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The Effect of Different Agricultural Wastes on Aroma Composition of Shiitake (*Lentinula edodes* (Berk.) Pegler) Mushroom

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ABSTRACT

Shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) popular as both edible and medicinal, is one of the most cultivated and consumed mushroom species in the world. Cultivation of this mushroom on different agricultural wastes has been experimented in many studies until today. Nevertheless, agricultural waste trials are going on as agricultural production patterns of the countries are different. In this study, volatile aroma composition of shiitake mushroom produced on different agricultural wastes (wheat stalk, wheat bran, peanut shell, corn cob and vine pruning waste) was compared. Oak sawdust was selected as control because of its common use in literature and substrate materials were mixed at different ratios. In addition, some substrate mixtures were prepared with poplar sawdust. Volatile compounds of the shiitake mushroom were determined by using headspace-solid phase microextraction (HS-SPME) technique combined with Gas Chromatography-Mass Spectrometer (GC-MS). At the end of study, 41 volatile compounds were detected and dimethyl trisulfide, benzaldehyde, dimethyl disulfide, 1-octen-3-ol and 3-octanone were found to be dominant volatile components. Flavour and fragrance of mushrooms are dependent on many volatile aroma compounds and their proportions. While the concentration of eight-carbon components and hydrocarbons was found the highest by using wood straw, amount of sulphur containing compounds and alcohols increased by agricultural wastes in this study. In addition, corn waste positively affected aldehyde compounds.

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Introduction

Lentinula edodes is known as “shiitake mushroom” and is one of the most cultivated mushroom species in the world. World total mushroom production is 8 993 280 tons and the ranking is as follows: China with 6 664 606 tons, USA with 416 050 tons, the Netherlands 300 000, Poland with 280 232 tons and Spain 166 250 (FAO, 2018). For shiitake mushroom, Royse (2014) reported that China is leader with over 4 million tons estimated and this corresponds to 90% of the total production. Li et al. (2019) emphasized that 70% of the shiitake mushroom production has been provided by China. In addition, China has been assessed as the most shiitake consuming country. Therefore, one may say that shiitake mushroom is one of the most produced and preferred mushroom species in the world. Although mushrooms have been known for both food and medicinal purposes since ancient times, with the increasing importance given to “functional foods”, use of mushrooms for medical purposes has become even more important nowadays. Today, studies on determining of chemical composition of mushrooms are intensively conducted. These studies have been accelerated by using new technologies and also with becoming more accessible and more affordable of these technologies. The most advantage of shiitake mushroom is that it is both edible and medicinal. Some mushroom species are assessed as medicinal; however they may not be suitable for direct consumption, as for example *Ganoderma lucidum*, which has hard, woody texture and is therefore used in the form of tea or as dry extracts for addition to various beverages. To benefit from shiitake mushroom, it can be consumed directly or it can also be used as instant products such as tea and pills.

