



Effect of Different Substrate Mixtures on Volatile Aroma Compounds and Antioxidant Activity of Maitake (*Grifola frondosa*) Mushroom

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ABSTRACT

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In the present study, it was aimed to determine the volatile aroma composition and antioxidant activity of Maitake mushroom grown in different substrate mixtures comparatively. Five different substrate mixtures except control were prepared. Total polyphenols and antioxidant activities were specified by Folin-Ciocalteu, Ferric Reducing Antioxidant Power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods. Furthermore, analyzes were carried out in both dried and frozen samples. Head Space Solid Phase Micro Extraction technique combined with Gas Chromatography-Mass Spectrometry (GC-MS) was used in the analysis of volatile compounds. In the present study, yield was obtained only from S4 (oak sawdust + wheat stalk + wheat bran at 1:1:1 ratios) and S5 (poplar sawdust + wheat stalk + wheat bran at 1:1:1 ratios) growing mixtures. Therefore, the studies have been continued by comparing only these two mixtures. While the yield in S4 mixture was 55.02 g 1 kg bag⁻¹, it was determined as 124.82 g 1 kg bag⁻¹ in S5 mixture. DPPH analysis results of frozen and dried samples were 7.99±0.08 and 8.19±0.05 µmol TE g⁻¹ DM (S4) and 8.07±0.09 and 8.20±0.06 µmol TE g DM⁻¹ (S5) respectively. FRAP results were 1.87±0.63 and 6.29±0.66 µmol TE g⁻¹ DM (S4) and 4.24±0.44 and 6.45±0.16 µmol TE g⁻¹ DM (S5) in the same order. In volatile aroma profile analysis, 22 and 32 compounds were detected in S4 and S5, respectively. Ketones were the most found compound groups and its ratio was 68.67% in S4 and 52.37% in S5. The highest percentage among ketones was obtained from 4-nonanone and 3-octanone compounds.

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Introduction

The value of *Grifola frondosa* (Dicks.) Gray has increased as the importance of this mushroom species was discovered and different expressions have been used to describe its importance over time. It has been named “Maitake” in Japan, “gray tree flower” or “chestnut mushroom” in China, “hen of the woods” or “sheep’s head mushroom” in the United States (Chang and Miles, 2004).

Consumers’ preferences for food products are related to the stimulation of senses such as sight, taste and smell before and during consumption. Together with many quality features (colour, shape, different chemical content, hardness etc.) flavour characteristics are effective in choosing food (Reineccius, 1994). Aroma of foods is formed as a result of joint action of many volatile compounds and each of them affects the aroma profile differently. These volatile substances, which are present in low concentrations and form the aroma of food, are called “aroma active compounds”. These aroma active

compounds, which are found at very low amounts such as ppm and ppb in foods, can still be perceived by the human nose. The aroma determination threshold values of each aroma active component are also different. Therefore, aroma analysis is imperative to distinguish every single one of them (Reineccius, 1994; Mayol and Acree, 2001).

Gas Chromatography-Olfactometry (GCO), which uses the human nose as a detector for the determination of aroma active compounds, is widely used (Fuller et al., 1964). Although the human nose has an aroma detection limit of 10-19 moles, many chemical detectors do not. GCO system has two parts: chromatographic as the first part, which includes sampling, extraction, separation and instrumental determination of the aroma components and the second part, which includes the quality, density, time-dependent changes and identification of the aroma compounds (Lopetcharat and McDaniel, 2005). The working principle of GCO can be defined as follows;

injecting of isolated aroma extract into the gas chromatography; chromatographically determining of aroma substances by dividing extract into two equal proportions at column exit and sending it to a chemical detector (FID or MS), determination of aroma qualities by sending to smelling unit. Three different parameters have been determined in GCO: perceived perception or quality of the flavouring agent (for example popcorn), aroma density and retention index of the aroma agent. The retention index of a volatile indicates the location of flavouring agent on the chromatogram. The odour density of the aroma substance is expressed according to a predetermined 9 or 10-point scale (Friedrich and Acree, 1998; Van Ruth, 2001; Hışıl, 2004; Lopetcharat and McDaniel, 2005). The most common methods used for collecting data in GCO: Dilution Analysis, Time-Intensity and Posterior Intensity (Güneşer and Yüceer, 2010).

Oxidation has an important place in providing the energy required for nutrition in living organisms. Besides, free radicals in the living body can play an important role in diseases such as cancer, arthritis and arteriosclerosis (Halliwell and Gutteridge, 1984). However, there is an important balance between free radicals and antioxidants. Maintaining this balance has been an essential element for living organisms. There are some synthetic antioxidants produced to reduce damage of free radicals in the living body. However, these antioxidants are suspected of causing liver damage. Therefore, it will be essential for people to use natural antioxidants that can protect from free radicals and prevent diseases, rather than synthetic antioxidants that cause such chronic and serious damage (Qi et al., 2005). Disruption of this balance causes cellular protein, DNA and lipids to be oxidizable and deterioration may occur. A large number of antioxidant substances have been isolated from various plant materials such as vegetables, fruits, cereals and herbs (Ramarathnam et al., 1995). In addition, mushrooms have shown antioxidant properties and this property of some mushroom species has been used for centuries.

