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The Technological and Biological Role of Oyster Mushrooms (*Pleurotus ostreatus* L.) in Some Functional Foods

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ABSTRACT

Oyster mushrooms belonging to the *Pleurotus* genus are healthy and sustainable, growing on agricultural waste substrates. In order to enhance the functional properties of burgers, the study incorporated oyster mushrooms into the recipe. The resulting oyster mushroom product underwent a comprehensive analysis of its physico-chemical properties and sensory evaluations, as well as oyster mushroom phytochemical screening, chemical composition phytochemical screening antioxidant, and antimicrobial activities. The study revealed that this mushroom is rich in various bioactive compounds with promising applications in both the food and pharmaceutical sectors. The results showed that it had a total phenolic content of 130.2 µg GAE/100g and a total flavonoid content of 250.3 µg RE/100g. The product was found to be a rich source of proteins (34.19%) and fibers (8.12%). Additionally, the *Pleurotus* extract was observed to have antimicrobial activity against some strains of pathogenic potential for humans. The impact of powdered oyster mushroom as a dietary supplementation on biochemical changes in rats with high cholesterol levels was studied. The results showed a significant decrease in lipid profile in comparison to control groups. A burger product enriched with oyster mushroom samples was also subjected to sensory evaluation, which indicated general acceptance of all attributes. In conclusion, the oyster mushroom is a nutritious food and a great source of antioxidants. This makes it a suitable ingredient to replace certain additives in meat products, making it a functional food option for those with hyperlipidemia.

Keywords: Oyster Mushroom, Functional Foods, Oyster Mushroom Burger, Hyperlipidemia, liver functions, weight gain.

1. Introduction

Functional foods are a highly nutritious category of foods renowned for their many health benefits. These specially-designed foods promote optimal health and help lower the risk of several diseases, including cardiovascular diseases, cancer, hyperlipidemia, osteoporosis, diabetes, and hypertension. With their multifunctional properties, they can act as anti-obesity, anti-diabetic, anti-cancer, and immune-boosting agents. They are also formulated to cure degenerative diseases and are a regular part of many people's diets. Additionally, functional foods are effective at managing chronic inflammatory disorders, making them an excellent choice for those who seek to maintain their health and wellness (Morris *et al*., 2017).

According to Yeung *et al*. (2018), the most popular topics of focus are nutraceuticals and functional foods such as prebiotics, probiotics, antioxidants, and phenolic contents. Recently, functional edible and medicinal mushrooms have gained recognition as a potential natural source for creating innovative functional foods, and dietary supplements.

There are many ways to use fresh and dried mushrooms, their powders, extracts, and compounds in food. By incorporating them into different types of foods, it's possible to create fortified functional foods that can be used instead of other ingredients like flour, meat, fat, salt, phosphates, nitrates, and

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antioxidants. This can help to extend the shelf-life of the food and make it more affordable to produce. Many different types of foods have already been studied to see how mushrooms can be incorporated, including bakery items, meat dishes, dairy products, fermented foods, fruits, vegetables, and beverages. These studies have shown that the resulting value-added products may have some changes in nutritional, sensory, textural, and pharmacological properties (Morris *et al*., 2017).

Oyster mushrooms, also known as Pleurotus mushrooms, have caught the attention of nutritionists and food technologists for their nutraceutical properties. With approximately 200 different species under the Pleurotus genus, these mushrooms are regarded as functional foods due to their high content of functional food ingredients. As a result of their numerous health benefits, the consumption of oyster mushrooms has recently increased. These mushrooms are considered as potential sources of bioactive components that can aid in the prevention and treatment of various lifestyle diseases (Yasar and Tosun, 2020).

Oyster mushrooms are popular due to their great taste and health benefits. They are rich in antioxidants, proteins, dietary fibers, vitamins, and minerals. They also contain high amounts of amino acids like methionine, cysteine, and aspartic acid. Oyster mushrooms have many health benefits, including anti-bacterial, anti-diabetic, anti-oxidant, anti-arthritic, anti-carcinogenic, and hepato-protective properties. They can also be used to address protein deficiencies in some countries (Vital *et al*., 2015).

Elevated cholesterol and triacylglycerol levels also weight gain are known risk factors for cardiovascular diseases. A number of animal studies have indicated that the consumption of oyster mushrooms (*Pleurotus ostreatus*) can positively influence the lipid profile. From the information given, it appears that oyster mushrooms are packed with nutrients and biologically active substances, for this reason, oyster mushrooms have been selected to serve as a substitute for some additives in meat products and enhance the functional properties of burgers. This makes it a feasible option for people with high blood lipids, and consequently, it may gain popularity among this group of individuals in the future.