Different agricultural wastes are used in the production of mushrooms. These wastes vary according to their availability in different countries. Each country prefers to use their own wastes of agricultural products in the cultivation of mushrooms. Different agricultural wastes have been investigated in studies on shiitake mushroom cultivation, such as hazelnut husk-wheat straw-wheat bran-beech sawdust (Özçelik and Pekşen, 2006), hazelnut husk-wheat straw-beech wood chip-wheat bran mixtures (Özçelik and Pekşen, 2007), wheat straw-corn cobs-oak wood sawdust mixtures (Philippoussis et al., 2007), cocoa husk-cotton waste-oak sawdust-wheat bran mixtures (Escobar et al., 2007), wheat straw-coir pith-poplar sawdust-teak sawdust-sal sawdust (Puri et al., 2011), eucalyptus sawdust-rice bran-wheat bran-soybean bran (Casaril et al., 2011), oak sawdust-almond bark-poplar-walnut shell-linter-residue of textile fibers-olive waste-residue of guar-corn residue-residue of sunflower seed-residue of cotton-residue of grape mixture (Sözbir, 2014) and chickpea straw-corn stalk-alfalfa hay-sunflower head residue (Atila, 2019). In addition to all these, it was reported the use of wheat straw (Delpech and Olivier, 1991; Mata and Savoie, 1998; Savoie et al., 2000; Mata and Gaitán-Hernández, 2004; Philippoussis et al., 2007; Sharma et al., 2013; Gaitán-Hernández et al., 2014), paddy straw (Puri, 2012), coffee residues (Mata and Gaitán-Hernández, 1994; Fan and Soccol, 2005), sunflower seeds hulls (Curvetto et al., 2004), vineyard pruning waste (Gaitán-Hernández et al., 2006), corn cobs (Philippoussis et al., 2007), sugar cane bagasse (Salmones et al., 1999), sawdust (Fan and Soccol, 2005; Royse and Sánchezi, 2007; Martínez-Guerrero et al., 2012) in a review study carried out on shiitake mushroom by Mata and Savoie (2018). Composition of these different agricultural wastes can affect taste and aroma of the mushrooms produced, as well as their quality and yield. Volatile aroma compounds are the major contributor to the characteristic mushroom flavour. The volatile compounds of shiitake mushroom have been studied by many researchers (Chen et al., 1984; Chen and Ho, 1986; Wu and Wang, 2000; Cho et al., 2003; Hiraide et al., 2004; Hiraide, 2006; Mata et al., 2014; Politowicz et al., 2018; Li et al., 2019). According to findings obtained from these studies, volatile components of shiitake mushroom are predominantly eight-carbon and sulphur compounds. There are many factors that affect the aroma content of mushrooms such as strains, substrate mixtures and mushroom maturity stage. Therefore, type and content of volatile compounds can vary significantly between two identical strains grown in different substrate mixtures. The aim of this study is to determine the effect of different agricultural substrate mixtures on the volatile aroma composition of shiitake mushroom.

Material and Methods

This study was carried out in Department of Horticulture (in Prof. Dr. Saadet BÜYÜKALACA Tissue Culture Laboratory and full climate-controlled mushroom growing room) of Cukurova University (Adana, Turkey). As spawn, strain “4320” obtained from Sylvan Cultivating Excellence was used.

Substrate Preparation

Poplar sawdust, wheat stalk, peanut shell, corn cob and vine pruning waste were selected as main ingredients. Wheat bran was added as additive materials (5%). Oak sawdust used commonly in shiitake mushroom cultivation (Stamets, 1993) was assessed as control in the experiments. The substrate materials were mixed in different ratios as indicated in Table 1.

Compost mixtures were filled into high temperature resistant polypropylene bags as 1 kg per bag. Sterilization of substrate materials was carried out in an autoclave at 121°C under 1.2 atm pressure for 90 minutes. Spawn inoculation was performed by mixing 50 g of spawn into per substrate bag in sterile bench. Substrate bags were placed in mushroom growing room set to 25±2°C temperature and 70–80% humidity for the first step aiming spawn development. After development of spawn, light was provided by using fluorescent lamps (300 lux) for 12 hours a day. Ventilation was performed 4-7 times per hour in the growing room to keep CO_2 rate below 1000 ppm and humidity was increased to 80-90%. Figure 1 shows shiitake mushroom cultivated in A5 substrate mixture.

Table 1. Content of different substrate mixtures used in *L. edodes* production

Substrate materials	SC
Oak sawdust (control)	C
3 oak sawdust + 1 wheat bran	A1
3 poplar sawdust + 1 wheat bran	A2
3 wheat stalk + 1 wheat bran	A3
1 oak sawdust + 1 poplar sawdust + 1 wheat bran	A4
1 oak sawdust + 1 wheat stalk + 1 wheat bran	A5
3 peanut shell + 1 wheat bran	A6
3 corn cob +1 wheat bran	A7
3 vine pruning waste+ 1 wheat bran	A8
1 oak sawdust + 1 peanut shell + 1 wheat bran	A9
1 oak sawdust + 1 corn cob + 1 wheat bran	A10
1 oak sawdust + 1 vine pruning waste + 1 wheat bran	A11