Although many studies have been carried out on the nutritional and medicinal value of Maitake mushroom (Chang and Miles, 2004; Hsieh and Yang, 2004; Tabata et al., 2004; Barreto et al., 2008; Svagelj et al., 2008; Montoya et al., 2012; Sato et al., 2017; Song et al., 2018), studies on volatile aroma compounds of this species are limited (Rapior et al., 1996; Zhou et al., 2015). Also, the aroma of this mushroom has been identified as “hydrocyanic acid, cherry laurel nut oil, then blue cheese, herbaceous, whey, moldy, wild hyacinth, cereal, hyacinth, yeast, cereals then citrus” based on previous studies organized by Jong and Birmingham (1993) (Badcock, 1939; Maga, 1976; Schindler and Schmid, 1982; Gallois et al., 1990).

Production of volatile compounds by mushrooms and obtaining of taste depends on the composition of the growing mixture, growing conditions, genetic diversity of strains and subjectivity of sensory perception. Modification of culture conditions, especially selection of nitrogen and carbon sources, affects the composition of mushroom odour profile at least quantitatively (Jong and Birmingham, 1993).

The aim of this study is to determine and compare the volatile aroma composition and antioxidant activity of Maitake mushroom obtained from different growing mixtures.

Material and Method

In the present study, WC 828 Maitake mushroom strain (Mushroom Spawn Lab of Penn State University, US) was used as the main stock. Then, mycelia propagation was performed using Potato Dextrose Agar (PDA) nutrient medium from these main petri dishes at the Prof. Dr. Saadet Büyükalaca laboratory of Çukurova University (Adana, Turkey). Wheat grains were preferred for obtaining spawn. Oak sawdust, poplar sawdust, wheat stalk and wheat bran were chosen in the preparation of mushroom growing mixtures.

Obtaining of Spawn

In the first stage of obtaining spawn, main stock of Maitake mushroom strain mycelia (WC 828 strain) was propagated in Potato Dextrose Agar (PDA) nutrient medium. For this purpose, 39 g L⁻¹ of instant PDA medium was weighed and pH was adjusted to 5.4. The prepared nutrient media were sterilized in an autoclave at 121°C and 1.2 atm for 15 minutes together with materials used. The nutrient media removed from the autoclave was cooled in the sterile bench for a while and then poured into sterile plastic petri dishes. Then, one of the Maitake mycelia stock was opened in the sterile bench and 1 cm pieces were taken using sterile forceps and scalpels. Cultures obtained were kept in an incubation room in dark condition until entire mycelia development in petri dishes. Following mycelia development, wheat grains were boiled and dried. Then, they were filled into large glass jars and sterilized in the autoclave at 121°C and 1.2 atm for 15 minutes. The bottles removed from the autoclave were placed in the sterile bench for cooling. The mycelia in each petri dish were divided into eight pieces and a piece was placed on each side of each wheat-filled jar. Then, the jars were shaken every day for obtaining homogeneous mycelia development in the jar.

Preparation of Substrate Mixtures

In the preparation of substrate mixtures; oak sawdust (OS), poplar sawdust (PS), wheat stalk (WS) and wheat bran (WB) were used by mixing at different proportions. In the experiments, 2 volumes of oak sawdust and 1 volume of wheat bran (2 OS + WB) known as successful in the cultivation of mushroom species were used as control.

Other mixtures were prepared as follows:

- C : 2 oak sawdust + 1 wheat bran (2 OS + WB)
- S1 : 2 poplar sawdust + 1 wheat bran (2 PS + WB)
- S2 : 2 wheat stalk + 1 wheat bran (2 WS + WB)
- S3 : 1 oak sawdust + 1 poplar sawdust + 1 wheat bran (OS + PS + WB)
- S4 : 1 oak sawdust + 1 wheat stalk + 1 wheat bran (OS + WS + WB)
- S5 : 1 poplar sawdust + 1 wheat stalk + 1 wheat bran (PS + WS + WB)

All materials were first grinded, then kept in water-filled containers for a certain time until their moisture content was appropriate (at least 70% moisture). After moistening process was completed, adjustments were performed by measuring of pH of the growing mixtures (6-7) and adding lime.

Sterilization and Mycelia Inoculation

The prepared substrate mixtures were filled into high temperature resistant polypropylene bags as 1 kg. The mouths of the bags were tied with rubber bands and were sterilized for 1 hour in the autoclave at 121°C and 1.2 atm for sterilization. Since experiments on obtaining spawn by wrapping mycelia to wheat grains were not successful, the mycelia inoculation process was carried out by cutting the mycelia developed in the PDA medium into pieces and placing these pieces into the growing mixtures directly with sterile forceps.