2. Material and Methods

The edible mushroom (*Plueotus ostreatus*) was provided by the Desert Research Center (DRC), Cairo, Egypt. The components needed for burger products were purchased from the local central markets in Cairo, Egypt. *In vivo* study was done in the Veterinary of Medicine, Benha University, Egypt.

2.1. Conversion of Fresh Edible Mushroom to Dried Powdered Form

The mushroom samples were sorted by hand; trimmed using a stainless knife and cleansed very well. The whole mushroom is cut into uniform sizes to facilitate the drying. The whole mushroom was dried in an oven at 65ºC for almost five hours. After drying, the whole mushroom was milled and sieved to pass through a 250 µm sieve. The mushroom powder obtained was packaged in polyethylene bags and used for further analysis. For proper extraction, 70% ethanol was utilized.

2.2. Determination of Proximate Compositions and Mineral Content

The contents of moisture, dry matter, protein, crude fiber, and the total amount of ash were measured by AOAC (2005). The fat content of the sample was determined by Pearson (1976). The Iron content was measured colorimetrically at 480nm according to the method (AOAC, 2005).

2.3. Phytochemicals screening

Alkaloids, steroid*s*, and flavonoids were determined by the method described by Haborn (1998). Saponins were determined by the method described by AOAC (2000). Phenol and terpenoids were determined by the method described by Person (1976).

2.4. Total phenolic content assay

To analyze the total phenolic content, we used Folin-Ciocalteu reagent as per Chun *et al.* (2003). Firstly, we mixed 0.5 ml of the extract with 0.5 ml of Folin-Ciocalteu reagent and kept the solution at 25ºC for 5-8 min. Then, we added 2 ml of sodium carbonate solution (7.5%) and adjusted the volume to 8 ml with water. After 2 hours, we measured the absorbance at 725 nm using Gallic acid as a standard for the calibration curve. The total phenolic content is expressed as mg Gallic acid equivalents per gram of the sample (mg/g).

2.5. Determination of total flavonoid content

To measure the total flavonoid content, a colorimetric assay was conducted using the method outlined by Zhishen *et al*. (1999). A 100-microliter extract was combined with 4 milliliters of distilled water. Next, 0.3 milliliters of 5% sodium nitrite was added, followed by 0.3 milliliters of 10% aluminum chloride after 5 minutes. In 6 minutes, 2 milliliters of 1 M sodium hydroxide was added to the mixture. The mixture was then quickly diluted with 3.3 milliliters of distilled water and thoroughly mixed. The absorbance was measured at 510 nm against a blank. Rutin was used as a calibration standard. The total flavonoid content of the extract was reported in mg rutin equivalents per gram of sample (mg/g).

2.6. DPPH scavenging activity

To measure the activity of free radical scavenging, we utilized the DPPH assay, which involved adding 5 ml of an 80 mM DPPH radical solution to 1 ml of extract solutions ranging from 0.375 to 3 mg/ml. After allowing the reaction to proceed for 30 minutes, we measured the absorbance at 515 nm using a spectrophotometer. From the plotted graph of radical scavenging activity against extract concentration, we calculated the IC_{50} value, which represents the concentration of sample needed to scavenge 50% of the DPPH free radical (Dharmishtha *et al*., 2009).

2.7. Antimicrobial activity

Samples of *Staphylococcus aureus ATCC3536 and Escherichia coli ATCC8739* were obtained from the Egyptian Microbial Culture Collection at the Cairo Microbiological Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. These strains were kept under sterile conditions and grown on Nutrient Agar (NA). To conduct the *in vitro* anti-microbial activity study, we dissolved 0.3 grams of mushroom extract sample in 0.1 ml (100 µl) of 100 % (DMSO) on the day of experimentation. For the anti-microbial sensitivity test using the paper disc diffusion method, we mixed the suspension with distilled water to create a volume of 1 ml (1000 µl). We prepared various concentrations of the suspension as described by Chetan *et al.* (2012).

2.8. *In vivo* study

Twenty-five male Sprague-Dawley albino rats, weighing 150 g at the beginning of the study, were kept in aluminum cages with screen bottoms. The rooms were maintained at a temperature of 25±1°C and had alternating cycles of light /darkness that lasted 12 hours. The rats were divided into two main groups: the normolipidemic (NC-L) group fed on a basal diet, which had five animals, and the hyperlipidemic group (HL), which had twenty animals. The HL group was further divided into four subgroups: positive control-HL (PC-HL) fed on a basal diet with 1% cholesterol, and three HL-treated groups (HL-O) fed on a basal diet with 1% cholesterol and 5%, 10%, and 20% powdered oyster mushroom [Table 1 represented basal diet ingredients as described by Alam *et al*. (2011)]. The experiment duration was 45 days, and the changes in body weight were recorded weekly.

