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Original article

Determination of the Effects of Different Tree Logs on Some Important Volatile Aroma Components in the Oyster Mushroom (*Pleurotus ostreatus* Jacq Kumm)

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Abstract

Oyster mushroom (*Pleurotus* spp.) is grown in significant quantities around the world. Compared to the *Agaricus* species, it is highly tolerant to hot and cold conditions, making it easier to cultivate. At the same time, their cultivation can be done in many different nutrient media. This provides advantages in the agricultural use of many waste materials or materials used for heating purposes. In addition, mushrooms have an important place in the agriculture and food sector, especially with their unique flavor. In the study, oyster mushroom (*Pleurotus ostreatus*) was grown in nutrient media prepared using logs of beech (*Fagus* spp.), chestnut (*Quercus* spp.), poplar (*Populus* spp.), linden (*Tilia* spp.) trees. The research was conducted in the mushroom cultivation facility at C.O.M.U., Faculty of Agriculture, Horticulture Department. In the study, aroma compounds were analyzed in mushrooms grown in the mentioned media. Under the conditions of our study, it was found that linden logs were not suitable for *Pleurotus ostreatus* production. Major aroma components were 3 octanone, 3-octanol, 1-octen-3-ol components known to create mushroom aroma, odor or taste. It has been determined that total rate of these components, which are important in the aroma industry, are at higher levels in the nutrient medium consisting of poplar tree logs.

Keywords: *Pleurotus Ostreatus*, Mushroom, Log Substrate, Volatile Aroma Components.

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INTRODUCTION

The oyster mushroom (Pleurotus ostreatus) is an important cultivated mushroom. In Türkiye, it is estimated that the production of cultivated mushroom (*Agaricus bisporus*) is 15% of the production of oyster mushroom (*Pleurotus ostreatus*) (Öztürk et al., 2019). In 2022, 65636 tons of cultivated mushrooms (*Agaricus bisborus*) and truffle mushrooms (*Tuber melanosporum*) were produced in Turkey (FAO, 2024).

Pleurotus mushrooms are recognized for their high protein content, along with a rich profile of vitamins and minerals. Additionally, they are characterized by low fat and cholesterol levels. This genus offers several advantages over other mushrooms, including reduced environmental control requirements during cultivation, a shorter growth cycle, and a lower incidence of damage to the fruiting bodies from various pests. Furthermore, agricultural wastes and other wastes can be used in its cultivation (Agarwal et al., 2017).

Oyster mushrooms can grow on fresh tree logs, which are generally inactive in nature, or on living trees, including some species. In other words, tree logs are one of the natural environment of oyster mushrooms. The cultivation of oyster mushrooms in a nutrient medium consisting of tree logs is a simpler technique than compost cultivation. However, there are not many references on the cultivation of oyster mushrooms in a nutrient medium consisting of tree logs.

Mushrooms occupy a significant position within the agricultural sector, particularly due to their distinctive aroma. Concurrently, their cultivation can be conducted in a multitude of nutritional substrates. This offers advantages in the utilisation of a multitude of waste or fuel materials in agricultural contexts. The utilisation of wood as a fuel source has the potential to result in elevated levels of carbon emissions, exceeding the required threshold. This situation has an adverse effect on the atmosphere. It is therefore crucial to assess these materials from an agricultural standpoint. Nevertheless, it is of significant importance to investigate the impact of these nutrient media on aroma.

Aroma components play an important role in the product's characteristic taste and smell. At the same time, the more distinctive the product's taste and smell, the more marketable it becomes. Mushrooms have long been used as food or food flavouring due to their unique and pleasant aroma compounds (Mau and Hwang, 1997). Essential oils, which are the origin of aromas, are used in two ways: to impart the aroma of the oil to the final product and to synthetically produce natural aroma components (Çalıkoğlu et al., 2006).