Mycelia Development and Harvest

Growing bags inoculated with mycelia were placed in mushroom growing rooms having 22±1°C of temperature and 70-80% of humidity for mycelia development. After the mycelia development was achieved, the temperature was reduced to 18±1°C. Following the fruiting body stage, the humidity was kept at 90-95% to prevent drying of the growing mixtures and 40-watt fluorescent lamps were used for 12 hours and 200 lux lighting was provided. When the mycelia development were observed in the growing mixtures (from 35 to 42 days), the bags were cut with a sterile scalpel in 5 cm wide to stimulate the formation of the primordia and also the mouths of bags were opened. When most of the mushrooms were the same size, samples were harvested.

Extraction of Bioactive Substances

For the extraction process, method of Bennett et al. (2011) was used in a modified form. 25 mL of 80% methanol was added onto one gram of mushroom sample. The mixture was shaken by vortex for 15 seconds, then kept in an ultrasonic water bath at 25°C (J.P. Selecta Ultrasons HD, Barcelona) for 20 minutes. Following this process, samples were centrifuged for 15 minutes in a centrifuge at 3500 rpm (Hettich Zentrifugen, Tuttlingen, Germany). Moreover, they were filtered with filter paper and made ready for spectrophotometric analysis. Relevant analyzes were carried out on the same day and tests were performed with four repetitions. 80% methanol was used as a control in all methods used except the FRAP method.

Total Phenolic Compound Determination

For determination of the total amount of phenolic compounds, 0.5 mL of Folin-Ciocalteu reagent was added to 0.5 mL of extract. Then, 3 mL of 10% Na₂CO₃ solution was added and the mixture was kept in the dark condition for 30 minutes. The total phenolic compounds of the samples whose absorbances were measured in the spectrophotometer (Shimadzu, UV 1800, Japan) at 760 nm were calculated as gallic acid equivalent (GAE) g⁻¹ dry matter (DM) (modified from Li et al., 2015).

Antioxidant Activity Determination (DPPH Method)

A solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) prepared at a concentration of 2 mL of 0.025 g L⁻¹ was added to 0.1 mL of mushroom extract and the samples were kept in the dark condition for 30 minutes. Readings were performed in the spectrophotometer (Shimadzu, UV 1800, Japan) at 519 nm and the results were expressed as μmol trolox equivalent (TE) g⁻¹ DM (modified from Aghraz et al., 2018).

Antioxidant Activity Determination (FRAP Method)

To prepare the FRAP (Ferric Reducing Antioxidant Power) reagent used in the experiment, 10 mmol L⁻¹ 2.5 mL TPTZ solution prepared in 40 mmol L⁻¹ HCl solution, 2.5 mL 20 mmol L⁻¹ FeCl₃ solution and 25 mL 0.1 mol L⁻¹ acetate buffer (pH=3.6) were used. 2 mL of FRAP reagent was added to 0.3 mL of extract and the volume was completed to 10 mL with distilled water. Absorbances of the samples kept in the dark condition for 10 minutes were measured in the Spectrophotometer (Shimadzu, UV 1800, Japan) at 593 nm. In addition, 2 mL of FRAP reagent was completed with 10 mL of distilled water and this solution was used as a blank sample. The results were expressed as μmol TE g⁻¹ DM (Szydłowska-Czeraniak et al., 2008).

Volatile Aroma Compounds Analysis

Volatile aroma composition was determined using Gas Chromatography Mass Spectrophotometer (GC/MS) combined with Head Space-Solid Phase Micro Extraction (HS-SPME) technique. Aroma analysis was carried out with the technique advanced by Palazzolo et al. (2017) containing minor modifications. For this, 5 g of Maitake mushroom samples were extracted at 30°C for 30 minutes. Analyzes were carried out using 1 cm 50/30 μm Divilbenzene Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) StableFlex (Supelco, Bellefonte, PA, USA) fiber at 270°C for 60 minutes. For the extraction, 50/30 μm fiber was used, which is recommended by many researchers because of its sensitivity and efficiency (Tian et al., 2016; Politowicz et al., 2018; Li et al., 2019). Volatile aroma compounds were determined by Agilent 7000 Triple Quatro MSD Mass Spectrometer equipped with Agilent 7890B brand gas chromatography. Separation of volatile compounds was performed using a DB-WAX capillary column (60 m × 0.25 mm × 0.5 μm) at 1.5 mL min⁻¹ flow rate of carrier gas (helium). The oven temperature was first at 40°C for 4 minutes, then increased to 90°C to 3°C per minute, then to 130°C at 4°C per minute and 240°C to 5°C per minute, finally at 240°C for 8 minutes. The same oven temperature schedule was applied for MSD. MSD conditions were as follows: ionization energy 70 Ev, mass range m/z 30-300 a.m.u, scan rate 2.0 scan/sec, interface temperature 250°C and source temperature 180°C. The diagnosis of peaks was performed by injecting a standard solution in the samples. In compounds not having standard, it was carried out by comparing their mass spectra with the mass spectra in computer memory (W9, NIST 11, flavour 2). Retention indices (RI) of all identified compounds were calculated using n-alkane series. All analyses were performed in triplicate (Selli et al., 2006; Cho et al., 2007).