Table 1: Basal diet ingredients

Ingredient	(%)
Wheat flour	50.00
Wheat bran	19.00
Rice powder	11.25
Egg white	10.00
Casein	8.00
Soya bean oil	1.00
Table salt	0.50
Mixture of vitamins	0.125
Mixture of menials	0.125

Blood samples were also obtained from a tail vein with a disposable plastic syringe. Serum was obtained from blood samples by centrifugation at 1500 rpm for 15 min at an ambient temperature for liver enzymes (ALT, AST) levels were measured enzymatically using commercially available assay kits. Plasma was collected into heparinized tubes, and prepared by centrifugation at 2500 rpm for 10 min at an ambient temperature for triglyceride (TG), total cholesterol (TC), high-density lipoprotein

cholesterol (HDL), low-density lipoprotein cholesterol (LDL) levels were measured enzymatically using commercially available assay kits. All the serum and plasma samples were stored under -20 °C before use.

2.9. Mushroom beef burger preparation and characterization

4 kg of minced meat was used in this study. Beed Burgers were prepared in 4 treatments (control and 3 treatments) according to the procedure described by Ali *et al*. (2011) with slight adjustments. The ingredients % include the following: Individual mixtures with 0, 5, 10, and 20% replacement levels were created by blending beef meat (70, 65, 60 and 50%) with dried mushroom, and the sheep fat was added as shortening (10%), corn starch (10 %), burger spices (5%), and table salt (5%). To achieve a homogeneous mixture, all ingredients were mixed for 5 minutes on medium speed in a food processor. The resulting mixture was shaped into circular patties measuring 12.5cm in diameter, 1.0 cm in thickness, and weighing approximately 50g for each. Prior to packaging in polyethylene bags, each patty was separated from the others using a layer of polyethylene and stored at -18°C. The burger samples were then grilled in a preheated oven at 163°C for 10 minutes, ensuring uniform browning without charring. Three replicates were taken from each batch and subjected to chemical composition [A.O.A.C. (2005)], physical properties [water holding capacity according to Denhetog- Meishchke *et al.* (1997), and shrinking according to the method of El-Akary (1986)], Caloric values (Kcal) by using the method of Watt and Mersil (1975) and organoleptic properties analysis (Crehan *et al.,* 2000).

2.10. Statistical analysis

The data from the study underwent statistical analysis. The Statistic version 9 Analytical Software (2008) computer program was used to determine significant differences in treatment means at the 5% level, utilizing the least significant difference (L.S.D). In cases where means were represented by the same letter, there were no significant differences at the $p \leq 0.05$ level, as per Duncan's multiple range tests outlined in Gomez and Gomez's (1984) methodology.

3. Results and Discussion

When creating herbal extracts such as *P. ostreatus*, it's crucial to take into account various factors that can impact the final product's quality. These factors involve the drying technique, solvent selection, and the ratio of solvents to solutes. Ethanol is widely utilized in extractions due to its ability to dissolve both polar and nonpolar substances. To improve its capacity to extract polar active compounds, water can be combined with the mixture to reduce the ethanol percentage. This study utilized a concentration of 70% ethanol.

3.1. Proximate, Mineral, and Phytochemical Composition of dried powdered *Pleurotus ostreatus* extract

Table (2) presents the results of the proximate analysis done on the oyster mushroom sample. The tested sample showed a moisture content of 41.40%, protein content of 20.42%, ash content of 1.55%, fat content of 3.50%, crude fiber content of 2.20%, and total carbohydrate content of 30.93%. These values were in agreement with those reported by Afiukwa *et al*. (2013). The high moisture content indicated that the mushroom was fresh, but it also made it susceptible to microbial growth and enzyme activity. Wong and Chye (2009) suggested that reducing the moisture content during processing could increase the nutrient concentration and extend the shelf life. The ash content of the oyster mushroom was relatively low (1.55%), but it support the diet, particularly with important minerals.

The fat content of oyster mushrooms is around 3.50%. Fat plays a crucial role in providing energy to muscles and body processes, regulating body temperature, and aiding in proper digestion and nutrient absorption. In comparison to the findings of Afiukwa *et al.* (2013), the fiber content of oyster mushrooms is relatively low at 2.20%. Fiber is essential for normalizing bowel movements, improving overall health, lowering cholesterol, controlling blood sugar levels, and supporting healthy weight (Alam *et al.,* 2007).

