	20.64
75% PS+20% OW+10% VSW	5	27.72 a	14.13	10.82	38.94
75% PS+25% W	8	7.25 cd	4.85	1.20	17.58
70% PS+20% W+10% VSW	4	22.40 ab	8.09	12.17	30.18

*Same letter shows no significant difference between groups

The maximum and minimum mean biological efficiencies were 29.50 and 7.25% for % PS + 45% W + 10% VSW and 75% PS + 25% W substrates, respectively. Moreover, 50% PS + 50% OW, 100% W, 50% PS + 50% W and 75% PS + 25% OW substrates had the same biological efficiency.

The results were somewhat disagree with other results where the maximum biological efficiency was 83.87% for 100% wheat bran as a control (Minstesnol *et al*., 2014).

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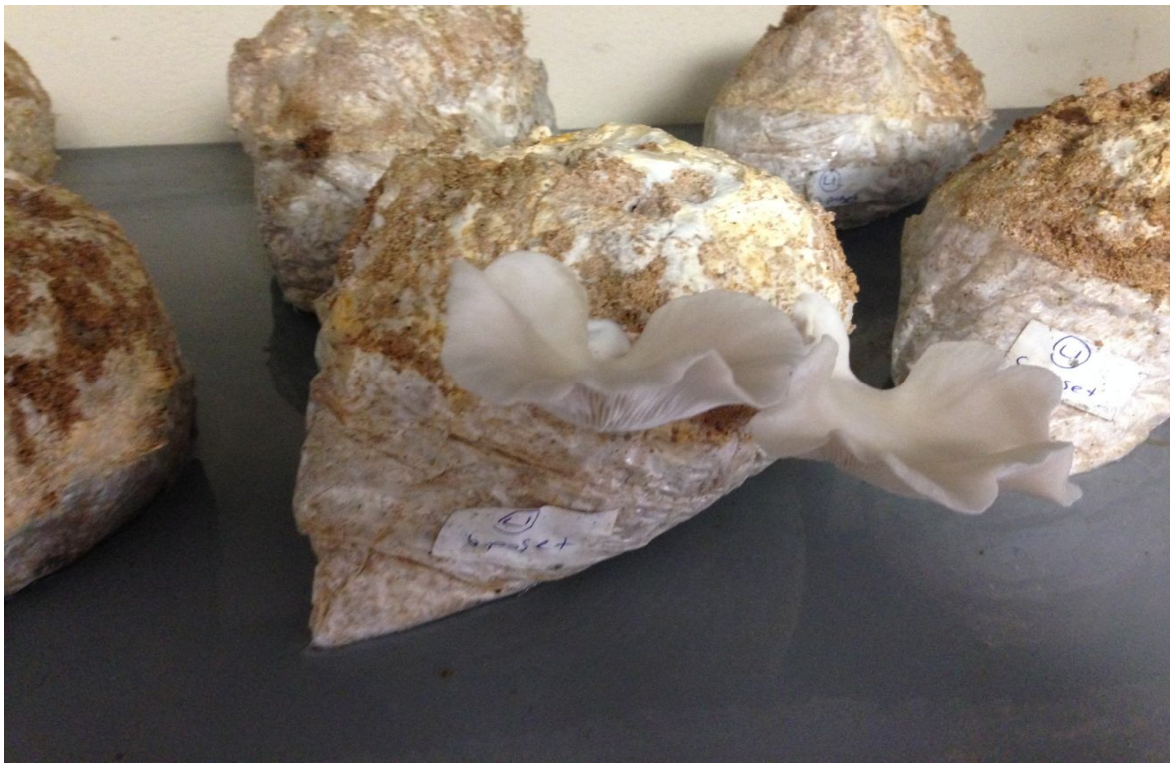

Figure 4.2. *Pleurotus ostreatus* grown on 100% PS substrate

Figure 4.3. *Pleurotus ostreatus* grown on 50% PS + 50% OW substrate

Figure 4.4. *Pleurotus ostreatus* grown on 45% PS + 45% OW + 10% VSW.

Figure 4.5. *Pleurotus ostreatus* grown on 100% W.

Figure 4.6. *Pleurotus ostreatus* grown on 50% PS + 50% W.

Figure 4.7. *Pleurotus ostreatus* grown on 45% PS + 45% W + 10% VSW.

Figure 4.8. *Pleurotus ostreatus* grown on 75% PS + 25% OW.

Figure 4.9. *Pleurotus ostreatus* grown on 70% PS + 20 % OW + 10% VSW.

Figure 4.10. *Pleurotus ostreatus* grown on 75% PS + 25% W.

Figure 4.11. *Pleurotus ostreatus* grown on 70% PS + 20 % W + 10% VSW.

4.8. Physical properties of *Pleuorotus ostreatus*

4.8.1. Weight of *Pleuorotus ostreatus* mushroom

The weights of *Pleurotus ostreatus* mushroom grown on various substrates are presented in Table 4.7. There were statistically significant differences between the mushroom weights of all substrates ($P \leq 0.05$).

Table 4.7. The weight of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Weight of Mushroom (g)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	20	9.11 d*	3.36	4.32	17.00
50% PS+50% OW	31	11.10 cd	6.18	1.06	29.89
45% PS+45% OW+10% VSW	29	17.38 cd	10.00	4.43	46.57
100% W	22	12.17 cd	8.70	2.96	43.83
50% PS+50% W	25	12.16 cd	9.29	4.61	45.86
45% PS+45% W+10% VSW	13	24.80 a	12.73	7.44	52.63
75%PS+25% OW	28	10.36 cd	3.61	4.74	19.58
70% PS+20% OW+10% VSW	22	23.42 ab	16.33	7.18	60.67
75% PS+25% W	18	10.37 cd	5.16	3.57	19.59
70% PS+20% W+10% VSW	11	29.25 a	25.51	4.62	98.35

•Same letter shows no significant difference between groups.

The maximum mushroom weight was observed on 70% P + 20% W + 10% VSW substrate (29.25 g), while the minimum mushroom weight was 9.11 g on 100% PS substrates. However, there were no significant differences between the other groups.

In another study (Ananbeh and Almomany, 2005),the maximum mushroom weight was (21.5 g) for the combination of 60% wheat straw+ 30% olive cake+ 5% wheat bran+ 5% gypsum substrate. However, authors recorded the minimum mushroom weight on 50% wheat straw + 40% olive cake + 5% wheat bran + 5% gypsum (11.67 g).

4.8.1. Cap thickness of *Pleurotus ostreatus*

The cap thickness of *Pleurotus ostreatus* grown on various substrates are presented in Table 4.8. There were statistically significant differences between the cap thickness of all substrates ($P \leq 0.05$).

Table 4.8. Cap thickness of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Cap Thickness (mm)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	20	62.01 c*	9.26	47.62	77.63
50% PS+50% OW	31	64.78 c	9.45	46.08	82.74
45% PS+45% OW+10% VSW	29	76.21 ab	17.42	47.40	125.38
100% W	22	62.62 c	10.28	45.99	81.76
50% PS+50% W	25	66.83 bc	15.97	45.54	101.74
45% PS+45% W+10% VSW	13	82.49 a	20.09	51.82	116.19
75%PS+25% OW	28	66.12 c	10.62	36.50	90.94
70% PS+20% OW+10% VSW	22	83.85 a	17.99	58.33	125.73
75% PS+25% W	18	68.29 bc	14.91	50.52	96.76
70% PS+20% W+10% VSW	11	82.43 bc	22.43	27.79	112.91

•Same letter shows no significant difference between groups

The maximum mean cap thickness was observed on 70% PS+20% OW+10% VSW substrate (83.85 mm), while the minimum mean cap thickness was 62.01 mm on 100% PS substrate. Moreover, 100% PS, 50% PS + 50% OW, 100% W, 75%PS + 25% OW, 50% PS + 50% W, 75% PS + 25% W, 70% PS + 20% W + 10% VSW, 45% PS + 45% W+10% VSW and 70% PS + 20% OW + 10% VSW substrates had the same cap thickness.

Chukwurah *et al*., (2013) also tested the thickness of mushrooms grown in farm substrates that were composed of two different types of agricultural wastes. Substrate combinations of palm kernel cake + saw dust and maize cob + palm kernel cake produced mushrooms with the thickest fruiting bodies.

4.8.2. Cap diameter of *Pleurotus ostrates*

The cap diameter of *Pleurotus ostreatus* grown on various substrates are given in Table 4.9. There were statistically significant differences between the cap diameters of all substrates ($P \leq 0.05$).

The maximum mean cap diameter was observed for 70% PS + 20% OW + 10% VSW substrate (8.23 mm), while the minimum mean cap diameter was 7.02 mm for 70% PS + 20% W + 10% VSW substrate. Moreover, 100% PS, 50% PS + 50% OW, 45% PS + 45% OW + 10% VSW, 100% W, 50 % PS + 50% W, 45% PS + 45% W + 10% VSW, 75% PS + 25% OW and 75% PS + 25% W substrates had the same cap diameter.

Ananbeh and Almomany (2005) found the maximum (7.43 mm) and minimum mean cap diameters (6.00 mm) when 100% wheat bran was used as a control substrate.

Table 4.9. Cap diameters of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Cap Diameter (mm)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	20	7.17 ab*	1.08	5.53	9.34
50% PS+50% OW	31	7.25 ab	1.28	5.06	10.99
45% PS+45% OW+10% VSW	29	7.28 ab	1.82	5.58	11.90
100% W	22	7.60 ab	1.90	4.61	12.58
50 % PS+50% W	25	7.31 ab	1.72	4.68	10.95
45% PS+45% W+10% VSW	13	7.52 ab	1.24	5.37	9.74
75% PS+25% OW	28	7.14 ab	0.97	5.28	9.55
70% PS+20% OW+10% VSW	22	8.23 a	2.42	5.68	14.97
75% PS+25% W	18	7.07 ab	1.48	4.82	10.35
70% PS+20% W+10% VSW	11	7.02 b	1.46	5.26	10.08

•Same letter shows no significant difference between groups.

4.8.3. Length and diameter of *Pleurotus ostrates* stalk

The stalk diameters of *Pleurotus ostreatus* grown on various substrates are presented in Table 4.10. There were statistically significant differences between the stalk diameters of all substrates ($P \leq 0.05$).

Table 4.10. Stalk diameters of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Stalk Diameter (mm)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	20	7.80 c*	1.34	5.06	9.60
50% PS+50% OW	31	8.91 bc	2.06	5.54	13.44
45% PS+45% OW+10% VSW	29	11.00 ab	5.51	6.37	27.92
100% W	22	10.20 ab	2.88	5.91	15.47
50% PS+50% W	25	9.68 bc	2.61	5.18	16.05
45% PS+45% W+10% VSW	13	11.04 ab	2.51	7.64	14.56
75% PS+25% OW	28	9.63 ab	1.61	6.45	12.84
70% PS+20% OW+10% VSW	22	11.93 a	2.89	8.73	19.29
75% PS+25% W	18	10.39 ab	2.81	6.06	16.96
70% PS+20% W+10% VSW	11	11.92 a	3.05	7.28	17.90

*Same letter shows no significant difference between groups

The stalk lengths of *Pleurotus ostreatus* grown on various substrates are presented in Table 4.11. There were statistically significant differences between the stalk lengths of all substrates ($P \leq 0.05$).

Table 4.11. Stalk lengths of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Stalk Length (mm)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100 % PS	20	28.67 ab*	7.94	10.02	43.35
50 %PS+50 % OW	31	40.26 a	11.71	21.98	62.41
45 % PS+45 % OW+10 % VSW	29	41.30 a	16.47	16.14	78.98
100 %W	22	37.11 ab	9.67	20.14	53.53
50 %PS+50 %W	25	36.74 ab	14.86	4.20	66.33
45 % PS+45 % W+10 % VSW	13	37.85 a	11.80	19.15	58.13
75 %PS+25 % OW	28	28.87 ab	9.44	10.13	50.20
70 % PS+20 % OW+10 %VSW	22	38.45 a	13.30	5.03	76.71
75 %PS+25 % W	18	36.84 ab	9.47	19.42	53.38
70 % PS+20 % W+10 %VSW	11	45.00 a	10.19	27.15	61.63

*Same letter shows no significant difference between groups

The maximum mean stalk diameter was (11.93 mm) for 70% PS + 20% OW + 10% VSW substrate, and the minimum mean stalk diameter was (7.80 mm) for 100% poplar sawdust substrate.