Mushrooms have an interesting taste and smell. They are characteristically a source of mushroom-like taste and aroma. The taste of mushrooms is generally related to their aroma. The most characteristic aroma compound is defined by C8 volatiles. Among the C8 compounds, the most important ones related to mushroom aroma are oct-1-en-3-ol, octan-3-ol, octan-3-on and oct-1-en-3-on (Moliszewska, 2014).

1-octen-3-ol, an aromatic compound found in high amounts in mushrooms, can be used in mosquito traps (Takken and Kline, 1989; Kline, 1994). It was determined that some defense genes in Arabidopsis thaliana were activated by 1-octen-3-ol treatment and tolerance against *Botrytis cinerea* increased (Kishimoto et al., 2007). The major volatile components in oyster mushroom (*Pleurotus ostreatus*) collected from their natural environment was determined as 3-Octanone 1-Octen-3-ol, 3-Octanol, m-Cresol in order from highest to lowest (Deveci et al., 2017) and in another study (Nyegue et al., 2003) they were octen-3-ol (59%), 3-octanol (5.8%) and octan-3-one (5.3%). In addition, it was stated that many of the volatile substances identified in the mushroom species (*P. ostreatus*, *T. shimperi*) included in the study (Nyegue et al., 2003) are widely used in the flavor industry. In a study (Venkateshwarlu et al, 1999), the major aroma components in *Pleurotus florida* species were 1-octen-3-ol (68.4±3.08%), 3-octanone (5.60±1.11%), n-pentanal (4.47±1.12%), 3-octanol (3.99±0.43%), n-octanol (2. 54±0.66%); in *Agaricus bisporus* species, 1-octen-3-ol (56.69±1.70%), 3-octanol (10.54±0.22%), 2-octen-1-ol (9.88±0.39%), benzyl alcohol (2.61±0.42%).

It was reported that the 1-Octen-3-ol component creates mushroom (Venkateshwarlu et al., 1999; Usami et al., 2014) and earthy (Anonymous, 2022c) odor perception and mushroom aroma (Anonymous, 2022c). In one study (Vlasenko et al., 2017) it was stated that the characteristic aroma of the 3-octanol component creates mushroom, oily, walnut, citrus perceptions. It was stated that the 3-octanol component creates nutty (Venkateshwarlu et al., 1999) and mushroom (Usami et al., 2014) odor perception. It was reported that the compound 3-octanone contributes to a perception of oily, herbaceous, and moldy (Vlasenko et al., 2017), mushroom-like (Anonymous, 2022e), sweet, mushroom-like (Venkateshwarlu et al., 1999) aromas. Additionally, 3-octanone has been associated with earthy, mushroom-like, and soil-like flavor perceptions (Anonymous, 2022e).

The aim of this study to evaluate oyster mushrooms obtained from various tree logs in terms of volatile aroma components and especially the volatile aroma components that give mushroom aroma and provide information about the production of oyster mushrooms using tree logs, a method that is easier compared to compost production.

MATERIALS and METHODS

Mycelium of oyster mushroom (*Pleurotus ostreatus*) and logs of diffrent tree logs were used as materials of the study. The substrates used in mushroom production consisted of the logs of beech (*Fagus* spp.), chestnut (*Castanea* spp.), poplar (*Populus* spp.) and linden (*Tilia* spp.) trees.

The cultivation to be carried out will involve:

1. Poplar log substrate

2. Beech log substrate

3. Linden log substrate

4. Chestnut log substrate was used.

Preparation of Nutrient Substrate Made from Logs:

Mycelia of oyster mushroom (*Pleurotus ostreatus*) and logs of trees were used as materials in the study. For the purpose of mycelial cultivation, the logs (with a weight of 9.5 kg) were cut at an average of one-quarter of their length and divided into two. The mycelia (up to 1% of the substrate) were mixed with sawdust and planted on the upper part of the large-sized log and moistened by spraying. Subsequently, the smaller log was positioned on the area where the mycelia had been planted. The smaller log was affixed to the larger log at its upper extremity. Subsequently, the entire log was subjected to a spraying process to ensure uniform moisture distribution. The edges of the junction section of the two logs where the mycelia were planted were sealed externally with duct tape.