The protein content of oyster mushrooms is high at 20.42%, which means that they are a rich source of protein from a nutritional standpoint. The protein content of oyster mushrooms is comparable to the 16.35% reported by Afiukwa *et al.* (2013). Proteins play a vital role in catalyzing chemical reactions in the body, regulating gene expression, forming the major structural elements of all cells,

regulating the immune system, and making up the major constituents of muscles. Oyster mushrooms are also a good source of carbohydrates, with a value of 30.93%. Carbohydrates are essential for providing energy to the body and brain. They are broken down into glucose, which is used as fuel by the body's cells, tissues, and organs (Khan *et al.,* 2013).

Table 2: Proximate composition of dried powdered *Pleurotus ostreatus* extract.

Components	(%)
Moisture	41.40
Protein	20.42
Ash	1.55
Fat	3.50
Crude Fiber	2.20
Carbohydrate	30.93

Table (3) shows that the oyster mushroom sample contains important mineral elements such as Ca, P, Fe, Na, K, Mg, and Zn. The sample contains all the mineral elements in substantial amounts. A study conducted by Afiukwa *et al.* (2013) revealed that the concentrations of Fe, Zn, and P were the lowest. Oyster mushrooms are a great source of calcium, with a concentration of 2.89 mg per 100g. This mineral is essential for the proper functioning of nerves, muscles, and bones in humans and other vertebrates. Although the phosphorus levels in oyster mushrooms are relatively low at 0.56, this mineral is still important for nucleic acid production, acid-base balance, and bone and tooth formation.

Oyster mushrooms also contain sodium and potassium, which are crucial for maintaining the balance of fluids in animal cells. The sample used in the study contained 21.64 mg of Na and 13.81 mg of K per 100g of oyster mushroom, suggesting that it could help lower blood pressure and maintain healthy bones, potentially reducing the risk of osteoporosis (Wani *et al.,* 2010). Although the oyster mushroom sample contained only a small amount of iron (0.86 mg per 100g), this mineral is still essential for the biosynthesis of hemoglobin and cytochromes, which are involved in cellular respiration. The concentration of magnesium and zinc in the oyster mushroom sample (12.45 mg and 1.20 mg per 100g, respectively) was similar to that detected in a previous study (Afiukwa *et al.,* 2013). These minerals are important co-factors for certain enzymes and are involved in numerous biochemical pathways.

Table 3: Mineral composition of dried powdered *Pleurotus ostreatus* extract

Mineral	(mg/100g)
Sodium (Na)	21.64
Calcium (Ca)	2.89
Potassium (K)	13.81
Magnesium (Mg)	12.45
Zinc (Zn)	1.20
Iron (Fe)	0.86
Phosphorus (P)	0.56

In Table (4), the phytochemical analysis of *Pleurotus ostreatus* mushroom shows the presence of alkaloids, saponins, steroids, phenols, terpenoids, and flavonoids. It was discovered that the edible mushroom variety contains important phytochemicals. The levels of these phytochemicals were found to be considerably lower than the safe limits reported by the World Health Organization, indicating that these mushrooms are safe for consumption. These mushrooms can also be a good source of natural antibiotics and antioxidants. It is safe to consume these types of mushrooms in large quantities without any toxic effects (Wandati *et al.,* 2013).

Pleurotus ostreatus mushrooms have phyto-constituents that are crucial to their medicinal properties. One such group is saponins, which are made up of steroids or terpenoids and have many pharmaceutical benefits, like anti-inflammatory and anti-diabetic effects. These mushrooms can be

helpful in treating inflammation-related diseases and managing diabetes. Terpenoids also have many pharmacological benefits, including anti-malarial, anti-inflammatory, and anti-cancer effects. Phenolic compounds are known for their antioxidant properties and have a wide range of medicinal benefits, including anti-cancer and anti-inflammatory effects. These mushrooms can be used to manage diseases caused by oxidative stress, as phenols and flavonoids have been found to have multiple antioxidant functions. Terpenoids are secondary metabolites that have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory, and anti-cancer among others (Hamzah *et al.,* (2013).

Table 4: Phytochemical screening of dried powdered *Pleurotus ostreatus* extract.

Phytochemicals	Qualitative screening
Alkaloids	++
Flavonoids	+++
Phenols	+++
Saponins	+
Steroids	+
Terpenoids	+

3.2. Total polyphenols content, antioxidant, and antimicrobial activities of dried powdered *Pleurotus ostreatus* extract

In recent years, there has been a growing interest in polyphenols, which are organic compounds found in plants and are believed to have a significant impact on health. Studies suggest that consuming polyphenols can regulate metabolism, weight, chronic diseases, and cell proliferation. The *Pleurotus ostreatus* mushroom is a good source of phenolic compounds, including flavonoids. The results from Table (5) show that the total phenolic content (TCP) was 1.76±0.09 mg GAE/g, and the total flavonoid content (TFC) was 2.81±0.02 mg GAE/g, which is similar to the findings reported by Oke & Aslim, (2011), ranging from 0.93 to 1.42 mg GAE/g.