The maximum mean stalk length was (45.00 mm) for 70% PS + 20% W + 10% VSW substrate, and the minimum mean stalk length was (208.67 mm) for 100 % PS. Moreover, 100% PS, 100% W, 50% PS + 50% W, 75% PS + 25% OW, 75 %PS + 25 % W, 50%PS + 50% OW, 45% PS + 45% OW + 10% VSW and 70% PS + 20% W + 10 %VSW substrates had the same stalk length.

The stalk length was increased possibly due to different substrate combinations. Earnshaw *et al*., (2012) studied the effects of different substrates on length and diameter of stalk. They found that results differed when first week mean was 0.0 mm with sugarcane + 10% wheat bran substrate was. The maximum stalk length was observed with sugarcane tops + 15% wheat bran substrate (20.3 mm). In the same study, the second week was resulted maximum stalk length (28.8 mm) and minimum stalk diameter (0.0 mm) with maize stover and cobs + 10% wheat bran.

4.8.4. Dry substrate of *Pleurotus ostreatus*

The dry substrates of *Pleurotus ostreatus* grown on various substrates are presented in Table 4.12. There was a significant difference between the various substrates ($P \leq 0.05$). The maximum mean dry substrate was 22.12 g for 50% PS + 50% W, while the minimum mean dry substrate was 7.93 g for 70% PS + 20% W + 10% VSW. Moreover, there were

no significant dry substrate differences between the other groups. Sharma *et al*., (2013) found the best dry substrate on rice straw. This was somewhat disagree with our study, since the mushroom fruit body decreases with rising incubation period. This is due to the microorganisms that use the available nutrients for their growth and metabolism.

Table 4.12. Dry substrate of *Pleurotus ostreatus* cultivated on various substrates.

Substrates	Dry Substrate (g)				
	Number	Mean	Std. Deviation	Minimum	Maximum
100% PS	20	18.07 ab*	11.18	7.50	52.62
50% PS+50 % OW	31	20.75 a	28.94	5.64	131.39
70% PS+20% OW+10% VSW	29	13.08 ab	6.80	0.35	30.27
100% W	22	15.23 ab	6.68	7.66	32.18
50% PS+50% W	25	22.12 a	15.32	8.17	82.31
70% PS+20% W+10% VSW	13	10.45 ab	9.64	3.88	35.59
75% PS+25% OW	28	14.94 ab	5.68	4.52	26.53
75% PS+20% OW+10% VSW	22	10.55 ab	3.68	1.77	15.14
75% PS+25% W	18	16.88 ab	10.98	8.64	56.61
70% PS+20% W+10% VSW	11	7.93 ab	6.38	2.25	22.38

*Same letter shows no significant difference between groups

4.8.5. Chemical composition (Nitrogen, Carbon, Protein) of fruiting bodies of *Pleurotus ostreatus* after harvesting stage

Table 4.13 illustrates nitrogen, carbon, and protein contents of fruiting body of *Pleurotus ostreatus* grown on various substrates. The maximum nitrogen content was detected on 100% OW (7.23%), while the minimum nitrogen was detected on 100% PS substrate (3.60%). However, maximum carbon concentration was on 100% PS (42.61%). In addition, protein content was the highest (45.19%) and lowest (22.48%) for 100% OW and 100% PS, respectively. Bonatti (2004) also studied the effects of banana and rice straws on *Pleurotus ostreatus* growth. The nitrogen, protein, and ash contents were 3.85 to 3.00%, 16.9 to 13.1% and 5.58 to 6.13% in banana to rice straws, respectively. Shelly, (2008) also found the protein and ash contents of *Pleurotus ostreatus* 24.03 and 7.27%, respectively.

Table 4.13. Nitrogen, carbon, C/N and protein of *Pleurotus ostreatus* cultivated on various substrates.

Substrate	Nitrogen (%)	Carbon (%)	Protein (%)
100% PS	3.60	42.61	22.48
100% OW	7.23	41.08	45.19
50%PS+50% O W	5.01	41.53	31.29
45% PS+45% OW+10% VSW	5.56	41.20	34.75
100% W	4.69	40.76	29.31
50% PS+50% W	4.71	40.66	29.45
45% PS+45% W+10% VSW	4.96	41.71	31.01
75% PS+25% OW	4.17	40.39	26.09
70% PS+20% OW+10% VSW	5.50	41.84	34.35
75% PS+25% W	3.81	40.17	23.79
70% PS+20% W+10% VSW	6.13	41.06	38.30

4.9. Chemical Composition of *Plerotus ostreatus*

4.9.1. Total tannin concentration of *Pleurotus ostreatus*

Table 4.14 shows tannin concentration of *Pleurotus ostreatus* chemically analyzed with mimosa calibration for calculating total tannin.

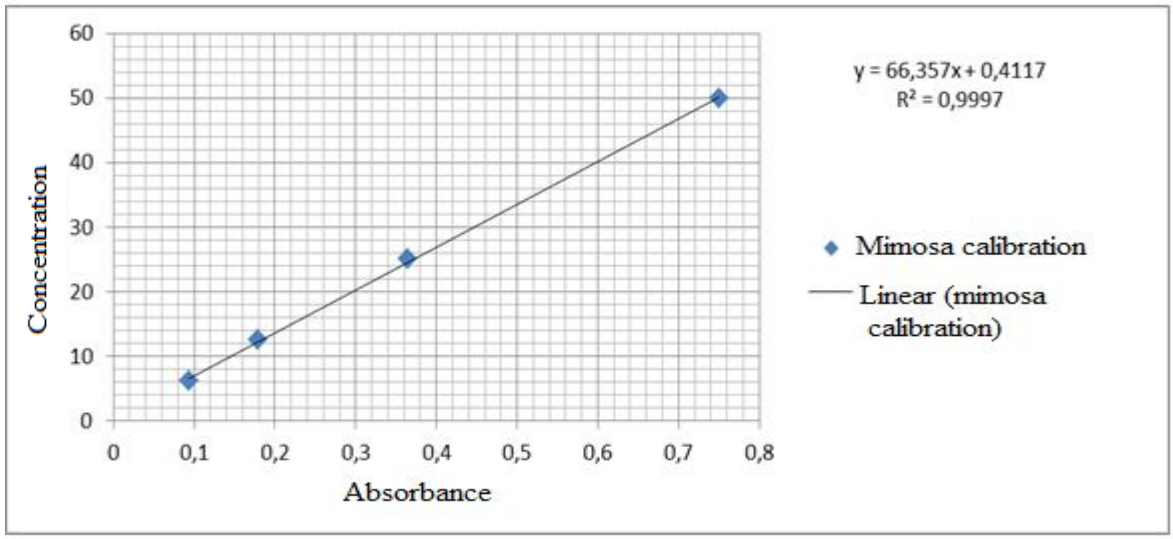

Figure 4.2. Total tannin concentration of *Pleurotus ostreatus* by mimosa calibration graphic.

Results indicated that *Pleurotus ostreatus* contained the highest tannin (6.18%) on 100% PS substrate, while the minimum tannin concentration was detected for on 70% PS + 20% OW + 10% VSW (3.66%). Fan (2006) indicated the feasibility of using 100% PS without any pretreatment for the cultivation of *Pleurotus ostreatus*.

Table 4.14. Total tannin concentration of *Pleurotus ostreatus* cultivated on various substrates

Substrate	ppm (mg/kg)
100% PS	6.18
50% PS+50% OW	4.06
45% PS+45% OW+10% VSW	4.46
100% W	3.86
50% PS+50% W	5.06
45% PS+45%W+10% VSW	4.19
75% PS+25% OW	4.26
70% PS+20% OW+10% VSW	3.66
75% PS+25% W	4.39

4.9.2. Sugar analysis of *Pleurotus ostreatus* by high performance liquid chromatogram (HPLC)

Table 4.15 illustrates two different sugar (sucrose, glucose) compositions of *Pleurotus ostreatus* grown on different substrates. The maximum sucrose concentration was on 50% PS + 50% OW substrate (88.035%), while the minimum concentration was on 100% PS substrate (65.84%). The maximum glucose concentration was on 100% PS substrate (26.54%), while the minimum concentration was on 70% PS + 20% OW + 10% VSW substrate (5.65%). Similarly, Shelly *et al*., (2008) found glucose concentration (40.3g/100g) close to ours. However, when the substrate combination contained cotton waste as a control, the amount of carbohydrate was 36.74% (Ashraf *et al*., 2013).

4.9.3. DPPH radical scavenging activity of *Pleurotus ostreatus* fruit extract

Table 4.16 present DPPH radical scavenging activity of *Pleurotus ostreatus* mushroom extract. All the samples were obtained from different substrates of different concentration with DPPH (1, 1-diphenyl pycril hydrazyl) free radical band capacities.

Antioxidants play important roles for maintaining human health due to their ability to scavenge free radical in the body. Table 4.16 shows that the DPPH percentage had a good amount of antioxidant activity (98.60%) containing 70%PS + 20% OW + 10% VSW substrate with 0.1 µl. Our results indicate that *Pleurotus ostreatus* possesses a good antioxidant potential, and consumptions of supplemented antioxidants from fruits and mushrooms in our diets is very important (Halliwell, 1996).

Table 4.15. Hydro-carbohydrate composition of *Pleurotus ostreatus*.

Substrate	Sucrose (%)	ppm (mg/kg)	Glucose (%)	ppm (mg/kg)
100% PS	65.84	1212.98	26.54	4577.35
50% PS+50% OW	70.72	1420.90	11.60	223.55
45%PS+45% OW+10% VSW	88.04	1620.49	4.53	83.26
100% W	85.53	1485.45	10.44	174.98
50% PS+ 50% W	79.98	1326.27	10.85	173.56
45% PS+45% W+ 10% VSW	79.61	1470.87	13.36	236.43
75% PS+25% OW	79.89	1511.38	13.31	237.90
70%PS+20% OW+10% VSW	85.43	1778.40	6.36	129.41
75% PS+ 25% W	72.56	1407.10	5.65	107.80
70%PS+20% OW+10% VSW	81.83	1529.66	7.20	131.08

Table 4.16. DPPH radical scavenging activity of *Pleuortus ostreatus* fruity bodies and BHT.

Substrates	*Pleuortus ostreatus*			BHT		
	DPPH Radical Scavenging (%)					
	Pleuortus ostreatus Extract Concentration (mL)					
	0.1	0.2	0.3	0.1	0.2	0.3
100% PS	73.50	62.32	61.70	92.86	94.3	93.3
50% PS+50% OW	59.23	60.04	64.45	92.86	94.3	93.3
45%PS+45% OW+10% VSW	64.23	63.94	67.03	92.86	94.3	93.3
100% W	74.61	74.24	74.46	92.86	94.3	93.3
50% PS+ 50% W	69.68	61.36	62.39	92.86	94.3	93.3
45% PS+45% W+ 10% VSW	61.36	61.00	61.22	92.86	94.3	93.3
75% PS+25% OW	59.89	61.95	60.19	92.86	94.3	93.3
70%PS+20% OW+10% VSW	98.60	86.75	90.21	92.86	94.3	93.3
75% PS+ 25% W	61.00	65.56	63.94	92.86	94.3	93.3
70%PS+20% OW+10% VSW	60.70	61.81	61.44	92.86	94.3	93.3

4.9.4. Lipid analysis by gas chromatography (GC-FID)

The simple free fatty acid concentration of *Pleuortus ostreatus* was ranged from 0.17 to 84.00% in dry weigh. Individually, fatty acids have been analyzed as methyl esters by gas-liquid chromatography or gas chromatography coupled to mass spectrophotometer. They are usually presented in relative terms, as percentage of total fatty acids.

As shown in Table 4.17, *Pleuortus ostreatus* grown on different substrates contained different fatty acids. When *Pleuortus ostreatus* was grown on 100% PS substrate, there were palmitic (5.65%), stearic (8.70%), oleic (33.41%) and linoleic acids (30.34%). However, lipid composition of *Pleuortus ostreatus* on 45% PS + 45% OW + 10% VSW substrate was palmitic 21.47%, stearic 8.61%, oleic 33.34%, linoleic 30.97%, alpha linolenic 1.55%, and behenic acids 1.13%. These results showed that *Pleurotus ostreatus* was containing the most fatty acids respectively; alpha-linolenic acid among them decreases the risk of heart disease by helping to maintain normal heart rhythm and heart pumping. It might also reduce blood clots. Although alpha-linolenic acid seems to benefit the cardiovascular system and reduce the risk of heart disease, several other studies also concluded this benefit (Ashraf *et al*., 2013; Hadar and Cohen-Arazi, 1986; Usda, 2010; Bautista Justo *et al*., 1998; Mostafa, E., 2012; Khanna and Garcha, 1981; Rashad *et al*., 2009; Shashirekha *et al*., 2005).