SC: Substrate code

Figure 1. *Lentinula edodes* (shiitake) cultivated in A5 substrate mixture

Chemicals used for Volatile Aroma Analyses

Retention indices were determined by using a mixture of n-alkane standards ranging from C8-C40 and obtained from Sigma Aldrich (Stenheim, Germany). While acetaldehyde (≥99.5% purity), 3-methylbutanal (≥99% purity), 3-octanone (≥98% purity), 3-octanol (≥98% purity), (E)-2-octenal (≥97% purity) and 1-octen-3-ol (≥98% purity) were provided from Merck (Darmstadt, Germany), dimethyl disulfide (≥98.5% purity), carbon disulphide (≥99% purity), 2-methyl-1-butanol (≥98% purity), 3-methyl-1-butanol (≥98.5% purity), 1-octen-3-one (≥97 purity), dimethyl trisulfide (≥98.5% purity), benzaldehyde (≥99% purity), 1-octanol (≥99.7% purity), benzyl alcohol (≥99.5% purity) and phenyl ethyl alcohol were obtained from Sigma Aldrich (Stenheim, Germany).

Determination of Volatile Compounds

HS-SPME technique combined with GC-MS was used to determine volatile compounds of the shiitake mushroom. Effects of different parameters such as extraction time (15, 30 and 45 minutes) and extraction temperature (30°C, 40°C, 50°C) were experimented for extraction efficiency. The highest peak area and the best extraction parameters (30 minutes of extraction time and 30°C extraction temperature) were applied based on detection number of peaks maintained consistency. Method of Palazzolo et al. (2017) was followed in preparation of samples with some small modifications. Fresh mushroom sample (5 g) was cut into slices, then placed into 20 mL vial with a PTFE/silicon septum (Supelco) and stored at 4°C until the analysis. The volatile compounds were extracted by using 1 cm 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) StableFlex fibre (Supelco, Bellefonte, PA, USA). According to recommendation of the supplier, fibre was conditioned (270°C for 60 min). For the extraction of volatile components, the fibre type 50/30 µm DVB/CAR/PDMS suggested by many researchers (Tian et al., 2016; Politowicz et al., 2018; Li et al., 2019) was used because of its high sensitivity and extraction efficiency. Analyses of volatile compounds were carried out by using a 7890B Gas Chromatography (GC) equipped with 7000 triple quatro Mass Selective (MS) detector (Agilent Technologies). A DB-WAX Capillary column (60 m lenght × 0.25 mm i.d. × 0.5 µm thickness) was the stationary phase and flow rate of carrier gas (helium) was 1.5 mL/min. The oven temperature was firstly kept at 40°C for 4 minutes, then increased to 90°C at 3°C/min, 130°C at 4°C/min and 240°C at 5°C/min and finally held at 240°C for 8 minutes. For the MSD, the same oven temperature program was applied. The MSD conditions were as follows: ionisation energy 70 Ev; mass range m/z 30-300 a.m.u; scan rate 2.0 scan/s; interface temperature 250°C and source temperature 180°C. The volatile compounds were identified by comparing the retention indices and their mass spectra from the DB-WAX column with those of a commercial spectra database (W9 N11.L, NIST98, flavour 2) and the internal library of instrument created from previous laboratory studies. The identification of some volatile compounds was confirmed by the injection of chemical standards into the GC-MS system under identical conditions. Retention indices for all volatile compounds detected were calculated using an n-alkane series. All analyses were performed in triplicates (Selli et al., 2006; Cho et al., 2007).

Statistical Analysis

The results of relative percentage (%) of volatile compounds among different substrate mixtures containing different agricultural wastes were compared by the analysis of variance JMP version 5.0.1 (SAS Institute Inc., Cary, NC). A least significant difference test was done to examine the differences among groups. Comparisons that yielded P≤0.05 were considered to be statistically significant.