Statistical Analysis

The % results of the volatile aroma compounds of Maitake mushrooms obtained in different substrate mixtures were determined in the JMP package program according to the random plot design.

Results and Discussion

In the present study, fruiting body formation was observed only in S4 (1 OS + 1 WS + 1 WB) and S5 (1 PS + 1 WS + 1 WB) growing mixtures. Therefore, only these two substrate mixtures could be compared in the study. While first flash mushroom yield was 55.02 g kg bag⁻¹ in the S4

mixture, it was determined as 124.82 g kg bag⁻¹ in the S5 mixture. In the study of Song et al. (2018), the first flash mushroom yield varied between 31.75 and 134.25 g bag⁻¹ (each bag 800 g). When we calculated yield values for 1000 g (1 kg) to compare with our study, the yield ranged from 39.68 to 167.81 g bag⁻¹. In our study, the yield was between 55.02 and 124.82 g bag⁻¹. Our results seem within an acceptable range.

Reasons for not obtaining yield from the other substrate mixtures (Control, S1, S2 and S3) during the study may be detailed as follows: (i) the cultivation of Maitake mushroom was the first experience for us (ii) temperature was above seasonal norms (This temperature increase should not be considered as affecting the growing room. Because our experiments were carried out in climate-controlled mushroom growing rooms. The temperature increases revealed insect/pest's problem. With the increase of temperature, pests were activated earlier. Pest entrance to the mushroom growing rooms can be explained by the fact that our mushroom growing rooms are located in the university building, which is an educational institution and there are rooms used for different aims in the corridor and student entrance to the corridor).

Total Phenolic Compounds

While the total bioactive amounts of the frozen and dry samples of S4 substrate mixture were 5.06±0.14 and 17.47±1.00 mg GAE g⁻¹ DM, respectively, they were 3.45 ± 0.07 and 29.26 ± 1.34 mg GAE g⁻¹ DM in S5 substrate mixture. When the results were examined, it was observed that the drying process increases the total amount of bioactive substance in both sample groups. In addition, it was observed that the effect of drying process on the phenolic content of mushrooms produced in S5 is more than S4. This situation reveals the fact that more phenolic compounds are synthesized or produced in S5 with the effect of heat.

When the literature on the total phenolic content of Maitake and different mushroom species was examined, Mau et al. (2002) found that the total phenol content in dried samples of 4 mushroom species (*Dictyophora indusiata*, *Grifola frondosa*, *Hericium erinaceus* and *Tricholoma giganteum*) ranged from 7.61 to 16.28 mg g⁻¹. Maitake mushroom ranked second with 12.31 mg g⁻¹. Mau et al. (2004) investigated the total phenol content of mycelia of freeze-dried *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mushroom species and they found the lowest content in Maitake mushroom with 1.59 mg g⁻¹ compared to the other two species. In a study carried out by Yeh et al. (2011), total phenol contents were determined between 15.28 and 42.30 mg g⁻¹ in ground mushroom powder samples belonging to two different strains of dried Maitake depending on the strains and extraction method. Shin and Lee (2014) investigated the effect of hydrothermal extraction on the total phenol content of Maitake mushroom and the total phenol content was between 12.16 and 13.61 mg GAE g⁻¹ Maitake powder. Yıldız et al. (2015) determined that the total phenolic amount of *G. frondosa* was higher than *Lentinula edodes* and *Hericium erinaceus* and less than *Ganoderma lucidum* and *Morchella esculenta*. Total phenolic contents of some mushroom species such as *Agaricus bisporus*, *Flammulina velutipes*, *Schizophyllum commune*, *G. frondosa*, *Hypsizygus tessellatus*, *Pleurotus eryngii* and *Pleurotus*

ostreatus obtained from market were detected separately in water and methanol extracts by Muhammad Ezzudin et al. (2019). Among nine mushroom species, the highest total phenolic content in both extracts was recorded in Maitake mushroom with 129.44 µg GAE g⁻¹. The results of total phenol and antioxidant activity analysis performed on 25 different mushroom species by Butkhuip et al. (2018) were presented in Table 1 and the phenolic content ranged between 0.72 g GAE kg⁻¹ dry weight (*Alpova trappei*) and 8.84 g GAE kg⁻¹ dry weight (*Termitomyces clypeatus*). Although this study (conducted on dried mushrooms) did not include Maitake mushroom, when we compared with the results of the dried samples of our study (results of both frozen and dried samples are presented in our study), values obtained by us in Maitake mushroom were higher than all data determined by Butkhuip et al. (2018) in 25 different mushroom species with 17.47±1.00 mg GAE g⁻¹ DM in S4 and 29.26±1.34 mg GAE g⁻¹ DM in S5. Furthermore, our results were higher than the most of data obtained in Maitake by different researchers such as 12.31 mg g⁻¹ (in dried samples) by Mau et al. (2002), 1.59 mg g⁻¹ (in freeze-dried samples) by Mau et al. (2004), 15.28 and 42.30 mg g⁻¹ (in dried samples) by Yeh et al. (2011) in two different strains and 12.16 and 13.61 mg GAE g⁻¹ Maitake powder (in dried samples) by Shin and Lee (2014).