4.9.5. Mineral analysis

Mineral composition of *Pleurotus ostreatus* cultivated on various substrates is presented in Table 4.18. The K, Cl, Ca, and S concentrations were higher (1.410, 0.031, 0.014, and 0.116%) in 100% PS than 100% W. The results of our study are also confirmed with the other researches (Demirbas, 2000; Çağlarır, 2007; Duzkali, 2014) where *Pleurotus ostreatus* fruit bodies contained different amounts of minerals.

Table 4.17. Mineral composition of *Pleurotus ostreatus* cultivated on various substrates.

substrates	Fe (%)	P (%)	Cl (%)	K (%)	Ca (%)	Zn (%)	S (%)	Mg (%)
100% PS	0.000	0.290	0.031	1.410	0.014	0.006	0.116	0.017
100% W	0.002	0.310	0.028	1.190	0.008	0.006	0.112	0.017

Table 4.18. Effect of fatty acid composition of *Pleurotus ostreatus* grown on various substrates.

Substrates	AP1 (%)	AP2 (%)	AP3 (%)	AP4 (%)	AP5 (%)	AP6 (%)	AP7 (%)	AP8 (%)	AP9 (%)	AP10 (%)	AP11 (%)	AP12 (%)	AP13 (%)	AP14 (%)	AP15 (%)
100% PS	20.61	0.90	8.00	33.41	30.34	1.34	1.14	0.95	1.205	-	-	-	-	-	-
50% PS+50% OW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45%PS+45% OW+10% VSW	21.47	0.97	8.61	33.34	30.97	1.55	1.13	-	0.51	-	-	-	-	-	-
100% W	20.94	0.68	8.63	84.00	33.16	1.07	1.04	-		-	-	-	-	-	-
50% PS+ 50% W	21.32	1.15	7.66	35.53	30.04	0.91	0.97	-	0.49	0.36	-	-	-	-	-
45% PS+45% W+ 10% VSW	20.99	1.05	8.11	35.07	30.13	1.02	1.14	-	0.61		-	-	-	-	-
75% PS+25% OW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70%PS+20% OW+10% VSW	19.96	-	6.06	32.69	38.81	0.68	-	0.34	-	-	0.20	0.16	0.53	0.59	-
75% PS+ 25% W	19.08	0.58	5.87	28.87	42.78	0.73	-	0.47	-	-	-	-	0.51	0.76	0.37
70%PS+20% W+10% VSW	18.51	0.47	5.80	30.34	42.46	0.35	-	-	0.17	1.24	-	-	-	0.663	-

AP1: Palmitic acid

AP2: Palmiteloic acid

AP3: Stearic acid

AP4: Oleic acid

AP5: Linoleic acid

AP6: Alfa Linolenic acid

AP7: Behenic acid

AP8: Eicosapentaenoic acid

AP9: Docosahexaenoic acid

AP10: Pentadecanoic acid

AP11: Arachidic acid

AP12: Heneicosanoic acid

AP13: Eicosatrienoic acid

AP14: Lignoceric acid

AP15: Nervonic acid

5. CONCLUSION

Although most fungal cultures prefer slightly acidic pH in the substrates for growth, the initial pH's of all substrates in the present study were between netural to acidic. However, after spawning and harvesting stage, pH was recorded acidic in all substrates respectively. High moisture content was recorded in 100% PS substrate.

The best harvesting time and total time were observed for mushrooms grown on 70% PS + 20% W + 10% VSW substrate. Thus, mixture substrate was the best spawn type among the three spawn types tested. The mixture substrate (70% PS + 20% OW + 10%VSW) was resulted the highest production.

The greatest biological efficiency, more mushroom weight and maximum cap diameter and thickness were observed with 45% PS + 45% W + 10% VSW, 70% PS + 20% W + 10% VSW and 70% PS + 20 %OW +10 % VSW substrates, respectively.

The highest mushroom stalk diameter was observed in 100% PS, while the stalk length was higher on 70% PS + 20% W + 10% VSW substrate. The maximum dry substrate was detected on 50% PS + 50% W substrate. After harvesting stage, the minimum nitrogen and maximum carbon concentrations were recorded on same substrate. Moreover, the minimum protein content was measured on 75% PS + 25% W substrate. The highest tannin concentration was detected in 100% PS, while sugar (sucrose + glucose) concentrations were maximum for sucrose in 50% PS + 50% OW and for glucose in 100% PS substrates.

A higher antioxidant property was observed in 75% PS + 25% OW substrate that is useful for heart disease and anti-cholesterol activity.

We have shown that *Pleurotus ostreatus* cultivation profiling techniques with different substrates may provide useful information for other *Pleurotus* spp.

This study reflects that mixture substrates are useful as a tool for mushroom cultivation. Therefore, for future studies, using different types of substrates, such as poplar sawdust, olive waste, walnut wood waste (*Juglans* spp), vicia sativa weed will improve the accuracy of resolution and accurate cultivation.

Mushroom cultivation is one of the efficient way by which residues can be recycled. *P. ostreatus* grown on different substrates are nutritious with high protein and

low lipid, and nitrogen content of the mushroom is affected by the cap and stalk proportions.

Further research programs using combination of two or more substrates for growing oyster mushroom (*P. ostreatus)* may provide more food, more jobs, better family incomes, and improved living standards.

The application of mixture substrates should also be encouraged to grow and use these species for its nutritional and medicinal benefits. and as a source of additional income. Therefore, farmers should be educated on mushroom cultivation of *P. ostreatus* with various substrates.

Using other agricultural and forestry wastes in the cultivation of *P. ostreatus* will encourage the mushroom production and help in the natural recycling of food, medicine and improvement of the environment.

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APPENDIX

Table A1. ANOA for harvesting time homogeneous subsets

		Subset for alpha = 0.05			
Parameters	N	d	c	b	a
70% PS+20% W+10 VSW	11	28.7273			
45% PS+45% OW+10% VSW	29	33.3448	33.3448		
100% W	22	33.7273	33.7273	33.7273	
50% PS+50% OW	31	39.2258	39.2258	39.2258	39.2258
45% PS+45% W+10% VSW	13	42.5385	42.5385	42.5385	42.5385
100% PS	20		45.2500	45.2500	45.2500
70% PS+20% W+10 VSW	22		45.9545	45.9545	45.9545
75% PS+25 W	18		46.6111	46.6111	46.6111
50% PS+50% W	25			49.2000	49.2000
75% PS+25 OW	28				50.1071
Sig.		.072	.096	.050	.176

Table A2. ANOA for total time homogeneous subsets

Parameters		Subset for alpha = 0.05			
	N	d	c	b	a
70% PS+20% W+10 VSW	11	47.7273			
45% PS+45% OW+10% VSW	29	51.3448	51.3448		
100% W	22	59.7273	59.7273	59.7273	
50% PS+50% OW	31		64.2258	64.2258	64.2258
100% PS	20			67.2500	67.2500
75% PS+25 W	18			72.6111	72.6111
45% PS+45% W+10% VSW	13			73.5385	73.5385
50% PS+50% W	25			74.2000	74.2000
75% PS+25 OW	28			75.1071	75.1071
70% PS+20% OW+10 VSW	22				76.9545
Sig.		.098	.075	.052	.111

Table A3. ANOA for weight of mushroom homogeneous subsets

Parameters	N	Subset for alpha = 0.05			
		d	c	b	a
100% PS	20	9.1056			
75% PS+25 OW	28	10.3569	10.3569		
75% PS+25 W	18	10.3689	10.3689		
50% PS+50% W	31	11.0964	11.0964		
50% PS+50% OW	25	12.1646	12.1646		
100% W	22	12.1659	12.1659		
45% PS+45% OW+10% VSW	29		17.3770	17.3770	
70% PS+20% OW+10 VSW	22			23.4181	23.4181
45% PS+45% W+10% VSW	13				24.8049
70% PS+20% W+10 VSW	11				29.2500
Sig.		.431	.064	.070	.098

Table A4. ANOA for cap thickness homogeneous subsets

Parameters		Subset for alpha = 0.05		
	N	c	b	a
100% PS	20	62.0053		
100% W	22	62.6209		
50% PS+50% OW	31	64.7794		
75% PS+25 OW	28	66.1214		
50% PS+50% W	25	66.8272	66.8272	
75% PS+25 W	18	68.2944	68.2944	
45% PS+45% OW+10% VSW	29		76.2078	76.2078
70% PS+20% W+10 VSW	11			82.4309
45% PS+45% W+10% VSW	13			82.4904
70% PS+20% OW+10 VSW	22			83.8491
Sig.		.246	.057	.137

Table A5. ANOA for cap diameter homogeneous subsets

		Subset for alpha = 0.05	
Parameters	N	b	a
70% PS+20% W+10 VSW	11	7.0218	
75% PS+25 W	18	7.0706	7.0706
75% PS+25 OW	28	7.1411	7.1411
100% PS	20	7.1715	7.1715
50% PS+50% OW	31	7.2503	7.2503
45% PS+45% OW+10% VSW	29	7.2793	7.2793
50% PS+50% W	25	7.3060	7.3060
45% PS+45% W+10% VSW	13	7.5200	7.5200
100% W	22	7.6041	7.6041
70% PS+20% OW+10 VSW	22		8.2332
Sig.		.347	.054

Table A6. ANOA for stalk diameter homogeneous subsets

		Subset for alpha = 0.05		
Parameters	N	c	b	a
100% PS	20	7.7960		
50% PS+50% OW	31	8.9139	8.9139	
75% PS+25 OW	28	9.6282	9.6282	
50% PS+50% W	25	9.6824	9.6824	
100% W	22		10.1955	10.1955
75% PS+25 W	18		10.3922	10.3922
45% PS+45% oW+10% VSW	29		11.0021	11.0021
45% PS+45% W+10% VSW	13		11.0346	11.0346
70% PS+20% W+10 VSW	11			11.9155
70% PS+20% OW+10 VSW	22			11.9314
Sig.		.073	.056	.116

Table A7. ANOA for length of stalk homogeneous subsets

		Subset for alpha = 0.05	
Parameters	N	b	a
100% PS	20	28.6685	
75% PS+25% OW	28	28.8729	
50% PS+50% W	25	36.7482	36.7482
75% PS+25% W	18	36.8428	36.8428
100% W	22	37.1100	37.1100
45% PS+45% W+10% VSW	13		37.8462
70% PS+20%O W+10 VSW	22		38.4478
50% PS+50% OW	31		40.2587
45% PS+45% OW+10% VSW	29		41.3014
70% PS+20% W+10 VSW	11		45.0009
Sig.		.050	.068

Table A8. ANOA for dry substrate homogeneous subsets

		Subset for alpha = 0.05		
Parameters	N	c	b	a
70% PS+20% W+10 VSW	11	7.9291		
45% PS+45% W+10% VSW	13	10.4469	10.4469	
70% PS+20%O W+10 VSW	22	10.5514	10.5514	
45% PS+45% OW+10% VSW	29	13.0817	13.0817	13.0817
75% PS+25% OW	28	14.9429	14.9429	14.9429
100% W	22	15.2355	15.2355	15.2355
75% PS+25% W	18	16.8839	16.8839	16.8839
100% PS	20		18.0685	18.0685
50% PS+50% OW	31			20.7523
50% PS+50% W	25			22.1216
Sig.		.081	.141	.078

Table A9. ANOA for total yield homogeneous subsets

		Subset for alpha = 0.05			
Parameters	N	d	c	b	a
75% PS+25% W	8	23.3301			
100% PS	7	26.0160			
75% PS+25% OW	8	36.2491	36.2491		
50% PS+50% W	8	38.0144	38.0144	38.0144	
100% W	7	38.2356	38.2356	38.2356	
50% PS+50% OW	8	42.9985	42.9985	42.9985	
70% PS+20% W+10 VSW	4		80.4375	80.4375	80.4375
45% PS+45% OW+10% VSW	6			83.9888	83.9888
70% PS+20%O W+10 VSW	5				103.0398
45% PS+45% W+10% VSW	3				107.4880
Sig.		.135	.064	.053	.247