The logs were placed in black plastic bags of a similar size. The openings of the bags were then sealed with a tape to prevent airflow. The logs were subsequently left in the mushroom production room, which was characterised by a damp and dark environment. In order to prevent the accumulation of excessive carbon dioxide, fifty holes were made in each bag with the aid of a needle. The logs remained in the plastic bags for a period of 35 days. A volume of 10 millilitres of water was administered into the bags on a 10-day schedule. After 35 days, the logs were extracted from the bags. Subsequently, the logs were placed in containers with an average height of 15 cm and a width of 30–40 cm. The containers were filled to a depth of approximately half their capacity with water for enabling the logs to absorb water and to create an humid environment.

During the micelle development stage, the temperature was maintained at a range of 23-25 °C, with a humidity level of 80-90%. During the mushroom formation phase, the temperature of the room was adjusted to a range of 13-17°C, with a humidity level of 80-90%.

The mushroom growing room is equipped with manual lighting and a window that can be closed in a light-proof way. Furthermore, the mushroom production room was illuminated for nine hours a day during cap formation, using a fluorescent lamp (300 lm/m^2) to encourage mushroom formation. The air in the mushroom production room was changed every day, and irrigation was conducted by pulverisation twice a day with the formation of primordia.

Trial Design and Statistical Evaluation:

During the statistical analysis process, the percentage values of the compounds that provide mushroom aroma were collected in the replicates of each substrates. Then the values were analyzed. The experiment was conducted in three replicates according to the randomised plot trial design, with three nutrient substrates in each replicate. The SAS.9 computer package programme was employed to perform statistical analysis on the data, with analysis of variance performed and a LSD ($P<0.05$) test used to compare the differences between the averages of the data.

Extraction of Aroma Profiles in Mushrooms (Liquid Extraction):

Aroma analyses were conducted using the Shimadzu QP2010 Plus Gas Chromatography-Mass Spectrometry system at C.O.M.U., Faculty of Agriculture, Department of Horticulture. To identify the aroma components present in harvested mushroom samples, Vichi et al. (2007), Sabatini and Marsilio (2008), Reboredo-Rodriguez et al. (2013), Ekinci et al. (2016) and Bozok et al. (2018) were modified and employed. The samples were prepared for analysis by the following extraction procedure: A total of 50 grams of the mushroom puree obtained via homogenization was treated with 100 milliliters of diethyl ether solvent in an Erlenmeyer flask. The solvent was then concentrated to 1 milliliter with the assistance of a centrifuge and concentrator. The operating conditions of the GC/MS device are provided below.

Carrier Gas: Helium, Column: polyethylene glycol (PEG) (30m x 0.25 mm x 0.25 µm), Injection block temperature: 280°C, Linear flow: 41cm/sec, Pressure: 70.3 kPa, Injection mode: Split (1:50), Oven temperature program: Initially 1 minute at 40°C, then 2 minutes at 200°C with a speed of 4°C/min, and finally 10 minutes at 250°C with a speed of 10°C/min. Total analysis time was 58 minutes., Detector: Mass spectrometer (MS), Library: Nist and Wiley, Ion temperature: 250 °C, Interphase temperature: 230 °C, Solvent Cut Time: 4 min, Scanned mass range and scan speed: 40-350 amu (m/z) and 666 amu/sec, Ionization energy: 70 eV

RESULTS and DISCUSSION

It was observed that the mycelium of the oyster mushroom was not grow on linden tree logs under the current environmental conditions (growth medium, environmental factors).

Under the conditions of our study, no yield could be obtained from linden logs. Based on the findings, it can be concluded that linden tree logs are not suitable for the cultivation of *Pleurotus ostreatus* under the production conditions employed in this study.