Result and Discussion

HS-SPME technique combined with GC-MS allowed to identification of 41 volatile compounds isolated from shiitake mushroom cultivated in different substrate mixtures (Table 2a, b). These compounds were classified into six categories in terms of chemical functionality: 13 sulphur containing components (V1, V3, V10, V19, V29, V30, V31, V32, V33, V38, V39, V40 and V41), 7 eight-carbon components (V16, V17, V20, V21, V23, V25 and V26), 9 aldehydes (V2, V4, V5, V6, V11, V24, V27, V34 and V37), 4 alcohols (V14, V15, V35 and V36), 5 hydrocarbons (V8, V9, V12, V13 and V22), 2 ketones (V18 and V28) and 1 other compound (V7). Total quantity of volatile compounds and volatile compound profile showed differences in shiitake mushroom fruiting bodies grown in different substrate mixtures.

In studies carried out by Wu and Wang (2000) and Politowicz et al. (2018); 1-octen-3-ol, 3-octanone, dimethyl disulfide and dimethyl trisulfide, 1,2,4-trithiolane and 1,2,3,5,6-pentathiepane were identified as major volatile components in fresh shiitake mushroom. When we assessed our finding in terms of those compounds, the highest 1-octen-3-ol content was determined in the substrate mixture C (oak sawdust) with 48.66% and it was followed by A7 (3 corn cob +1 wheat bran) with 12.92%, A2 (3 poplar sawdust + 1 wheat bran) with 12.07%, A1 (3 oak sawdust + 1 wheat bran) with 9.25% and A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 7.57%. This compound was found to be 20.2% by the Politowicz et al. (2018) in the shiitake mushroom samples obtained from a company. The analyses of mushroom samples obtained from A5 substrate mixture (1 oak sawdust + 1 wheat stalk + 1 wheat bran) resulted with the highest amount of 3-octanone with 12.05%. The substrate mixtures A3 (3 wheat stalk + 1 wheat bran), A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran), A1 (3 oak sawdust + 1 wheat bran), A2 (3 poplar sawdust + 1 wheat bran) and C (oak sawdust) were considerably rich in terms of this volatile with 11.90%, 10.41%, 10.35%, 8.25% and 8.17%, respectively.

The ranking for 3-octanol was as follows: A1 (3 oak sawdust + 1 wheat bran) with 6.75%, A3 (3 wheat stalk + 1 wheat bran) with 6.37%, A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 6.20%, A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) with 6.07% and A2 (3 poplar sawdust + 1 wheat bran) with 4.73%. Content of 3-octanol changed between 0.97% and 6.75% among substrate mixtures.