Table A10. ANOA for biological efficiency

		Subset for alpha = 0.05			
Parameters	N	1	2	3	4
75% PS+25% W	8	7.2544	7.2544		
100% PS	7	9.0011	9.0011		
75% PS+25% OW	8	10.6078	10.6078	10.6078	
50% PS+50% W	7	10.7676	10.7676	10.7676	
100% W	8	11.0636	11.0636	11.0636	
50% PS+50% OW	8	11.8163	11.8163	11.8163	
70% PS+20% W+10 VSW	6		20.5387	20.5387	20.5387
45% PS+45% OW+10% VSW	4			22.3991	22.3991
70% PS+20%O W+10 VSW	5				27.1959
45% PS+45% W+10% VSW	3				29.5013
Sig.		.116	.050	.079	.165

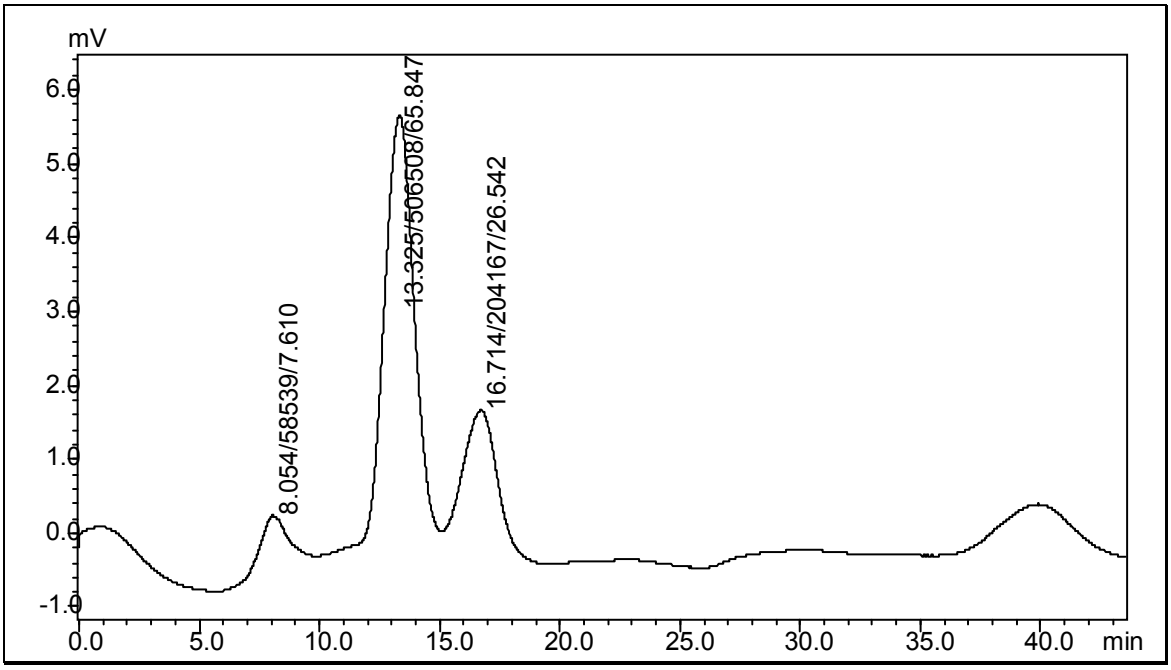


Figure B1. HPLC Chromatogram of *Pleurotus ostreatus* of 100% PS sugar amount (sucrose+glucose).

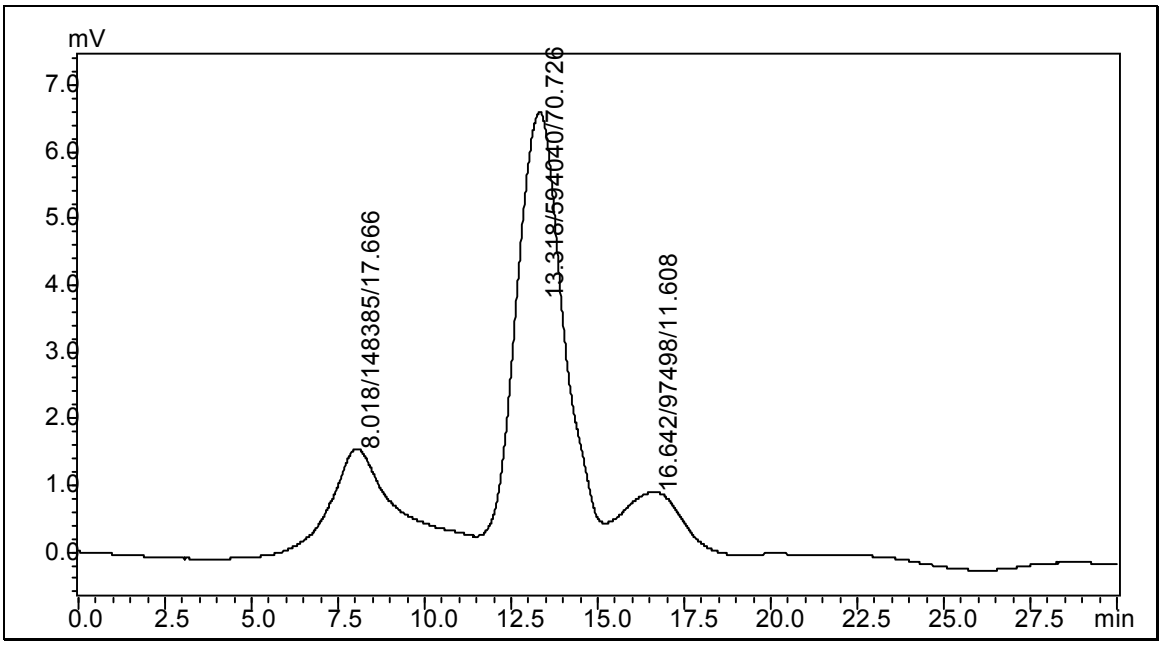


Figure B2. HPLC Chromatography of *Pleurotus ostreatus* o 50% PS + 50% OW sugar amount (sucrose + glucose).

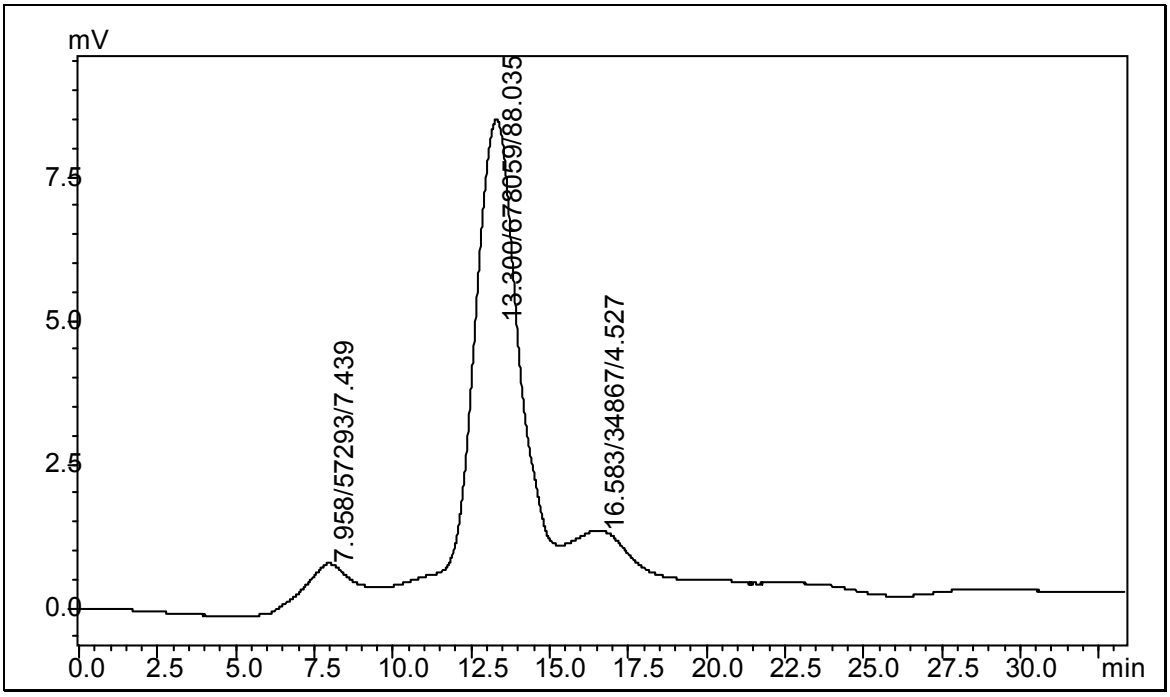


Figure B3. HPLC Chromatography of *Pleurotus ostreatus* of 45% PS + 45 % OW + 10 % VSW sugar amount of (sucrose + glucose).

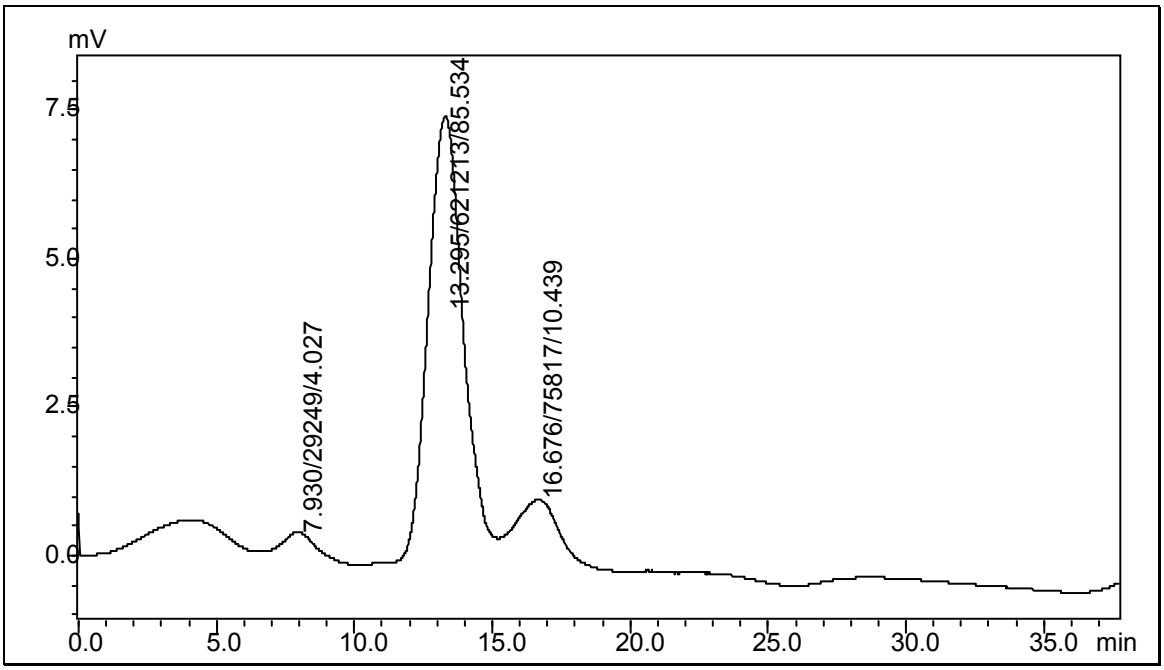


Figure B4. HPLC Chromatography of *Pleurotus ostreatus* of 100% W sugar amount (sucrose + glucose).

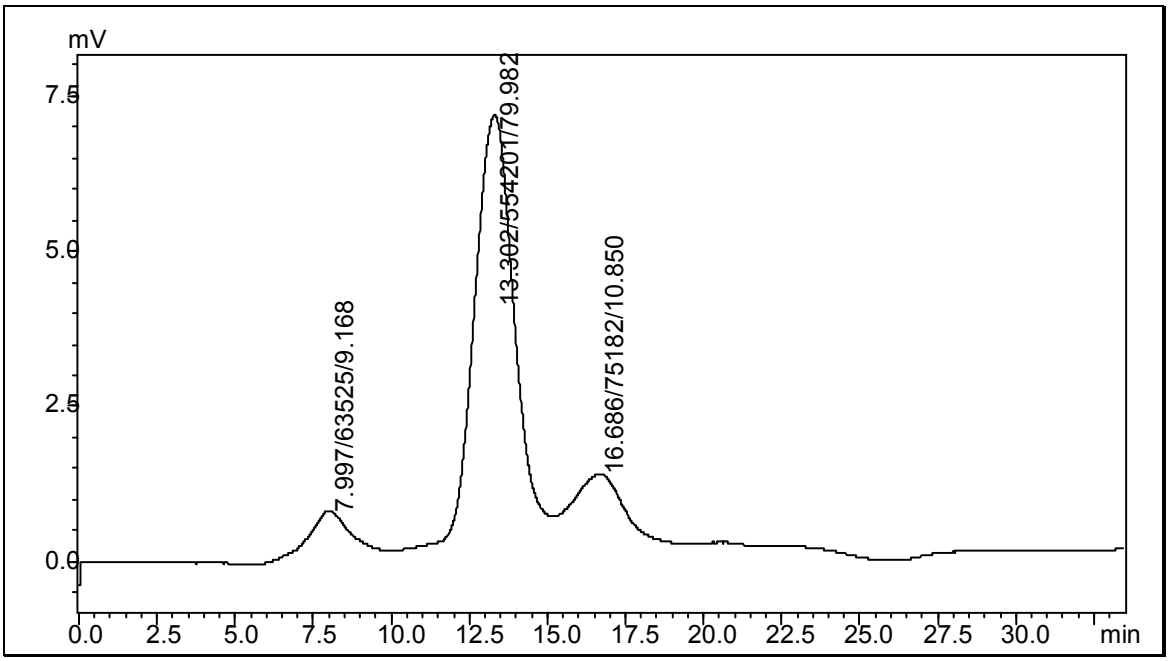


Figure B5. HPLC Chromatography of *Pleurotus ostreatus* of 50% PS + 50% W sugar amount (sucrose + glucose).