Antioxidant Activity

When the results of antioxidant activity analysis performed with different methods were evaluated, it was observed that obtained data by the DPPH method were higher than those obtained by the FRAP method in all samples. This proves that the amount of antioxidant molecules that can reduce the hydrogen ion in *G. frondosa* is higher than the antioxidants that reduce iron. DPPH analysis results of frozen and dry samples for S4 and S5 mixtures were 7.99±0.08 and 8.19±0.05 µmol TE g⁻¹ DM (S4) and 8.07±0.09 and 8.20±0.06 µmol TE g⁻¹ DM (S5). FRAP results were calculated in the same order as follows; 1.87±0.63 and 6.29±0.66 µmol TE g⁻¹ DM (S4) and 4.24±0.44 and 6.45±0.16 µmol TE g⁻¹ DM (S5). Our findings showed that the antioxidant activity of samples grown in S5 are higher than those in S4. In addition, while the drying process did not have a significant enhancing effect on bioactives capable of reducing hydrogen ion, it was determined that these bioactives increased approximately 2-fold in terms of those that reduce the iron ion.

When the previous studies were examined, it was concluded that *G. frondosa* was richer in antioxidants that could reduce iron than *G. lucidum*, *M. esculenta*, *L. edodes* and *H. erinaceus* (Yıldız et al., 2015). In a study carried out in *Dictyophora indusiata*, *G. frondosa*, *H. erinaceus* and *Tricholoma giganteum* by Mau et al. (2002), scavenging effects on DPPH radicals at 6.4 mg mL⁻¹ were found to be 92.1% in *D. raindusiata* and 63.2% in other mushroom species. At 40 mg mL⁻¹, scavenging effects were recorded as 75.0%, 69.4%, 39.6% and 47.4% for *D. raindusiata*, *H. erinaceus*, *G. frondosa* and *T. giganteum*, respectively. Chelating effect on iron ions at 24 mg mL⁻¹ was detected as 91.9% for *D. raindusiata* and between 46.4 and 52.0% for other mushroom species. Mau et al. (2004) determined scavenging effects of mycelia of *G. frondosa*, *M. esculenta* and *T. albuminosus* on DPPH radicals at 10 mg mL⁻¹ between 78.8 and 94.1%.

Table 1. Results of Total phenolic compounds and antioxidant activity analysis performed on different mushroom species by Butkhupet et al. (2018)

Species	Total phenol (g GAE kg ⁻¹ DM)	DPPH (%)	FRAP (g Fe (II) kg ⁻¹ DM)
<i>Amanita princeps</i>	1.68	59.40	3.01
<i>A. hamibapa</i>	8.53	71.75	7.54
<i>Lactarius volemus</i>	3.61	66.75	0.92
<i>Russula luteotacta</i>	4.63	81.24	7.53
<i>R. emetica</i>	1.71	46.31	0.20
<i>R. alboareolata</i>	4.68	62.71	2.66
<i>R. galochooides</i>	2.38	69.76	3.86
<i>R. cyanoxantha</i>	2.91	78.74	3.03
<i>R. nigricans</i>	2.31	51.90	0.32
<i>R. densifolia</i>	5.62	55.43	2.14
<i>R. delica</i>	4.83	75.72	3.74
<i>R. violeipes</i>	2.26	63.59	3.34
<i>Termitomyces fuliginosus</i>	6.31	72.34	4.47
<i>T. clypeatus</i>	8.84	83.07	9.79
<i>Tricholoma crassum</i>	2.56	64.18	0.37
<i>Volvariella volvacea</i>	8.49	86.60	8.42
<i>Astraeus hygrometricus</i>	2.33	45.72	0.41
<i>Alpova trappei</i>	0.72	36.75	0.24
<i>Auricularia auricula</i>	0.95	40.94	0.10
<i>Cantharellus cibarius</i>	1.41	64.10	1.94
<i>Craterellus aureus</i>	2.34	59.91	4.12
<i>Lentinula squarrosulas</i>	5.42	71.68	2.64
<i>L. polychrous</i>	5.35	85.43	3.91
<i>L. edodes</i>	2.21	63.44	2.50
<i>L. giganteus</i>	1.46	56.90	3.65