Several mushroom species, including *P. ostreatus*, have been found to contain polyphenols, which are major antioxidant components (Gąsecka *et al.,* 2016). The antioxidant activity of *P. ostreatus* was evaluated using the DPPH scavenging method, which showed a recording of 51.42±2.0%. This method is commonly used to quickly evaluate the antioxidant activity of specific compounds or extracts. DPPH is a stable, free radical that reacts with antioxidant molecules, resulting in a pale color in alcohols (Chorvathova *et al.,* 1993). The activity of *P. ostreatus* ethanolic extract suggests the presence of antioxidant components that can quickly react with DPPH radicals. The IC_{50} values for 50% inhibition of DPPH free radicals were found to be 47.7±1.8 mg/ml (Table 5), which is consistent with other studies that have found potent antioxidant activity against DPPH radicals in ethanolic extracts of *Pleurotus* spp. (Fernandez-Panchon *et al.,* 2008).

Table 5: Total polyphenols content and DPPH scavenging activity of dried powdered *Pleurotus ostreatus* extract

TPC mg GAE/g sample	TFC mg RE /g sample	DPPH scavenging activity (%)	IC_{50} (mg/ml)
1.76±0.09	2.81±0.02	51.42±2.0	47.7±1.8

Various studies have suggested that mushrooms may be a promising source of antimicrobials to combat a variety of pathogens, such as pathogenic bacteria, fungi, and viruses (Masri *et al.,* 2017). To determine the potential antimicrobial activity of a prepared extract, the agar-well diffusion method was used to test it against two foodborne bacterial pathogens. Initial findings showed that the 70% ethanolic extract of *Pleurotus ostreatus* (100 ml) demonstrated good antibacterial activity. Specifically, the ethanolic extract (70%) exhibited high antibacterial activity against Gram-negative bacteria, with inhibition zones ranging from 16 to 24.5 mm for the *E. coli* strain. Meanwhile, the mean inhibition zone of growth for the tested Gram-positive bacteria (*Staph aureus*) ranged from 17 to 22.60 mm, compared to the control Amikacin (30 µl) (Table 6).

According to research by Vaz *et al.,* (2011), certain phenolic compounds are linked to antioxidant activity, including radical scavenging activity. A study of *Pleurotus ostreatus* extract revealed that it contains phytochemicals that not only exhibit antioxidant activity but also antimicrobial activity. The antimicrobial and antioxidant properties of the extract are attributed to the presence of phenolic and flavonoid compounds. The extract of this mushroom showed high antimicrobial activity due to its high TPC and TFC content, making it a potential natural antimicrobial. Therefore, the 70% ethanolic extract of this mushroom can be used to promote health and prevent illness.

Table 6: Antimicrobial activity of dried powdered *Pleurotus ostreatus* extract.

Drug	*Escherichia coli*	*Staphyllococcus aureus*
Amikacin (30 µl) [antibacterial control]	28.30 mm	25.20 mm
Dried powdered *Pleurotus ostreatus* extract (100 ml)	16 to 24.5 mm	17 to 22.60 mm

3.3. Effect of dried powdered *Pleurotus ostreatus* extract on body weight gain, relative liver weight, and biochemical profiles in a hyperlipidemic *in vivo* model.

Weight gain and obesity can increase the chances of developing high cholesterol. Treating obesity can help reduce the risk of hyperlipidemia and its complications. High levels of cholesterol are often linked to several risk factors, including a diet rich in saturated fats and salty foods like fast food. To address this issue, a recent study suggests replacing some of these ingredients with healthier alternatives such as mushrooms. Mushrooms can be used as a snack or in various recipes that are widely enjoyed. As modern medicine continues to advance, traditional dietary practices like incorporating mushrooms into our meals play an essential role in maintaining good health.

The study, represented in Table (7), explores the impact of dried powdered *Pleurotus ostreatus* extract on weight gain or obesity in a hyperlipidemic rat model. The group of rats fed on a basal diet and powdered oyster mushroom (HL-O) showed a significant decrease ($p \leq 0.05$) in all oyster mushroom concentrations used during the experiment duration. The 20% oyster mushroom concentration (initial stage =149.4gm, final stage =169.2gm) demonstrated the most significant decrease in weight gain when compared to the control groups. Additionally, there was a significant decrease ($p \leq 0.05$) in the relative weight of liver organs at the final stage of the experiment, especially with the 20% oyster mushroom concentration (2.95gm) when compared to the control groups. The obtained results were supported by research, which revealed that feeding hypercholesterolemic rats *P. ostreatus, P. sajor-caju, and P. florida* mushrooms significantly decreased body weight by 17.36%, 23.37%, and 24.13%, respectively (Lee *et al.,* 2009).