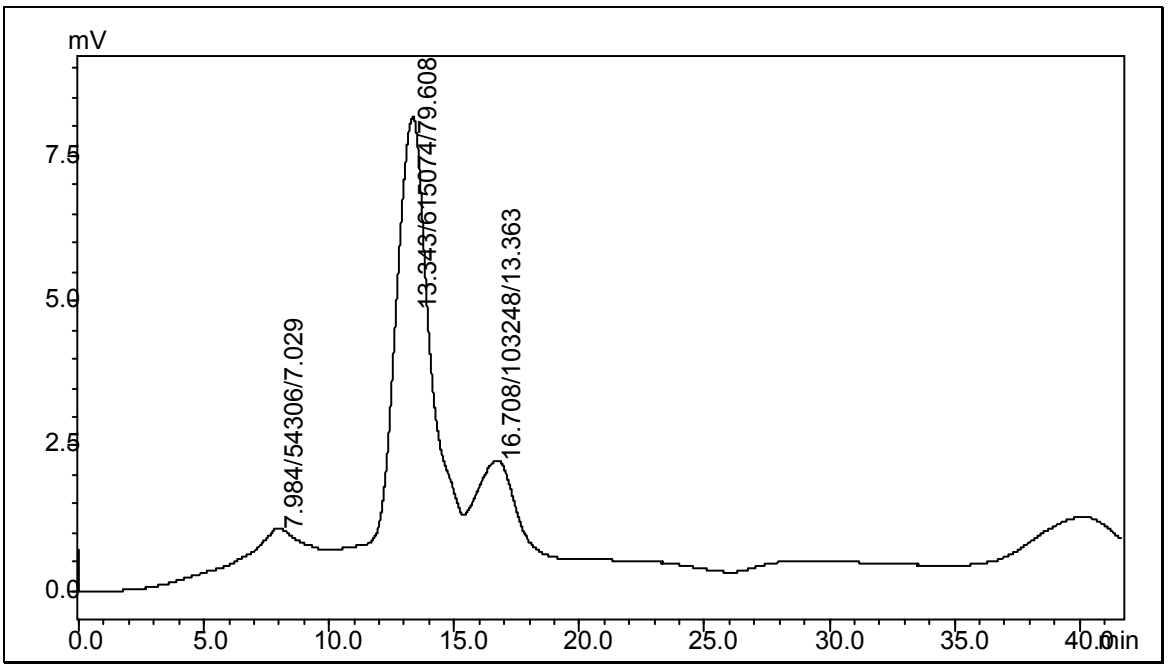


Figure B6. HPLC Chromatography of *Pleurotus ostreatus* of 45% PS + 45% W + 10% VSW sugar amount (sucrose + glucose).

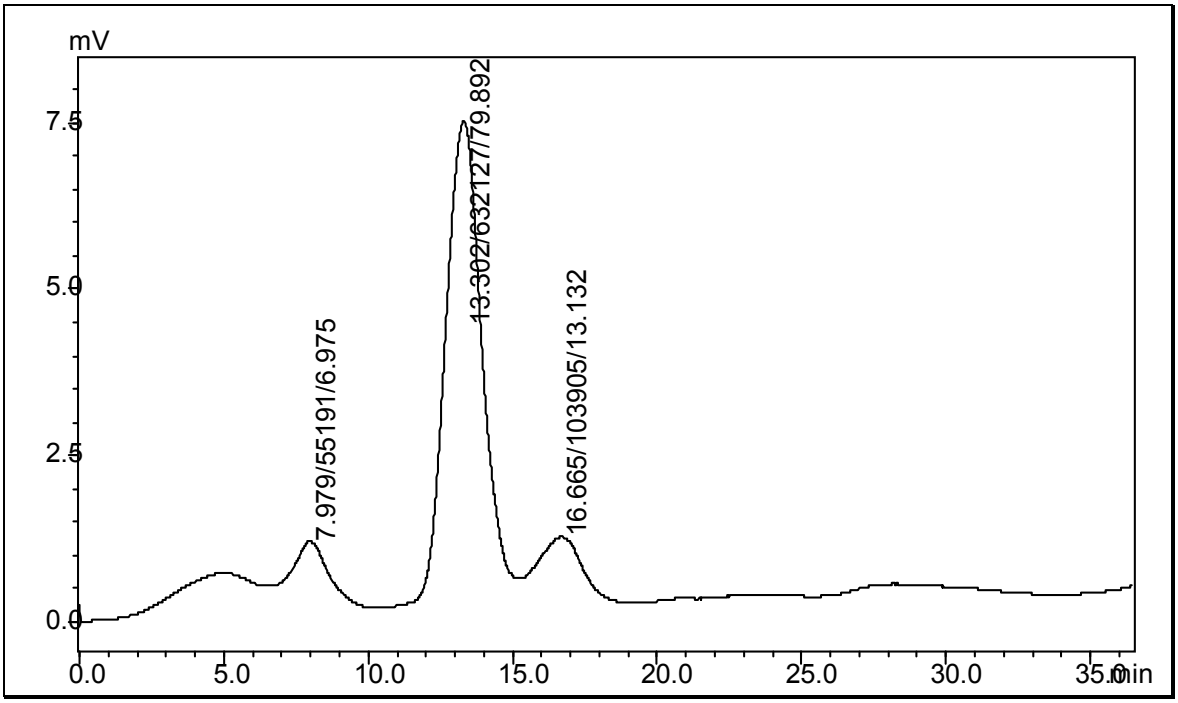


Figure B7. HPLC Chromatography of *Pleurotus ostreatus* of 75% PS + 25% OW sugar amount (sucrose + glucose).

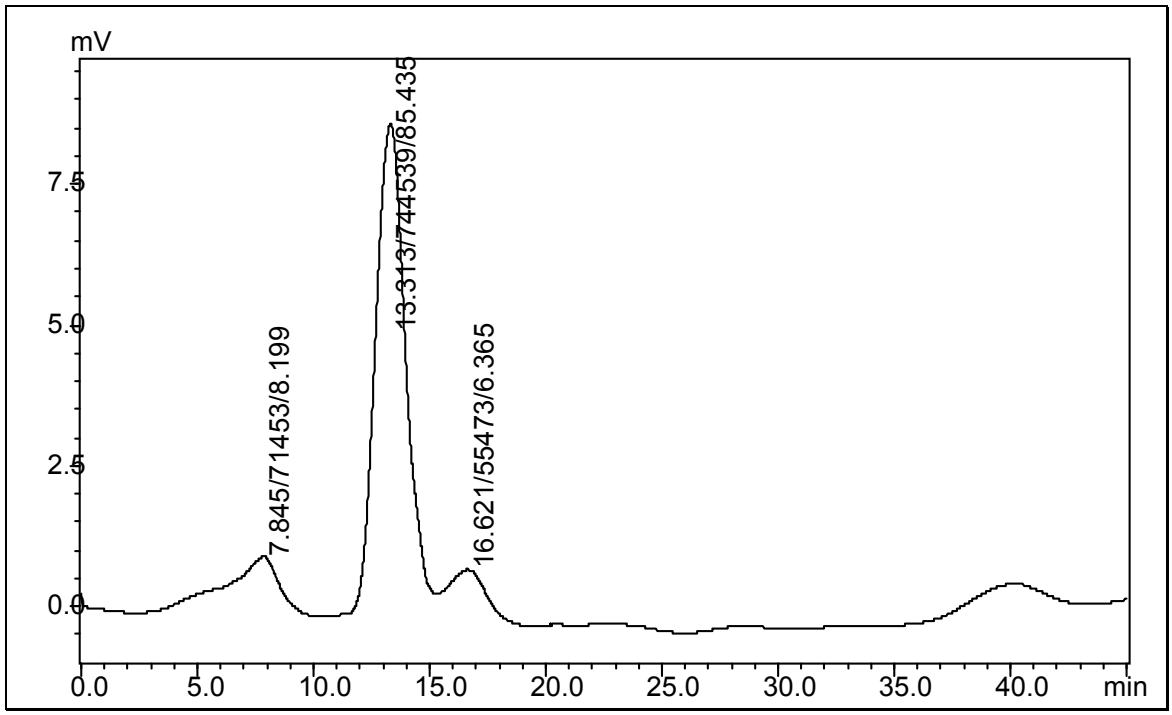


Figure.B8. HPLC Chromatography of *Pleurotus ostreatus* of 70% PS + 20% OW + 10% VSW sugar amount (sucrose + glucose).

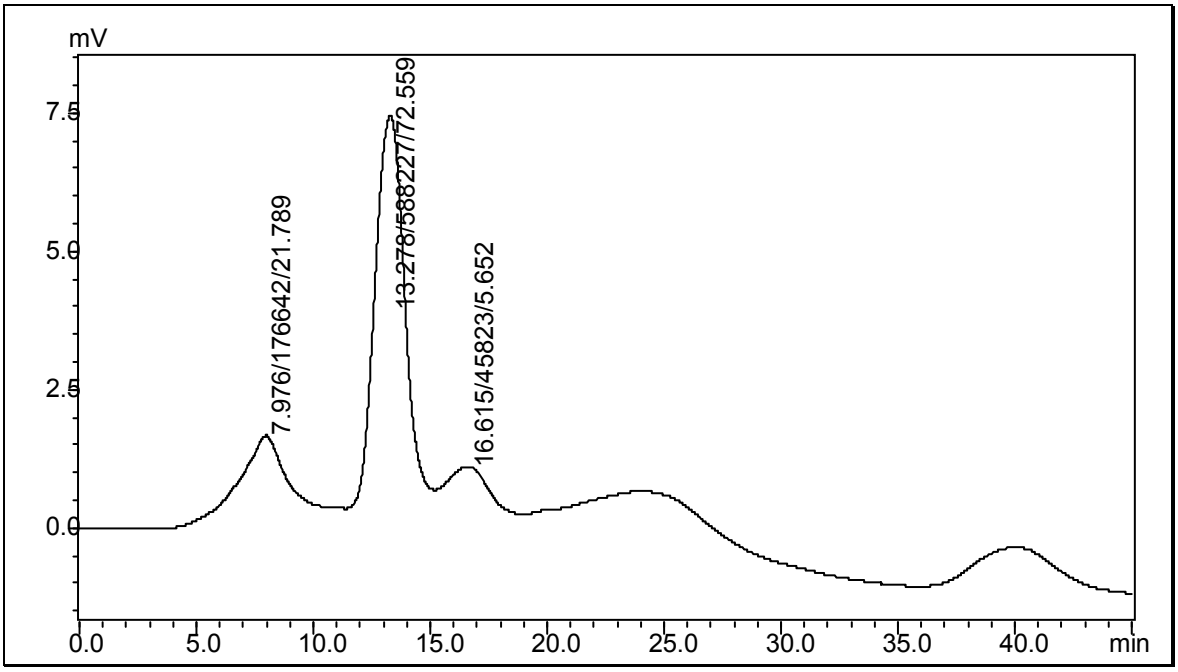


Figure B9. HPLC Chromatography of *Pleurotus ostreatus* of 75% PS + 25% W sugar amount (sucrose + glucose).