dw: dry weight

These three species did not have a scavenging effect on hydroxyl radicals. Chelating effect on iron ions was found to be high at 10 mg mL⁻¹ (90.3-94.4%). In a study conducted by Yeh et al. (2011) on the effect of different extraction methods on antioxidant activity in two strains of Maitake, while DPPH results were found to be 3.51 mg mL⁻¹ in ethanol extract, 19.42 mg mL⁻¹ in cold water and 17.36 mg mL⁻¹ in hot water in T1 strain, they were 5.07, 10.25 and 9.27 mg mL⁻¹ in ethanol, cold and hot water, respectively in T2 strain. Shin and Lee (2014) investigated the effect of hydrothermal extraction on the antioxidant activity of Maitake mushroom and DPPH values were determined as 37.13% at 121°C in 30 minutes, 55.36% in 60 minutes, 46.76% in 30 minutes at 130°C, 62.04% in 60 minutes, 46.81% at 140°C in 30 minutes, 57.19% in 60 minutes, 53.29% in 30 minutes at 150°C and 65.85% in 60 minutes. The antioxidant activity was determined in some mushroom species (*A. bisporus*, *F. velutipes*, *S. commune*, *G. frondosa*, *H. tessellatus*, *P. eryngii* and *P. ostreatus*) obtained from market in water and methanol extract separately using DPPH and FRAP methods by Muhammad Ezzudin et al. (2019). EC50 value of DPPH reducing activity could not be determined for Maitake mushroom in water. However, it was detected as 39.46 and 14.63 mg mL⁻¹ in FRAP method in water and methanol extract, respectively. When the study conducted by Butkhupet et al. (2018) on 25 mushroom species was examined (Table 1), DPPH values were between 40.94% (*Auricularia auricula*) and 86.60% (*Volvariella volvacea*) and FRAP values varied between 0.10 g Fe (II) kg⁻¹ dry weight (*A. auricula*) and 9.79 g Fe (II) kg⁻¹ dry weight (*Termitomyces clypeatus*). Comparing the literature data with the values of our study seems difficult since obtained data were in

different units. However, in our study and also in the literature, a significant level of antioxidant activity was detected in *G. frondosa* mushroom.

Volatile Aroma Analysis

In the experiment, 22 and 32 volatile components were detected in S4 (1 OS + 1 WS + 1 WB) and S5 (1 PS + 1 WS + 1 WB) growing mixtures (Table 2). These volatile compounds were classified as aldehyde, hydrocarbon, alcohol, ketone, volatile acid, ester, terpene and other compounds. Volatiles obtained from mushroom samples grown in S4 were 2.51% aldehyde, 5.51% hydrocarbon, 19.69% alcohol, 68.62% ketone, 0.79% volatile acid, 0.20% ester, 0.32% terpene and 0.84% other components. These percentages were 13.06% aldehyde, 3.21% hydrocarbon, 25.49% alcohol, 52.37% ketone, 2.06% volatile acid, 0.21% ester, 0.32% terpene and 2.32% other components in S5. Hydrocarbons and ketones were detected at a higher rate in S4, while aldehydes, alcohols, volatile acids and other components were higher in S5. Only 1 compound was detected for ester and terpene each and the ratio was the same or very close.

There is not much information in the literature regarding the volatile aroma components of Maitake mushroom. In a study carried out in frozen Maitake mushrooms by Rapior et al. (1996); 1-octen-3-ol, 3-methylbutan-2-one, methyl 2,4-dihydroxybenzoate, heptane, δ -cadinene, benzyl alcohol and benzyl aldehyde were detected. Since we could not reach the full text of the study, we do not have any information about the content of growing mixtures used in the cultivation of mushroom. In another study conducted by Zhou et al. (2015) on dry Maitake samples obtained from the market, 1-octen-3-ol,

nerolidol, spathulenol, α -cadinol, 2-octanone, 1-octen-3-one, 2-nonanone, 2-decanone, 3-nonen-2-one, 2-undecanone, 3-decen-2-one, octanal, (E)-2-heptenal, nonanal, 2-octenal, (2E, 4E)-2,4-decadienal, 1-isoamyl-2-formyl pyrrol, 1-(2-methylbutyl)-2-formyl, benzaldehyde, benzaldehyde, 4-methyl, phenylacetaldehyde, anethole, naphthalene, 2-methyl-butylated, hydroxytoluene, N-ethylpropionanilide, ortho-cresol 2,4-di-tert-butylphenol, γ -muurolene, cedran-8-ol, α -copaene, (3E) -3-ethyl-2-methyl-1,3-hexadiene, octylformate, diethylphthalate, hexanedioicacid, bis (2-ethylhexyl) ester and DL-menthol compounds have been identified. Likewise, since samples

were obtained from the market, there is no information about mixtures in which they are grown.

Among the volatiles obtained from these two studies and the compounds detected in our study, the common ones were mostly 1-octen-3-ol and benzyl aldehyde. However, interestingly, these two volatiles were detected at a low percentage in our study. It is known that the volatile aroma components of cultivated mushrooms vary according to strains, growing mixtures, which development stage of mushroom used in analysis, whether the samples are frozen, dried or fresh and method used.