Table 7: Effect of oral administration of dried powdered *Pleurotus ostreatus* at different concentrations on body weight gain and relative weight of the liver organs by gm *in vivo*.

Parameters	NC-L	PC-HL	HL-O (%) per day		
			5%	10%	20%
Body weight gain/gm					
Initial stage	$149.8^{b} \pm 9.9$	$153.5^{a} \pm 4.6$	$151.2^{a} \pm 4.8$	$150.9^{ab} \pm 4.9$	$149.4^{b} \pm 9.7$
Final stage	$170.7^{b} \pm 10.5$	$177.1^{a} \pm 11.5$	$172.4^{ab} \pm 11.2$	$171.6^{b} \pm 10.3$	$169.2^{c} \pm 9.3$
Relative weight of liver organs (final)/gm					
Liver	$3.33^{b} \pm 0.59$	$4.15^{a} \pm 1.29$	$3.34^{b} \pm 0.6$	$3.25^{b} \pm 0.55$	$2.95^{c} \pm 0.5$

NC-L: normolipidemic group, **PC-HL:** hyperlipidemic positive control group, **HL-O:** hyperlipidemic group fed on a basal diet+ powdered oyster mushroom. Values represent the mean ± SD, Values in the same row that do not share a common superscript are significantly different at $p \leq 0.05$.

The data presented in Table (8) displays the impact of dried *Pleurotus ostreatus* extract on biochemical profiles, including the lipid profile and liver enzymes. The HL-O group showed a significant decrease ($p \leq 0.05$) in cholesterol, triglycerides, and total lipid levels across all concentrations of oyster mushroom extract used, compared to the control groups. Additionally, the levels of HDL and LDL showed significant values ($p \leq 0.05$) at a 20% concentration of *Pleurotus ostreatus* extract (81.5 mg/dl and 12. mg/dl, respectively) compared to the control group. Agrawal

(2010) confirmed that the oyster mushroom diet effectively prevented hyperlipidemia progression and cholesterol accumulation in rat liver induced by a cholesterol diet which matched our results.

In terms of liver enzymes, the HL-O group showed a significant decrease ($p \leq 0.05$) in AST levels across all oyster mushroom concentrations compared to the control group. ALT levels recorded significant values ($p \leq 0.05$) at 10% and 20% concentrations of *Pleurotus ostreatus* extract (35.85 U/L and 31.51 U/L, respectively) compared to the control group. This is because *P. Ostreatus* contains antioxidant phytochemicals and other bioactive compounds such as phenolic components, flavonoids, terpenes etc (Rahimah *et al.,* 2019). According to a study by Waktola *et al.* (2020), *P. Ostreatus* possesses significant antioxidant properties that can help in combating the harmful effects of hyperglycemia-induced free radicals. Additionally, consuming *P. Ostreatus* was found to markedly decrease the levels of liver damage markers compared to the positive control.

Table 8: Effect of *Pleurotus ostreatus* extract on biochemical profiles in hypercholesterolemic *in vivo* model.

Parameters	NC-L	PC-HL	HL-O (%)		
			5%	10%	20%
Total cholesterol(mg/dl)	102.06 ^{b} ±9.52	120.6 ^{a} ± 10.3	85.2 ^{c} ± 8.6	83.5 ^{c} ± 8.15	82.82 ± 8.03^{c}
Triglycerides (mg/dl)	63.8 ^{b} ± 11.3	87.2 ^{a} ± 12.8	59.3 ^{bc} ± 6.8	51.3^{c} ± 6.03	50.2^{c} ± 5.02
HDL-cholesterol (mg/dl)	42.2 ^{b} ± 2.2	37.6 ^{c}± 2.9	52.31 ^{ab} ± 2.6	77.22^{a} ± 5.2	81.5 ^{a} ± 3.1
LDL-cholesterol (mg/dl)	17.0 ^{ab}±5.8	20.8 ^{a}±2.3	15.4 ^{b} ±4.9	14.9^{b} ±4.1	12.6 ^{c} ±3.1
Total lipids (mg/dl)	357.8 ^{ab} ± 9.9	423.3 ^{a}±4.8	315.0^{b} ±10.4	303.14 ^{c} ±11.26	300.5 ^{c} ±11.13
AST activity (U/L)	41. 80 ^{b} ±18.3	44.40 ^{a}± 14.4	37.61^{bc} ± 9.3	31.44 ^{c} ± 6.4	28.36 ^{c} ± 3.3
ALT activity (U/L)	40.44 ^{b} ± 3.95	50.07 ^{a} ±12.02	39.84 ^{b} ± 6.44	35.85^{bc} ± 3.35	31.51^{c}± 4.01