Table 2a. Volatiles and their amount detected in different substrate mixtures

Code	Volatile Compounds	RT	RI	ID	C	A1	A2	A3	A4
V1	Methanethiol	3,4	675	RI,MS	nd	nd	nd	nd	nd
V2	Acetaldehyde	3,5	716	RI,MS,std	0.48^{d}	0.65^{b}	0.71^{a}	0.70^{a}	0.54^{c}
V3	Carbon disulfide	3,7	745	RI,MS,std	13.32^{a}	6.58^{bc}	5.84^{c}	6.70^{b}	1.60^{f}
V4	Butanal	4,5	904	RI,MS	nd	0.16	1.71	0	0.13
V5	2-Methylbutanal	6,2	916	RI,MS	nd	0.52^{b}	0.47^{b}	0.50^{b}	0.69^{a}
V6	3-Methylbutanal	6,3	930	RI,MS,std	nd	0.74^{de}	1.03^{c}	0.67^{ef}	1.32^{b}
V7	Formamide	6,9	940	RI,MS	nd	2.63^{b}	3.06^{a}	2.12^{d}	1.99^{d}
V8	trans-1,3-octadiene	7,4	954	RI,MS	0.35^{a}	nd	0.23^{b}	nd	nd
V9	cis-1,3-octadiene	7,4	958	RI,MS	0.94^{a}	nd	0.20^{b}	nd	nd
V10	Dimethyl disulfide	11,5	1047	RI,MS,std	2.40ı	8.07^{g}	10.43^{e}	8.14^{g}	10.49^{e}
V11	Hexanal	11,8	1077	RI,MS	0.88^{a}	0.30^{c}	0.32^{c}	0.26^{c}	0.25^{c}
V12	Ethylbenzene	13,6	1083	RI,MS	nd	nd	nd	nd	0.08^{b}
V13	p-Xylene	14,2	1128	RI,MS	nd	0.17^{d}	$0{,}14^{e}$	0.29^{b}	0.30^{b}
V14	2-Methyl-1-butanol	17,6	1132	RI,MS,std	nd	nd	0.55^{a}	nd	0.08^{b}
V15	3-Methyl-1-butanol	17,6	1207	RI,MS,std	nd	0.1^{e}	0.16^{c}	0.12^{d}	0.17^{c}
V16	3-Octanone	19,6	1211	RI,MS,std	8.17^{c}	10.35^{b}	8.25^{c}	11.89^{a}	10.41^{b}
V17	1-octen-3-one	21,6	1259	RI,MS,std	0.85^{a}	0.51^{b}	0.28^{c}	0.34^{c}	0.18^{d}
V18	4-Nonanone	22,7	1263	RI,MS	0.43^{fg}	2.48^{b}	2.01^{c}	3.43^{a}	3.52^{a}
V19	Dimethyl trisulfide	24,9	1279	RI,MS,std	1.42^{h}	10.23^{g}	17.38^{e}	10.30^{g}	14.14^{f}
V20	3-octanol	25,6	1303	RI,MS,std	1.92^{d}	6.75^{a}	4.73^{c}	6.37^{cab}	6.07^{b}
V21	2-octenal, (E)-	26,8	1355	RI,MS,std	1.17^{a}	0.07^{b}	nd	nd	nd
V22	1,3-dichlorobenzene	27,3	1372	RI,MS	0.28^{a}	0.09^{c}	0.08^{c}	0.15^{b}	0.14^{b}
V23	1-octen-3-ol	27,7	1400	RI,MS,std	48.67^{a}	9.25^{c}	12.07^{b}	3.48^{e}	4.26^{e}
V24	Benzaldehyde	30,1	1414	RI,MS,std	1.59^{h}	13.94^{ef}	11.10^{g}	16.12^{c}	14.62^{d}
V25	1-octanol	31,4	1426	RI,MS,std	2.32^{a}	0.39^{bc}	0.39^{bc}	0.36^{bc}	0.22^{cd}
V26	trans-2-octen-1-ol	33,5	1493	RI,MS	1.69^{a}	0.21^{b}	0.15^{b}	nd	0.13^{b}
V27	Benzeneacetaldehyde	34,5	1528	RI,MS	0.88^{f}	7.06^{c}	4.72^{d}	6.24^{c}	3.73^{e}
V28	Acetophenone	35,0	1584	RI,MS	0.69^{bc}	0.96^{a}	0.92^{a}	0.58^{c}	0.72^{b}
V29	Methyl (methylthio)methyl disulfide	35,5	1611	RI,MS	0.84^{g}	3.23^{ef}	2.56^{f}	2.87^{ef}	5.82^{d}
V30	Benzyl methyl sulfide	35,7	1622	RI,MS	nd	nd	nd	nd	nd
V31	1,3,5-trithiane	36,5	1634	RI,MS	nd	nd	nd	nd	0.23^{c}
V32	Dimethyl tetrasulfide	38,6	1640	RI,MS	nd	0.14^{b}	0.13^{bc}	nd	0.11^{c}
V33	1,2,4-trithiolane	38,8	1659	RI,MS	5.43^{c}	6.06^{b}	4.09^{d}	6.19^{b}	8.12^{a}
V34	2-phenylpropenal	40,4	1714	RI,MS	0.27^{de}	2.0^{b}	0.53^{c}	2.63^{a}	0.20^{def}
V35	Benzyl alcohol	42,7	1722	RI,MS,std	0.32^{f}	2.53^{de}	2.26^{e}	2.98^{d}	3.10^{d}
V36	Phenylethyl alcohol	43,6	1769	RI,MS,std	nd	0.27^{gh}	0.22^{h}	0.70^{e}	0.57^{f}
V37	2-phenyl-2-butenal	44,1	1847	RI,MS	nd	0.36^{ef}	0.21^{ef}	0.45^{de}	0.84^{d}
V38	Methylthio(methylthio)methyl sulfone	45,9	1881	RI,MS	nd	0.09^{cd}	0.12^{bc}	0.17^{bc}	0.21^{b}
V39	2,4,5-trithiahexane 2,2-dioxide	45,9	1897	RI,MS	0.60^{e}	2.02^{d}	2.07^{d}	3.71^{c}	3.75^{c}
V40	1,2,4,5-tetrathiane	50,7	1970	RI,MS	1.26^{b}	0.87^{d}	0.63^{e}	0.94^{c}	0.66^{e}
V41	Methane, tris(methylthio)-	51,2	2218	RI,MS	nd	$0{,}24^{e}$	0.22^{e}	0.60^{d}	0.62^{d}