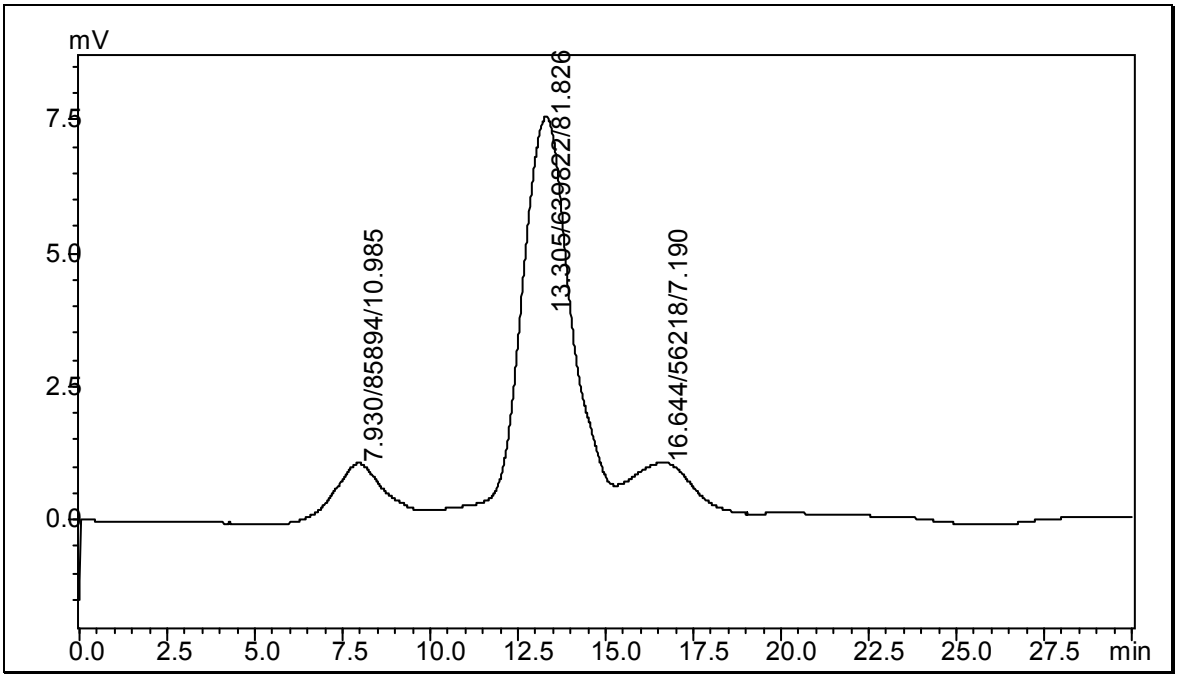


Figure.B10. HPLC Chromatography of *Pleurotus ostreatus* of 70% PS + 20% W+ 10% VSW sugar amount (sucrose + glucose).

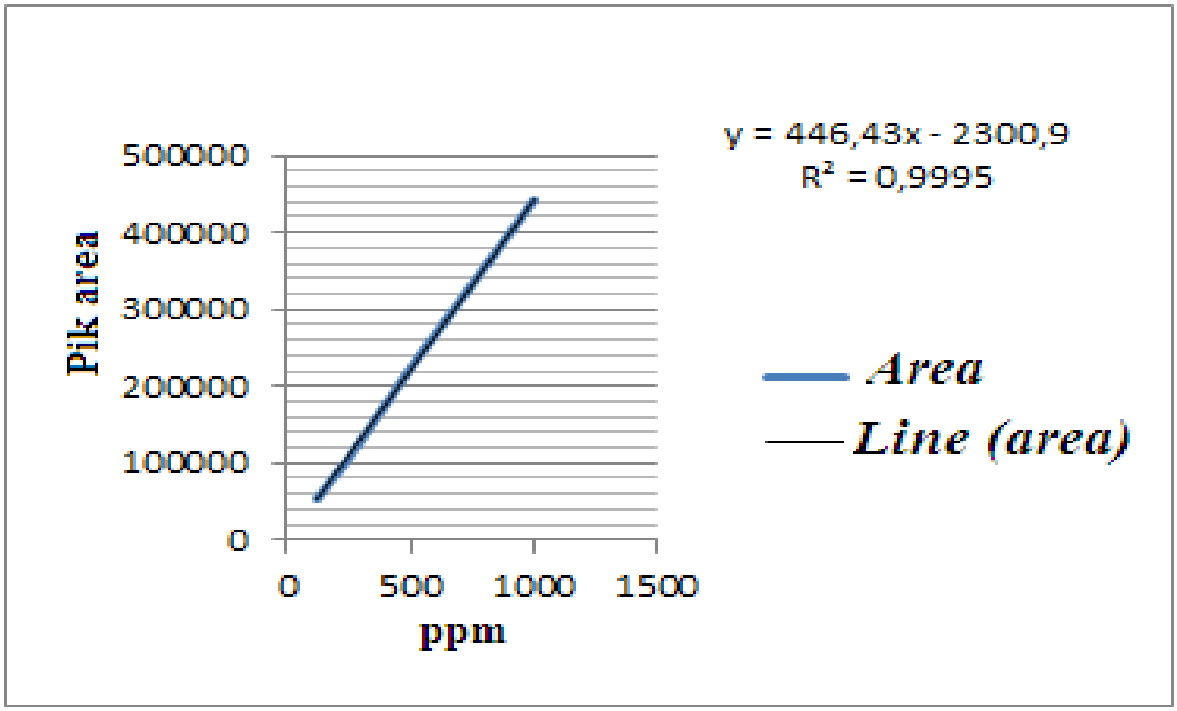


Figure B11. Glucose used in reference to the calibration graph.

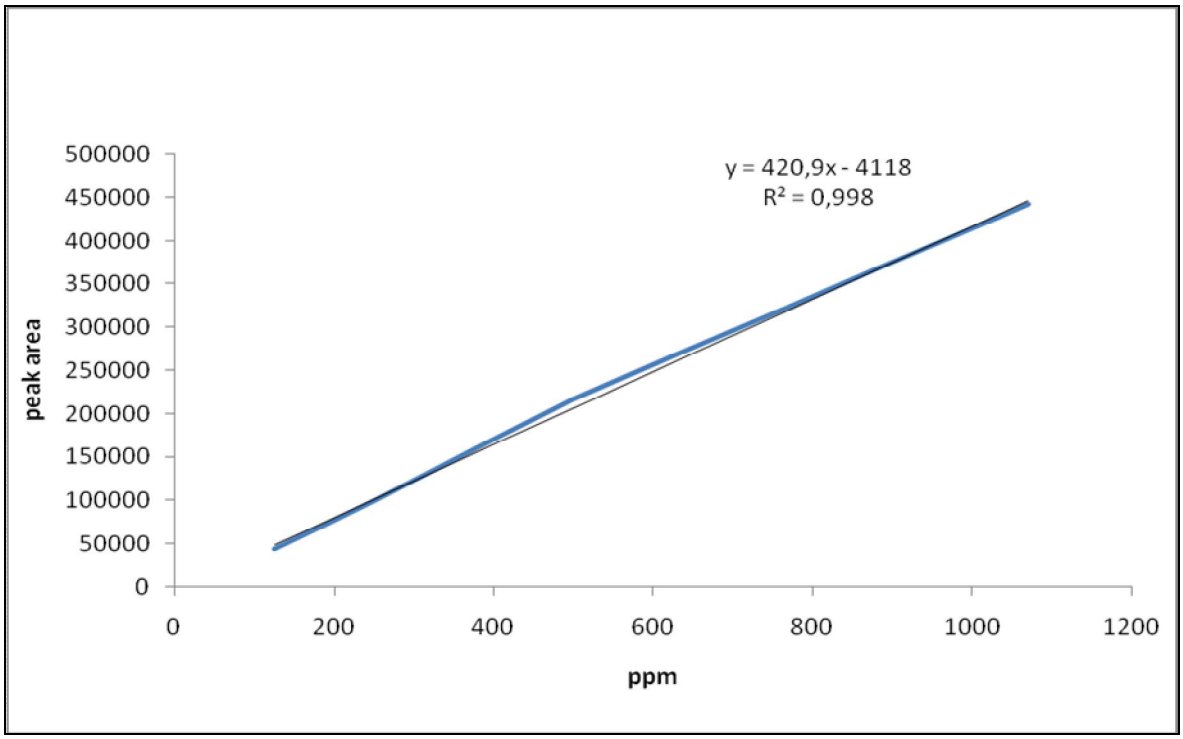
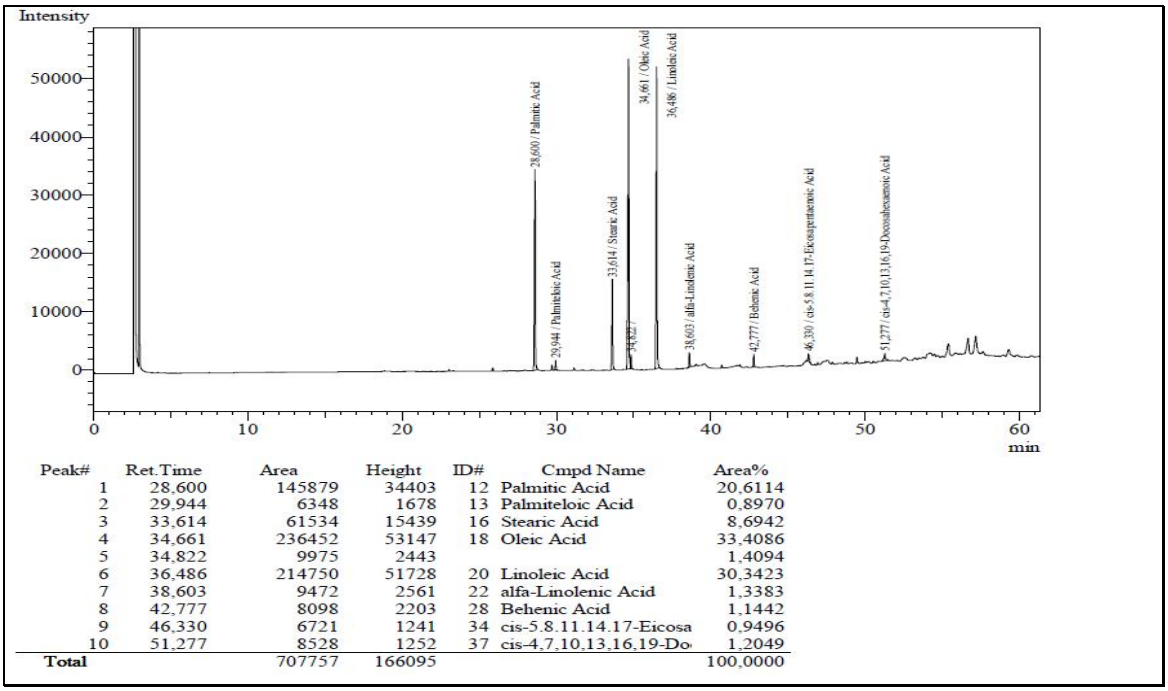
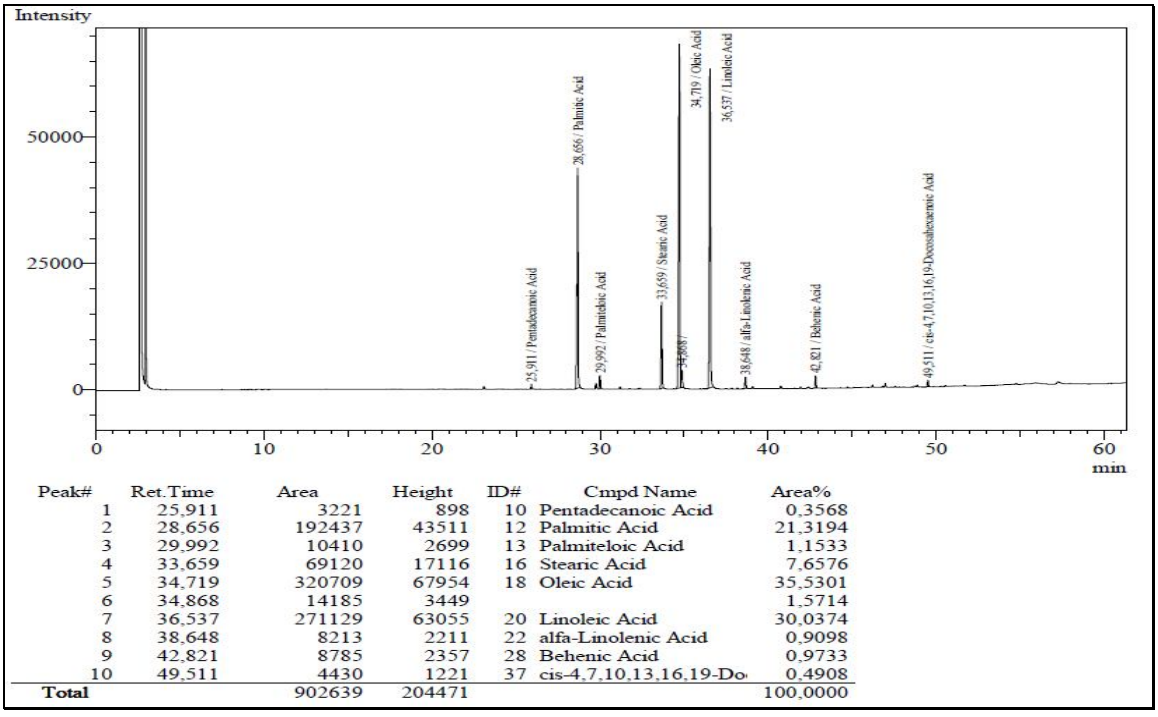


Figure B12. Sucrose used in reference to the calibration chromatogram.