The major aroma components of mushrooms collected from substrates made of poplar tree logs were, in descending order, 3-octanone, 3-octanol, and 1-octen-3-ol. For mushrooms collected from substrates made of beech logs, the major aroma components, in descending order, were 3-octanol, 3-octanone, and 1-octen-3-ol. In mushrooms collected from substrates made of chestnut tree logs, the major aroma components, in descending order, were 3-octanol, 3-octanone, and 1-octen-3-ol (Table 1).

It has been reported that the aroma type of the 1-octen-3-ol component is mushroom (Anonymous, 2022c; Usami et al., 2014; Venkateshwarlu et al., 1999). It has been stated that the 3-Octanone component creates oily, herbaceous, moldy aroma perceptions in one study (Vlasenko et al., 2017), and sweet, mushroom odor perceptions in another study (Venkateshwarlu et al., 1999). In addition, it has been mentioned that the aroma type is mushroom (Anonymous, 2022e). It has been stated that the characteristic aroma of the 3-Octanol component creates mushroom, oily, walnut, citrus perceptions (Vlasenko et al., 2017) and nutty odor perception (Venkateshwarlu et al., 1999).

Table 1. Effects of different substrates obtained from poplar (*Populus* spp.), beech (*Fagus* spp.), chestnut (*Quercus* spp.) tree logs on aroma components (%) in oyster mushroom (*Pleurotus ostreatus*).

Percentages of Volatile Components of Mushrooms Grown on Logs of Poplar Trees	%
Dodecane	1.92
3-Octanone	44.77
3-Octanol	29.61
1-Octen-3-ol	19.97
Octadecane	2.27
Heptadecane	1.46
Total	100
Percentages of Volatile Components of Mushrooms Grown on Logs of Beech Trees	%
Dodecane	4.01
3-Octanone	27.26
3-Octanol	45.12
Tetradecane	7.13
1-Octen-3-ol	11.89
Octadecane	4.59
Total	100
Percentages of Volatile Components of Mushrooms Grown on Logs of Chestnut Trees	%
Hexanal	7.76
Dodecane	3.47
3-Octanone	16.56
3-Octanol	41.45
Tetradecane	2
1-Octen-3-ol	13.86
Decane	4.62
Octadecane	4.13
Heptadecane	3.69
Tridecanol	2.46
Total	100

Table 2. Statistical comparison of 1-octen-3-ol, 3-octanone, 3-octanol components (%).

	1-octen-3-ol	3-octanone	3-octanol
Percentages of Volatile Components of Mushrooms Grown on Logs of Poplar Trees (%)	19.97 A	44.77 A	29.61 B
Percentages of Volatile Components of Mushrooms Grown on Logs of Beech Trees (%)	11.89 B	27.26 B	45.12 A
Percentages of Volatile Components of Mushrooms Grown on Logs of Chestnut Trees (%)	13.86 AB	16.56 C	41.45 A
LSD (P<0,05) =	7.2538	7.3874	5.7493

Table 3. Statistical comparison of total content of 1-octen-3-ol, 3-octanone, 3-octanol components (%).

Total Content of 1-Octen-3-Ol, 3-Octanone, 3-Octanol Components	%
Percentages of Volatile Components of Mushrooms Grown on Logs of Poplar Trees (%)	94.35 A
Percentages of Volatile Components of Mushrooms Grown on Logs of Beech Trees (%)	84.27 B
Percentages of Volatile Components of Mushrooms Grown on Logs of Chestnut Trees (%)	71.87 C
LSD (P<0,05) =	9.7595

Significant statistical differences ($P < 0.05$) were observed in the total percentages of 3-octanone, 3-octanol, and 1-octen-3-ol among the different substrates. The total percentage of these major aroma components (3-octanone, 3-octanol, 1-octen-3-ol) was found to be 94.35% in mushrooms collected from substrates made of poplar tree logs. In mushrooms collected from substrates made of beech logs, this percentage was 84.27%. For mushrooms collected from substrates made of chestnut tree logs, the percentage was 71.87% (Table 2).