The results of these three important compounds showed that the eight-carbon components are higher in the substrate mixtures including oak-poplar sawdust and wheat stalk except substrate A7 containing corn cob. When we considered that eight-carbon volatiles are usually high in wild mushroom species collected from forest (Taşkın, 2013; Taşkın et al., 2013; Bozok et al., 2015; Bozok et al., 2018; Taşkın et al., 2019), it seems quite normal that these compounds were found in higher amount in mushrooms grown on substrate mixtures containing wood sawdust than in those containing agricultural wastes.

A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran), A10 (1 oak sawdust + 1 corn cob + 1 wheat bran), A9 (1 oak sawdust + 1 peanut shell + 1 wheat bran), A6 (3 peanut shell + 1 wheat bran), A8 (3 vine pruning waste+ 1 wheat bran), A3 (3 wheat stalk + 1 wheat bran), A2 (3 poplar sawdust + 1 wheat bran), A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) were rich in terms of dimethyl disulfide with 17.39%, 16.07%, 14.95%, 11.36%, 10.95%, 10.49%, 10.43% and 9.34%, respectively and amount of this volatile ranged between 2.39% and 17.39% in all substrate mixtures tested. For dimethyl trisulfide, A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran) contained the highest amount with 38.96% and followed by A8 (3 vine pruning waste+ 1 wheat bran) with 34.24%, A9 (1 oak sawdust + 1 peanut shell + 1 wheat bran) with 32.55%, A10 (1 oak sawdust + 1 corn cob + 1 wheat bran) with 27.62% and A7 (3 corn cob +1 wheat bran) with 22.71%. For all substrates, it ranged from 1.41% to 38.96%. In contrast to the eight-carbon volatiles, sulphur containing compounds were higher in the substrate mixtures supplemented with agricultural wastes.

Maximum amount of 1,2,4-trithiolane was detected in A4 substrate (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) with 8.12% and it was followed by A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 7.75%, A6 (3 peanut shell + 1 wheat bran) with 7.59%, A3 (3 wheat stalk + 1 wheat bran) with 6.19% and A1 (3 oak sawdust + 1 wheat bran) with 6.06%. The amount of 1,2,4-trithiolane varied between 0.70% and 8.12%.