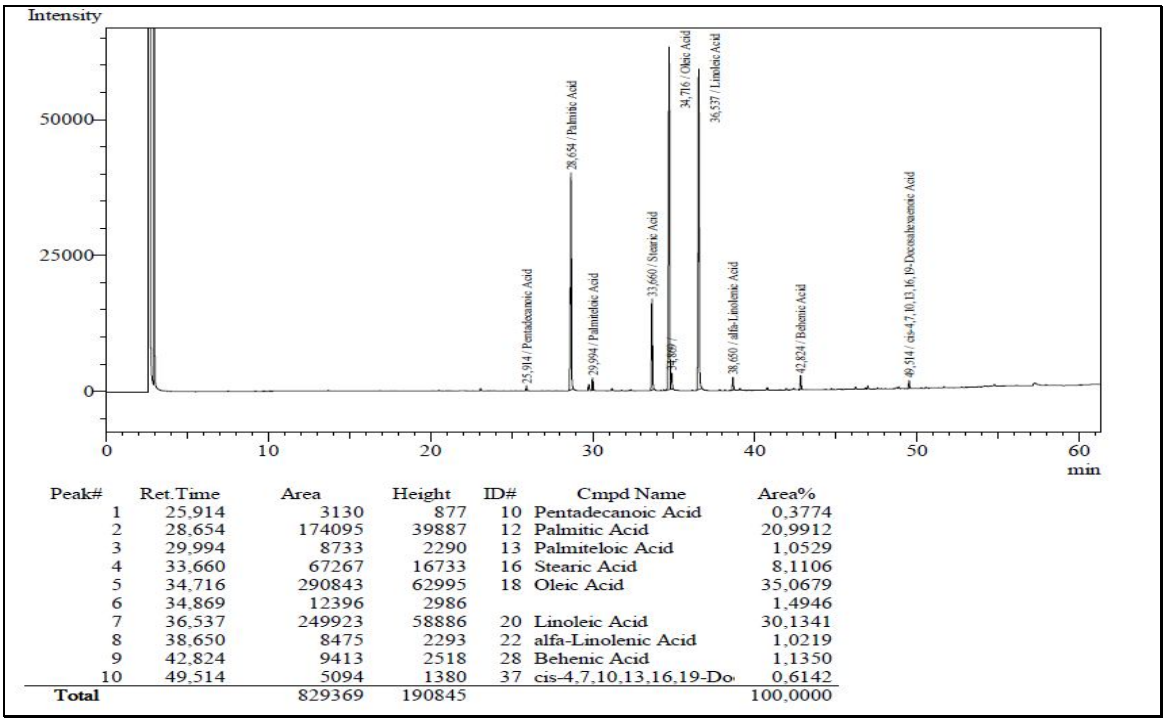
Peak#	Ret.Time	Area	Height	ID#	Cmpd Name	Area%
1	28,600	145879	34403	12	Palmitic Acid	20,6114
2	29,944	6348	1678	13	Palmiteloic Acid	0,8970
3	33,614	61534	15439	16	Stearic Acid	8,6942
4	34,661	236452	53147	18	Oleic Acid	33,4086
5	34,822	9975	2443			1,4094
6	36,486	214750	51728	20	Linoleic Acid	30,3423
7	38,603	9472	2561	22	alfa-Linolenic Acid	1,3383
8	42,777	8098	2203	28	Behenic Acid	1,1442
9	46,330	6721	1241	34	cis-5.8.11.14.17-Eicosa	0,9496
10	51,277	8528	1252	37	cis-4,7,10,13,16,19-Do	1,2049
Total		707757	166095			100,0000

Figure B13. GC-FID chromatogram of *Pleurotus ostratus* of 100% PS.



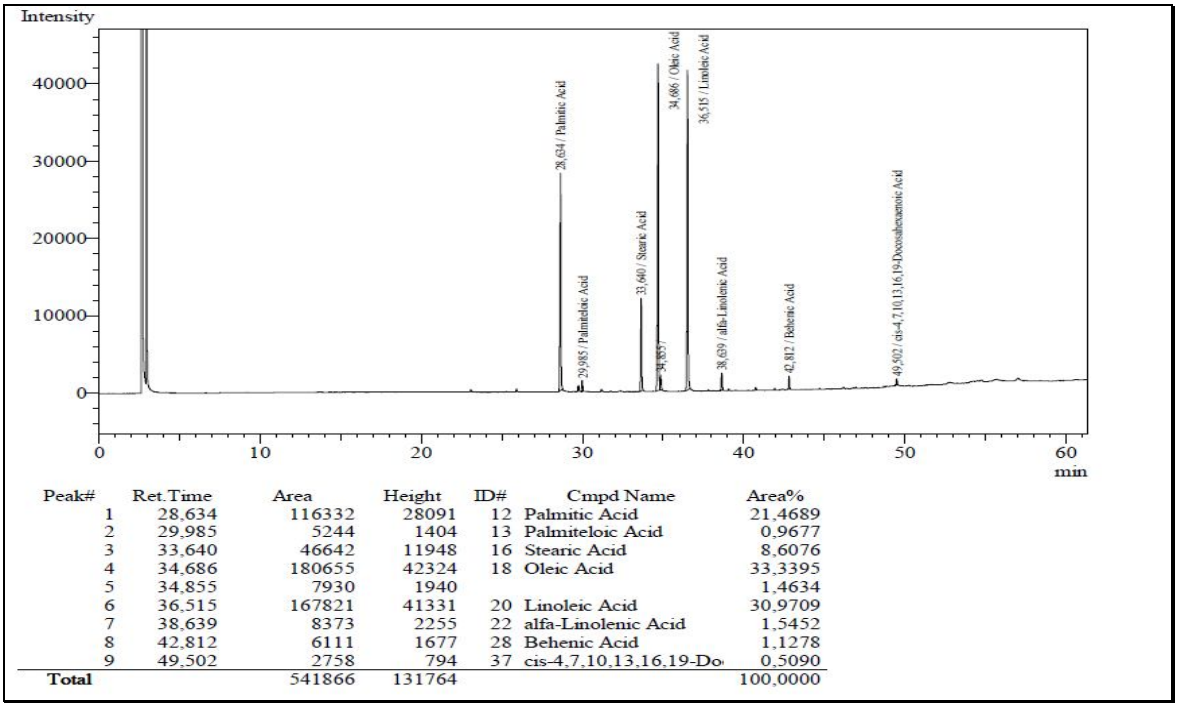
Peak#	Ret.Time	Area	Height	ID#	Cmpd Name	Area%
1	25,911	3221	898	10	Pentadecanoic Acid	0,3568
2	28,656	192437	43511	12	Palmitic Acid	21,3194
3	29,992	10410	2699	13	Palmiteloic Acid	1,1533
4	33,659	69120	17116	16	Stearic Acid	7,6576
5	34,719	320709	67954	18	Oleic Acid	35,5301
6	34,868	14185	3449			1,5714
7	36,537	271129	63055	20	Linoleic Acid	30,0374
8	38,648	8213	2211	22	alfa-Linolenic Acid	0,9098
9	42,821	8785	2357	28	Behenic Acid	0,9733
10	49,511	4430	1221	37	cis-4,7,10,13,16,19-Do	0,4908
Total		902639	204471			100,0000

Figure B14. GC-FID chromatogram of *Pleurotus ostratus* of 50% PS + 50% OW.



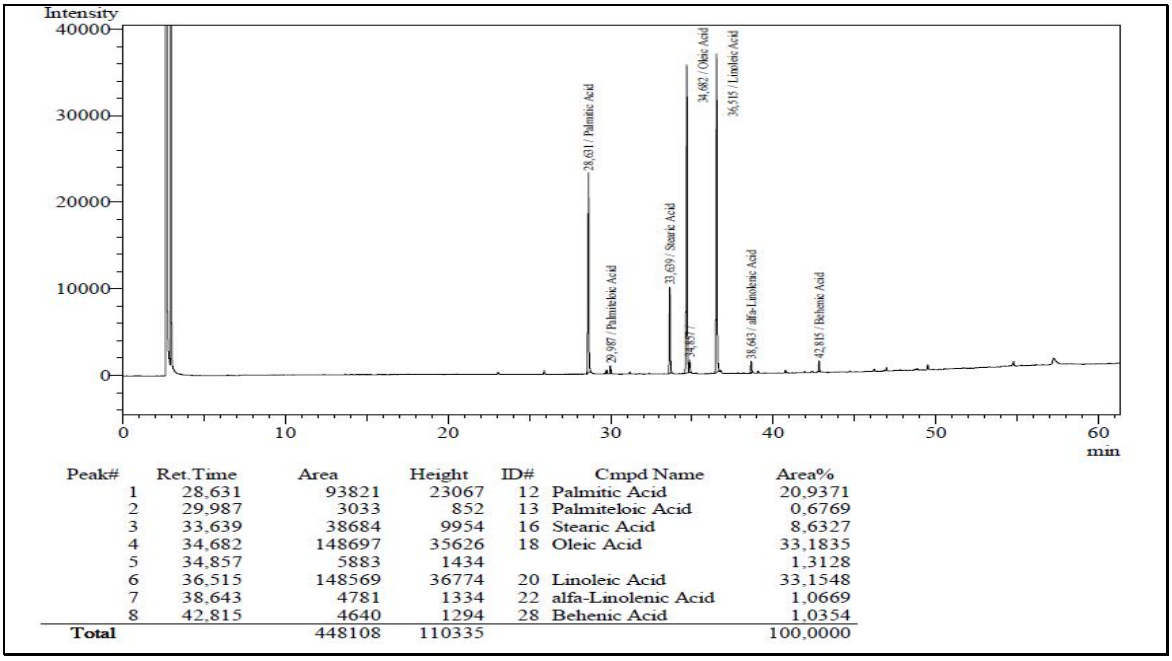
Peak#	Ret.Time	Area	Height	ID#	Cmpd Name	Area%
1	25,914	3130	877	10	Pentadecanoic Acid	0,3774
2	28,654	174095	39887	12	Palmitic Acid	20,9912
3	29,994	8733	2290	13	Palmiteloic Acid	1,0529
4	33,660	67267	16733	16	Stearic Acid	8,1106
5	34,716	290843	62995	18	Oleic Acid	35,0679
6	34,869	12396	2986			1,4946
7	36,537	249923	58886	20	Linoleic Acid	30,1341
8	38,650	8475	2293	22	alfa-Linolenic Acid	1,0219
9	42,824	9413	2518	28	Behenic Acid	1,1350
10	49,514	5094	1380	37	cis-4,7,10,13,16,19-Do	0,6142
Total		829369	190845			100,0000

Figure B15. GC-FID chromatogram of *Pleurotus ostratus* of 45 % PS + 45% OW + 10% VSW.