NC-L: normolipidemic group, **PC-HL:** hyperlipidemic positive control group, **HL-O:** hyperlipidemic group fed on a basal diet+ powdered oyster mushroom. Values represent the mean ± SD, Values in the same row that do not share a common superscript are significantly different at $p \leq 0.05$

As mentioned before, obesity is not just a matter of beauty concern, it is a medical concern that increases the risk of various diseases and health problems, such as heart disease, diabetes, high blood pressure, and certain cancers. Obesity often results from consuming more calories than are burned through exercise and daily activities. When a person's body mass index (BMI) is 25 or higher, excessive body fat increases the risk of serious health problems. The primary treatment involves making lifestyle changes, including diet and exercise. Childhood obesity is associated with a higher risk of obesity, premature death, and disability in adulthood. Additionally, obesity may cause some hormonal imbalances that are linked to several detrimental health problems which lead to cardiovascular abnormalities and insulin resistance (Soliman, 2019).

Various bioactive compounds in mushrooms make them a popular food, nutraceutical, and medicine for preventing oxidative stress. Mushrooms are a valuable source of protein and fiber while remaining low in fat and calories, making them a beneficial addition to one's diet for essential vitamins and minerals (Roman et al., 2006). The slogan "Medicines and foods have a common origin" is exemplified in mushrooms, which are both a functional food and a source of medicinal benefits recognized in China, Korea, and Japan. Mushrooms are considered a special type of nutraceutical due to their various medicinal properties, such as anticancer, antibiotic, antiviral, immune-stimulating, anti-hypersensitive, and blood lipid-lowering effects (Yang *et al*., 2002). Additionally, mushrooms contain high levels of protein, greater than many legume sources like soybeans and peanuts, and provide all the necessary amino acids required in a human diet (Bárbara *et al.,* 2008; Sadler, 2003). For these reasons, *P. Ostreatus* could be used as a substitutional ingredient, especially in fast foods like burgers.

3.4. Oyster mushroom beef burger characterization

Food sensory testing is the objective evaluation of food using human senses. Table (9) shows the impact of replacing beef with oyster mushroom powder on the sensory qualities of beef burgers. The level of dried mushroom used affected all of the properties examined. The burger's color, taste, flavor, juiciness, and overall acceptability were all heightened. The parameters showed that when dried mushrooms were used as a replacement for beef in burgers, there was an increase in certain aspects

except for taste and overall acceptability. The 20% mushroom beef burger had the lowest values for these factors (8.79 ^{ab} ±1.09 for taste and 9.46 ^{a} ±1.08 for overall acceptability). El-Refai *et al.* (2014) reported that increasing dried mushroom to 12% decreased the overall acceptability of beef patties, these results matched the current one. Based on the sensory data, it was found that beef burgers containing 5%, 10%, and 20% dried oyster mushroom were not significantly different from the control beef burgers in terms of all properties (*P>0.05*). However, beef burgers containing 5%, 10%, and 20% mushroom powder had slightly higher scores for color and flavor but were still not significantly different from the control. The amount of mushroom powder had a direct relationship with how juicy the beef burger was. The higher water-holding capacity of oyster mushrooms may be responsible for this increase in juiciness (Wan Rosli *et al.,* 2011). The productivity and best results were achieved without any unfavorable changes in sensory properties due to the addition of dried mushrooms.

Table 9: Organoleptic properties of mushroom oyster beef burger

Treatments	Color	Taste	Flavor	Juiciness	Overall Acceptability
BB (control) 0% DOM	9.38 ^{a} ±0.91	9.36 ^{a} ±0.79	9.18 ^{a} ±1.05	8.53 ^{ab} ±1.10	9.76 ^{a} ±1.13
BB with 5% DOM	9.43 ^{a} ±1.09	9.41 ^{a} ±0.89	9.56 ^{a} ±1.40	8.71 ^{ab} ±0.89	9.69 ^{a} ±0.97
BB with 10% DOM	9.47 ^{a} ±0.75	9.46 ^{a} ±1.03	10.09 ^{a} ±1.25	9.05 ^{a} ±1.05	9.66 ^{a} ±1.09
BB with 20% DOM	9.51 ^{a} ±0.72	8.79 ^{ab} ±1.09	10.10 ^{a} ±1.23	9.28 ^{a} ±1.18	9.46 ^{a} ±1.08

Values in the same column with the same letter are not significantly different at ($p \leq 0.05$).
BB= Beef Burger, DOM= dried oyster mushroom