Table 2. Volatile aroma compositions of Maitake mushroom samples obtained from two different growing mixtures

Volatile compounds	RI	S4	S5
Aldehyde			
Acetaldehyde	716	0.63 b	1.45 a
Pentanal	929	nd	0.37
3-methyl-butanal	930	1.09 b	6.02 b
Hexanal	1077	0	0.31
Octanal	1291	0	0.38
Benzaldehyde	1525	0.79 b	2.12 a
Benzeneacetaldehyde	1612	0	0.38
α -ethylidenbenzeneacetaldehyde	1939	0	2.03
Total		2.51	13.06
Hydrocarbon			
1-octene	833	0.38 b	0.44 a
Ethylbenzene	1083	0.77 a	0.59 b
p-xylene	1128	3.01 a	1.55 b
o-xylene	1185	0.48 a	0.20 b
1,3-dichlorobenzene	1418	0.87 a	0.43 b
Toplam		5.51	3.21
Alcohol			
3-methyl-1-butanol	1207	9.71 b	13.47 a
1-hexanol	1359	0.72 b	1.33 a
3-octanol	1394	4.01 a	3.06 b
1-octen-3-ol	1427	0.42 b	0.95 a
1-octyn-3-ol	1532	-	0.44
3-(methylthio)-1-propanol	1723	0.55 b	0.81 a
Phenylethyl alcohol	1923	4.28 b	5.88 a
Total		19.69	25.94
Ketone			
3-octanone	1211	32.59 a	22.80 b
Acetoin	1280	0.86 a	1.44 a
4-nonanone	1356	35.17 a	28.13 b
Total		68.62	52.37
Volatile acid			
Acetic acid	1423	0.79 b	1.63 a
3-methylbutanoic acid	1691	-	0.22
3-hydroxydodecanoic acid	2000	0	0.21
Total		0.79	2.06
Ester			
2,5-octadecadiynoic acid, methyl ester	2047	0.20 a	0.21 a
Terpene			
trans-geranylacetone	1865	0.32 a	0.32 a
Other compounds			
2-propenoic acid, 3-[2-(aminocarbonyl)phenyl]-	1420	-	0.24
2,7-dimethyl-4,5-octanediol	1446	0.27 b	1.23 a
Methyl N-hydroxybenzenecarboximidoate	1734	0.57 a	0.65 a
Methyl 8-[(1R,2R)-2-[(1S,2R)-2 hexylcyclopropyl]cyclopropyl]octanoate	2031	0	0.2
Total		0.84	2.32

nd: not determined

Table 3. Taste and fragrance definitions of compounds obtained from two different growing mixtures in Maitake mushroom in the study

Aroma compounds	Description	Reference
Aldehyde		
Acetaldehyde	sweet,pungent	Curioni ve Bosset (2002)
Pentanal	chemical	Curioni ve Bosset (2002)
3-methyl-butanal	malty	Zhang ve ark (2018)
Hexanal	woody, vegetable	Li ve ark (2019), Cho ve ark (2007)
Octanal	flavour, cut grass like	
Benzaldehyde	cucumber, citrus plastic	Aisala ve ark (2019)
Benzeneacetaldehyde	almond, fruity, nuty	Li ve ark (2019)
α -ethylidenbenzeneacetaldehyde	-	
Hydrocarbon	-	
1-octene	-	
Ethylbenzene	-	
p-xylene	rotten, onion	Aisala ve ark (2019)
o-xylene	-	
1,3-dichlorobenzene	-	
Alcohol		
3-Methyl-1-butanol	cheese	Culleré ve ark (2010)
1-hexanol	green	Cho ve ark (2007)
3-octanol	earthy, mushroom-like, herbal, buttery	Xu ve ark (2019), Cho ve ark (2007)
1-octen-3-ol	earthy, mushroom-like, green	Xu ve ark (2019), Cho ve ark (2007)
1-octyn-3-ol	-	
3-(methylthio)-1-propanol	heated onion	Zhang ve ark (2018)
Phenylethyl alcohol	floral, sweet	Cho ve ark (2007)
Ketone		
3-octanone	mushroom, soil, potato	Aisala ve ark (2019)
Acetoin	buttery, creamy, moldy	Pionnier ve Hugelshofer (2006)
4-nonanone	-	
Volatile acid		
Acetic acid	sour	Zhang ve ark (2018)
3-methylbutanoic acid	sweaty	Zhang ve ark (2018)
3-hydroxydodecanoic acid	-	
Ester		
2,5-octadecadiynoic acid, methyl ester	-	
Terpene		
trans-geranylacetone	moldy, leaves, plastic	Aisala ve ark (2019)
Other compounds		
2-propenoic acid, 3-[2-(aminocarbonyl)phenyl]-	-	
2,7-dimethyl-4,5-octanediol	-	
Methyl N-hydroxybenzenecarboximidoate	-	
Methyl 8-[(1R,2R)-2-[(1S,2R)-2-hexylcyclopropyl]cyclopropyl]octanoate	-	

There are many studies in the literature examining these parameters. For instance, Cho et al. (2003) investigated volatile aroma components in Shiitake mushroom (*Lentinula edodes*) at different maturity stages, including young, mature and old mushrooms and differences were identified. These differences were both in the number of compounds and in the decrease or increase of certain groups of compounds. Li et al. (2019) examined the effect of growing mixtures consisting of different agricultural wastes on the volatile aroma composition of Shiitake mushroom and again differences were observed in both the number of compounds and their ratios. Wu and Wang (2000) compared the aroma components of fresh and dried

Shiitake mushroom and found differences. Politowicz et al. (2018) found that drying techniques are effective on aroma components in Shiitake mushroom. Therefore, differences obtained from our study may have occurred due to these reasons. Within our knowledge, there is no study on the effect of growing mixtures on the volatile profile in Maitake mushroom grown in different growing substrate mixtures.