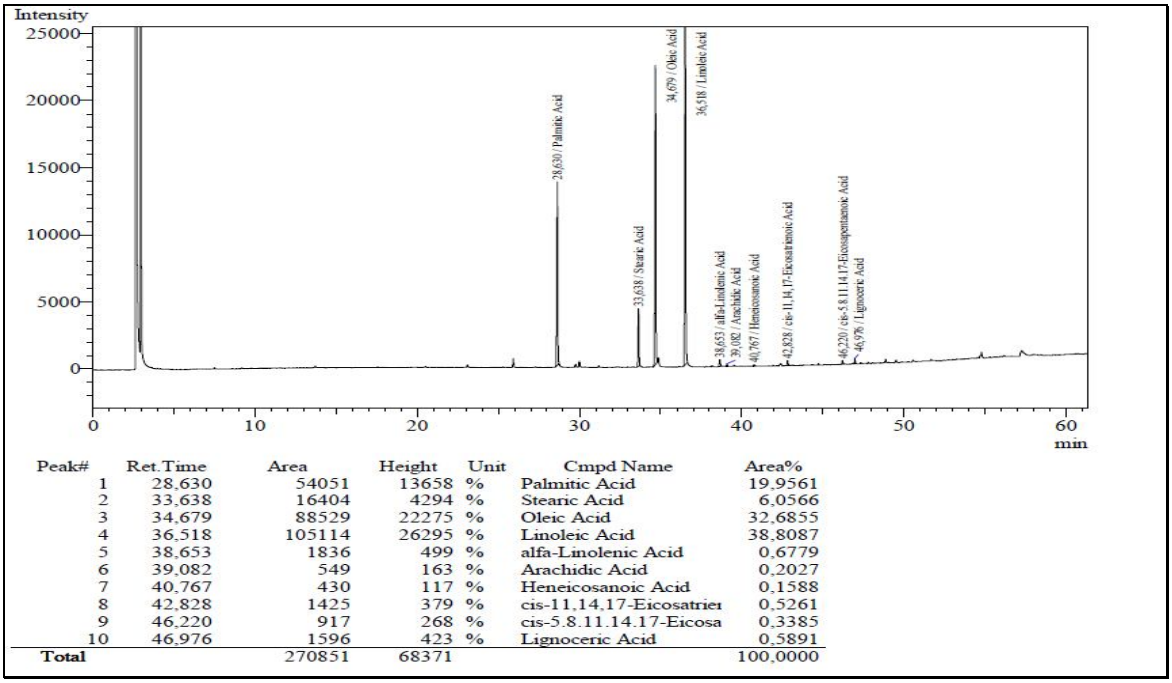
Peak#	Ret.Time	Area	Height	ID#	Cmpd Name	Area%
1	28,634	116332	28091	12	Palmitic Acid	21,4689
2	29,985	5244	1404	13	Palmiteloic Acid	0,9677
3	33,640	46642	11948	16	Stearic Acid	8,6076
4	34,686	180655	42324	18	Oleic Acid	33,3395
5	34,855	7930	1940			1,4634
6	36,515	167821	41331	20	Linoleic Acid	30,9709
7	38,639	8373	2255	22	alfa-Linolenic Acid	1,5452
8	42,812	6111	1677	28	Behenic Acid	1,1278
9	49,502	2758	794	37	cis-4,7,10,13,16,19-Do	0,5090
Total		541866	131764			100,0000

Figure B16. *Pleurotus ostratus* of 100% W lipid analyses by GC-FID chromatogram.



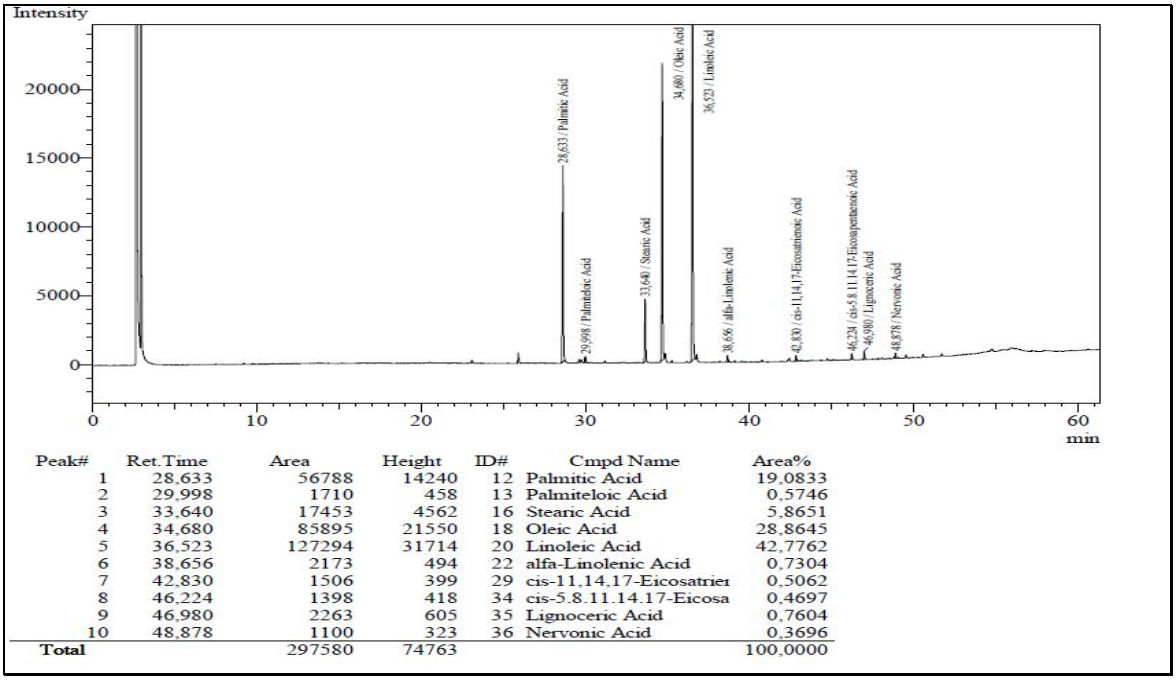
Peak#	Ret.Time	Area	Height	ID#	Cmpd Name	Area%
1	28,631	93821	23067	12	Palmitic Acid	20,9371
2	29,987	3033	852	13	Palmiteloic Acid	0,6769
3	33,639	38684	9954	16	Stearic Acid	8,6327
4	34,682	148697	35626	18	Oleic Acid	33,1835
5	34,857	5883	1434			1,3128
6	36,515	148569	36774	20	Linoleic Acid	33,1548
7	38,643	4781	1334	22	alfa-Linolenic Acid	1,0669
8	42,815	4640	1294	28	Behenic Acid	1,0354
Total		448108	110335			100,0000

Figure B17. GC-FID chromatogram of *Pleurotus ostratus* of 50% PS + 50% W.



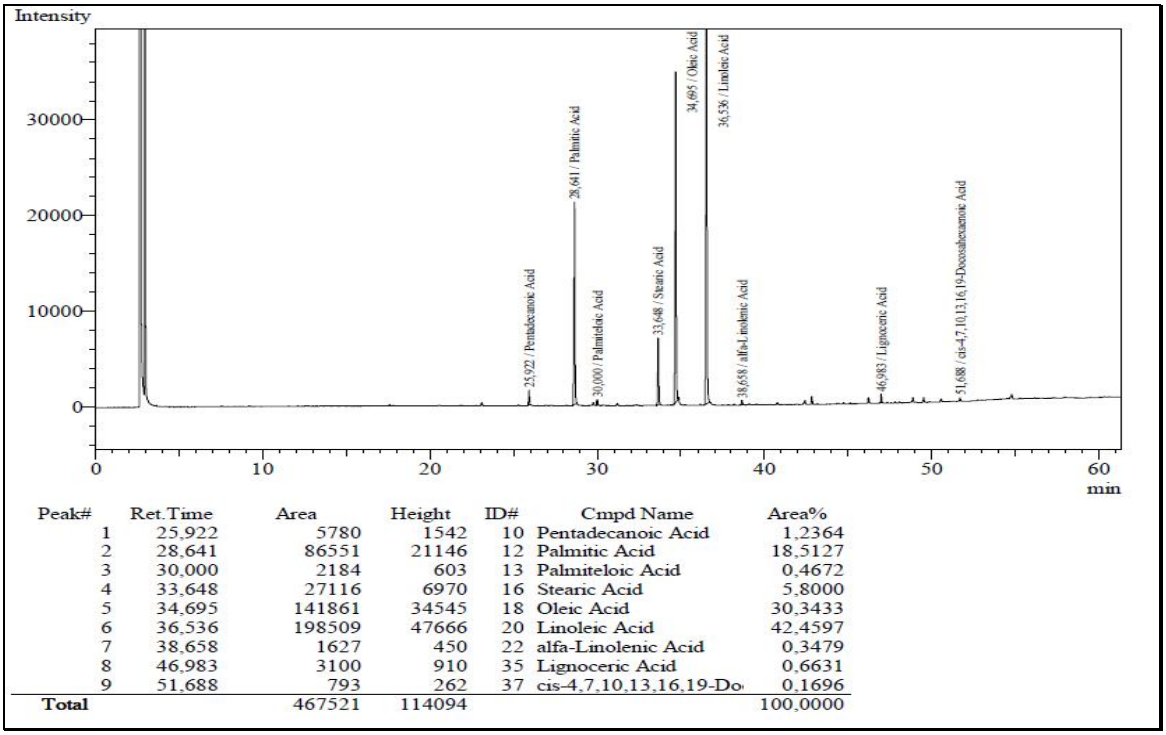
Peak#	Ret.Time	Area	Height	Unit	Cmpd Name	Area%
1	28,630	54051	13658	%	Palmitic Acid	19,9561
2	33,638	16404	4294	%	Stearic Acid	6,0566
3	34,679	88529	22275	%	Oleic Acid	32,6855
4	36,518	105114	26295	%	Linoleic Acid	38,8087
5	38,653	1836	499	%	alfa-Linolenic Acid	0,6779
6	39,082	549	163	%	Arachidic Acid	0,2027
7	40,767	430	117	%	Heneicosanoic Acid	0,1588
8	42,828	1425	379	%	cis-11,14,17-Eicosatrien	0,5261
9	46,220	917	268	%	cis-5.8.11.14.17-Eicosa	0,3385
10	46,976	1596	423	%	Lignoceric Acid	0,5891
Total		270851	68371			100,0000

Figure B18. GC-FID chromatogram of *Pleurotus ostratus* of 75% PS + 25% OW.



Peak#	Ret.Time	Area	Height	ID#	Cmpd Name	Area%
1	28,633	56788	14240	12	Palmitic Acid	19,0833
2	29,998	1710	458	13	Palmiteloic Acid	0,5746
3	33,640	17453	4562	16	Stearic Acid	5,8651
4	34,680	85895	21550	18	Oleic Acid	28,8645
5	36,523	127294	31714	20	Linoleic Acid	42,7762
6	38,656	2173	494	22	alfa-Linolenic Acid	0,7304
7	42,830	1506	399	29	cis-11,14,17-Eicosatrien	0,5062
8	46,224	1398	418	34	cis-5.8.11.14.17-Eicosa	0,4697
9	46,980	2263	605	35	Lignoceric Acid	0,7604
10	48,878	1100	323	36	Nervonic Acid	0,3696
Total		297580	74763			100,0000

Figure B19. GC-FID chromatogram of *Pleurotus ostratus* of 70% PS + 20% OW + 10% VSW.



Peak#	Ret.Time	Area	Height	ID#	Cmpd Name	Area%
1	25,922	5780	1542	10	Pentadecanoic Acid	1,2364
2	28,641	86551	21146	12	Palmitic Acid	18,5127
3	30,000	2184	603	13	Palmiteloic Acid	0,4672
4	33,648	27116	6970	16	Stearic Acid	5,8000
5	34,695	141861	34545	18	Oleic Acid	30,3433
6	36,536	198509	47666	20	Linoleic Acid	42,4597
7	38,658	1627	450	22	alfa-Linolenic Acid	0,3479
8	46,983	3100	910	35	Lignoceric Acid	0,6631
9	51,688	793	262	37	cis-4,7,10,13,16,19-Do	0,1696
Total		467521	114094			100,0000

Figure B20. GC-FID chromatogram of *Pleurotus ostratus* of 75% PS + 25% W.

CURRICULUM VITAE

Personal Identity

Name, Surname:	Lina Farhad Hussein BAZYANI
Nationality:	Iraqi
Date and place of birth:	3 June, 1985 Sulaymania, Iraq
Marital Status:	Married
E-mail:	linahussein8598@yahoo.com.

Educational Background

Degree	Place of Education	Date of Graduate
B.S.c	College of Agriculture/Forestry Department Dohuk University, Iraq	2006 -2007

Publications

KANAT, G. H., DÜZKALE, G. S., BEŞIKCI, N., GÖÇERI, A., **BAZYANI, L. F. H**., ALMA, M. H., (2014). Farklı Ortamlarda Yetiştirilmiş Pleurotus Ostreatus'un Ekstraksiyonu ve Analizi, 3rd International Non-wood Forest Products Symposium, 8 May 2014, Kahramanmaraş.