Significant statistical differences ($P<0.05$) were observed in the comparison of major aroma components among the substrates. The compound 1-octen-3-ol, associated with mushroom aroma, was found at the highest concentration in poplar tree logs and at the lowest concentration in beech tree logs. This finding is also significant for the aroma industry. It is seen that mushrooms obtained from poplar tree logs had highest total proportion of major components (3-octanone, 3-octanol, 1-octen-3-ol) related to mushroom aroma (Anonymous, 2022c; Usami et al., 2014; Venkateshwarlu et al., 1999; Anonymous, 2022e; Vlasenko et al., 2017). This supports that poplar tree logs had highest mushroom aroma perception (Table 2, Table 3).

While 3-octanone was the main component in mushrooms obtained from poplar tree logs, 3-octanol was the main component in mushrooms obtained from beech and chestnut tree logs. From this point of view, it can be said that mushrooms obtained from poplar tree logs showed mushroom aroma type (Anonymous, 2022e) as well as oily, herbaceous, moldy aroma (3-octanone) perceptions (Vlasenko et al., 2017). Mushrooms obtained from beech and chestnut logs showed mushroom, oily, walnut, citrus aroma (3-octanol) perceptions (Vlasenko et al., 2017) and nutty odor (3-octanol) perception (Venkateshwarlu et al., 1999).

Nyegue et al. (2003) reported that the major aromatic component in oyster mushrooms (*Pleurotus ostreatus*) is octen-3-ol (59%). They identified the second major component as 3-octanol (5.8%) and the third as octan-3-one (5.3%). In our study, 3-octanol, which is the major component in mushrooms grown on beech and chestnut logs, is also among the major components identified in oyster mushrooms (*Pleurotus ostreatus*) in the studies by Nyegue et al. (2003) and Deveci et al. (2017). In the study by Deveci et al. (2017), the major component in wild-collected *Pleurotus ostreatus* mushrooms was identified as 3-octanone. The fact that 3-octanone is also the predominant component in mushrooms cultivated on poplar logs in our study shows a similarity to the findings of Deveci et al. (2017).

In the study by Vlasenko et al. (2017), the oyster mushroom (*Pleurotus ostreatus)* was cultivated in sunflower husk and barley straw substrates. It was observed that the synthesis of volatile components occurred to a greater extent in mushrooms cultivated using sunflower husks compared to those cultivated using barley straw. At the same time, in the flavor attributes test conducted in the study (Vlasenko et al., 2017), it was stated that mushrooms grown using sunflower husk had a more pronounced mushroom aroma compared to mushrooms grown using barley straw. The study (Vlasenko et al., 2017) exemplifies that different substrates affect the aroma in oyster mushroom (*Pleurotus ostreatus*) cultivation.

Conclusion

In the present data, it was observed that 3-octanone, 3-octanol, and 1-octen-3-ol, which are compounds responsible for mushroom aroma, scent and taste, are major components in mushrooms obtained from all three types of logs.

The total percentage of components related to mushroom aroma (3-octanone, 3-octanol, 1-octen-3-ol) were observed in mushrooms harvested from poplar logs, whereas the lowest percentages were found in mushrooms harvested from chestnut logs. The highest percentage of the mushroom aroma-related compound 1-octen-3-ol were observed in mushrooms harvested from poplar logs, whereas the lowest percentages were found in mushrooms harvested from beech logs. This suggests that mushrooms cultivated on poplar logs possess the richest mushroom aroma in our study. Additionally, the principal component in mushrooms grown on poplar logs was identified as 3-octanone, whereas in mushrooms grown on beech and chestnut logs, the major component was 3-octanol. This indicates that mushrooms harvested from poplar logs not only exhibit a strong mushroom aroma but also generate oily, herbaceous, and moldy (3-octanone) aroma perceptions, whereas mushrooms harvested from beech and chestnut logs impart mushroom aroma along with oily, nutty, and citrusy aroma perceptions (3-octanol).

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