Among the compounds obtained in this study, 3-octanone, octanal, 3-octanol and 1-octen-3-ol are eight-carbon compounds. Their ratios were 37.02% and 27.19% in S4 and S5 mixtures, respectively. Eight carbon compounds are important in forming of taste and smell of

mushrooms. The percentage of eight-carbon compounds, especially 1-octen-3-ol, has been found to be high is generally in wild mushrooms (Taşkın, 2013; Taşkın et al., 2013; Bozok et al., 2015; 2018; Taşkın et al., 2019). However, there is information in the literature that this compound could not be detected in some wild mushroom species, albeit rarely. For example, Taşkın et al. (2019) detected high 1-octen-3-ol in wild-collected cedar mushroom (*Tricholoma anatolicum*), however they could not record 1-octen-3-ol in *T. caligatum* examined in the same study.

1-octen-3-ol is also called mushroom alcohol. In this presented study, 1-octen-3-ol was found to be at low level. However another eight-carbon compound 3-octanone was found to be high. In the comparison of growing mixtures, a higher result was obtained from S4 mixture containing oak sawdust for eight-carbon compounds than S5 containing poplar sawdust. Similar results were observed by Baktemur et al. (2020), who investigated the effects of different agricultural wastes on the aroma composition of Shiitake mushroom. It was determined that growing mixtures containing oak sawdust produce a higher number of eight-carbon compounds than those containing poplar sawdust. Another compound obtained at a high percentage in the study was 4-nonanone classified a ketone. The ratio of this compound was found as 35.17% and 28.13% in the S4 containing oak sawdust and S5 containing poplar sawdust, respectively. Similar results were also determined by Baktemur et al. (2020). Oak sawdust has been found to be more successful than poplar sawdust. The taste and odour definitions of the compounds obtained in this study were presented in Table 3.

Conclusion

The findings obtained from the study were summarized below:

- Yield values were 55.02 g kg bag⁻¹ and 124.82 g kg bag⁻¹ in S4 and S5 growing mixtures, respectively.
- Total bioactive amounts of frozen and dry samples obtained from S4 were calculated as 5.06±0.14 and 17.47±1.00 mg GAE g⁻¹ DM, respectively, and 3.45±0.07 and 29.26±1.34 mg GAE g⁻¹ DM in S5, respectively. It is seen that values increase with drying in both growing mixtures.
- DPPH analysis data of frozen and dry samples for S4 and S5 were found as 7.99±0.08 and 8.19±0.05 µmol TE g⁻¹ DM (S4) and 8.07±0.09 and 8.20±0.06 µmol TE g⁻¹ DM (S5). FRAP results were determined as 1.87±0.63 and 6.29±0.66 µmol TE g⁻¹ DM (S4) and 4.24±0.44 and 6.45±0.16 µmol TE g⁻¹ DM (S5). Antioxidant activity findings of samples grown in S5 were higher than in S4. In the antioxidant activity analysis methods, data obtained by DPPH method were higher than those obtained by FRAP method. While the drying process did not show a significant enhancing effect on bioactives that can reduce the hydrogen ion, those that reduce the iron ion increased about 2-fold.
- In S4 and S5 growing mixtures, 22 and 32 volatile aroma components were detected, respectively.

While aldehydes, alcohols, volatile acids and other components were detected higher in S5, hydrocarbons and ketones were found to be higher in S4. Eight-carbon compounds were 37.02% and 27.19% in S4 and S5, respectively. 3-octanone was found to be the highest eight-carbon compound and also the second highest for all compounds obtained from the study. The compound determined at the highest percentage was 4-nonanone (35.17% in S4, and 28.13% in S5). The most common compound group was detected to be ketone, which includes 3-octanone and 4-nonanone. In the growing mixtures comparison, S4 containing oak sawdust was more efficient for eight-carbon compounds than the S5 containing poplar sawdust.

Maitake mushroom is almost an unknown species in Turkey. Learning the cultivation of this species, revealing its nutritional value and medical importance will be beneficial for the producers and consumers of Turkey. The confidence of consumers in food products, whose nutritional value and medical importance have been proven by scientific studies, rises. Diversity of cultivated mushroom species in the country may be increased via the introduction and wide cultivation of new mushroom species. With this study, the antioxidant activity and volatile aroma composition of Maitake mushroom have been revealed. At the same time, first experiences have been gained in terms of cultivation.

The recommendations after this study can be listed as increasing number and types of agricultural wastes that can be used for different regions of Turkey and revealing nutritional value and medical importance of Maitake mushroom using different analyzes.

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