The health, well-being, and safety of consumers depend on their understanding of the chemical and biochemical compositions of the foods they consume. Additionally, it is essential for quality control, determining nutritional value, detecting adulteration, and adhering to legal requirements. Table (10) displays the chemical composition results of beef burgers made with dried mushrooms. The moisture content of the cooked beef burgers varied between (60.07% and 65.01%), while the control had 56.15%. Among them, the beef burger containing 20% dried mushroom had the highest moisture content (65.01%). These results are consistent with Mansour, (2003), in which cooked beef patties formulated with hydrated potato flakes had significantly higher moisture content than the control ($p \leq 0.05$). The mushroom's ability to hold water, like a potato, may explain this. Moreover, a cooked beef burger without dried mushroom had the highest protein value (15.85%), while the beef burger with 20% dried mushroom had the lowest (15.83%). The concentration of protein decreased proportionally with the level of mushroom powder used in the beef burger. This difference was significant ($P \leq 0.05$) when compared to the control. These findings are consistent with previous research by Wan Rosli *et al.* (2011).

Additionally, the data showed that the fat content of the beef burger decreased as the level of dried mushroom increased. The lowest fat content (2.77%) was observed in the beef burger with 20% dried mushroom. This decrease in fat content was also significant ($p \leq 0.05$) with increasing levels of dried mushroom, which aligns with previous research by Mansour (2003). Carbohydrates were the predominant macronutrient in the study. Burgers with 10%, 20%, and 30% dried mushrooms had less carbohydrate content than the control beef burger. According to Table 10, adding dried mushrooms to beef burgers resulted in a decrease in caloric value. The beef burger with 20% dried mushroom had the lowest caloric value at 212.54 K.cal/100g, which can be attributed to its low-fat content. These findings are consistent with Eldemery, (2017) research, which showed that adding non-fat materials to beef burgers decreased their energy values.

The Food Industry heavily relies on Physical Analysis Methods to evaluate various physical attributes of their products such as color, viscosity, weight, thickness, granulation size, and texture. This discussion will specifically focus on the significance of measuring Shrinkage (%) and Water Holding Capacity (%) in food products. The study showed that the treatment of beef patties containing 20% dried mushrooms had the highest value for the water holding capacity (57.45%), whereas the control had the lowest value. The results indicate that increasing the levels of dried mushroom significantly ($p \leq 0.05$) increased water holding capacity, accounting for the decreased weight loss during burger cooking. El-Refai *et al.* (2014) explained that mushroom proteins could store water and form networks

with their functional properties. The shrinkage values of the beef burger with dried mushroom formula increased ($p \leq 0.05$) when compared to the control treatment. The binding and stabilizing properties of dried mushroom components, which held the meat particles together and resisted changes in the product's shape, maybe the reason for the shrinkage of the size and shape of cooked beef burgers formulated with dried mushrooms during cooking. Increasing the amount of dried mushroom in burger formulations also reduced shrinkage. Even though this effect of the cooking characteristic was greater in the control, it varied significantly ($p \leq 0.05$) across all treatments.

Table 10: Physico-chemical characteristics of oyster mushroom beef burger**.**

Treatments	Moisture %	Crude protein %	Crude fat%	Carbs %	Caloric value (K.cal/ 100g)	Water holding capacity (%)	Shrinkage (%)
BB (control) 0% DOM	56.15^{c} ±0.18	15.85^{d} ±0.33	1.76^{b} ±0.01	11.17^{a} ±0.34	273.88	39.72^{d} ±1.12	29.40^{a} ±1.00
BB with 5% DOM	60.07^{b} ±0.29	16.92^{bc} ±0.31	2.46^{a} ±1.00	9.35^{b} ±0.06	247.85	45.45^{c} ±1.21	25.40^{b} ±1.01
BB with 10% DOM	62.17^{ab} ±0.31	17.86^{b} ±0.39	2.46^{a} ±1.00	8.18^{c} ±0.04	231.95	49.17^{b} ±1.32	24.30^{b} ±1.01
BB with 20% DOM	65.01^{a} ± 0.67	15.83^{d} ±0.33	2.77^{a} ±1.00	6.82^{d} ±0.10	212.54	57.45^{a} ±1.02	20.35^{c} ±0.16

Values in the same column with the same letter are not significantly different at ($p \leq 0.05$).
BB= Beef Burger, DOM= dried oyster mushroom

4. Conclusion

In conclusion, dried mushrooms can serve as a functional food or as a viable substitute for meat products in the fast food industry, particularly for individuals who are overweight or have high blood lipid levels. Our study has shown that dried mushrooms can effectively lower blood fat levels and improve liver function. To confirm the quality of the product, the examining of the chemical, physical, and sensory characteristics of beef burgers that had been permeated with dried mushrooms. The addition of dried mushrooms in meat products can also help to reduce production costs without losing the quality of the final product.

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