Table 2b. Volatiles and their amount detected in different substrate mixtures

Code	Volatile Compounds	A5	A6	A7	A8	A9	A10	A11	Sig.
V1	Methanethiol	nd	0.97	nd	nd	nd	nd	nd	*
V2	Acetaldehyde	0.72^{a}	0.11^{g}	0.26^{e}	0.21^{f}	0.19^{f}	0.13^{g}	nd	*
V3	Carbon disulfide	4.48^{d}	0.47^{c}	6.99^{b}	2.65^{e}	1.77^{f}	2.60^{e}	3.79^{d}	*
V4	Butanal	0.13	nd	nd	nd	nd	nd	nd	*
V5	2-Methylbutanal	0.72^{a}	nd	0.31^{c}	0.41^{bc}	nd	nd	nd	*
V6	3-Methylbutanal	1.46^{a}	nd	0.56^{g}	0.70^{def}	0.60^{fg}	0.77^{d}	0.76^{de}	*
V7	Formamide	2.34^{c}	1.53^{ef}	1.73^{e}	1.42^{fg}	1.13^{h}	1.22^{gh}	1.53^{ef}	*
V8	trans-1,3-octadiene	nd	nd	nd	nd	nd	nd	nd	*
V9	cis-1,3-octadiene	nd	nd	nd	nd	nd	nd	nd	*
V10	Dimethyl disulfide	9.34^{f}	11.36^{d}	5.70^{h}	10.95^{de}	14.95^{c}	16.07^{b}	17.39^{a}	*
V11	Hexanal	0.49^{b}	nd	nd	nd	nd	nd	nd	*
V12	Ethylbenzene	0.09^{a}	nd	nd	nd	nd	nd	nd	*
V13	p-Xylene	0.33^{a}	0.15^{de}	0.15^{e}	nd	nd	nd	0.23^{c}	*
V14	2-Methyl-1-butanol	0.10^{b}	nd	nd	nd	nd	nd	nd	*
V15	3-Methyl-1-butanol	0.19^{b}	nd	nd	nd	nd	0.13^{d}	0.25^{a}	*
V16	3-Octanone	12.05^{a}	2.10^{h}	5.74^{d}	4.19^{e}	2.85^{fg}	2.35^{gh}	3.43^{f}	*
V17	1-octen-3-one	0.30^{c}	nd	nd	nd	nd	nd	nd	*
V18	4-Nonanone	3.70^{a}	0.74^{ef}	0.33^{g}	0.59^{efg}	0.63^{efg}	0.83^{e}	1.20^{d}	*
V19	Dimethyl trisulfide	14.02^{f}	14.59^{f}	22.71^{d}	34.24^{b}	32.55^{b}	27.62^{c}	38.96^{a}	*
V20	3-octanol	6.20^{b}	0.93^{g}	1.41^{ef}	1.83^{de}	0.97^{g}	1.08^{fg}	1.65^{de}	*
V21	2-octenal, (E)-	0.07^{b}	nd	nd	nd	nd	nd	nd	*
V22	1,3-dichlorobenzene	0.15^{b}	nd	nd	nd	nd	nd	nd	*
V23	1-octen-3-ol	7.57^{d}	0.36^{g}	12.92^{b}	4.22^{e}	2.00^{f}	1.69^{f}	1.32^{fg}	*
V24	Benzaldehyde	10.52^{g}	14.53^{de}	21.76^{a}	17.79^{b}	16.79^{c}	16.27^{c}	13.57^{f}	*
V25	1-octanol	0.40^{bc}	0.12^{cd}	0.60^{b}	nd	nd	nd	nd	*
V26	trans-2-octen-1-ol	0.22^{b}	nd	0.33^{b}	0.18^{b}	nd	nd	nd	*
V27	Benzeneacetaldehyde	3.24^{e}	10.12^{a}	8.35^{b}	3.87^{de}	1.12^{f}	0.94^{f}	0.21^{f}	*
V28	Acetophenone	0.60^{bc}	nd	0.31^{d}	nd	nd	nd	nd	*
V29	Methyl (methylthio)methyl disulfide	3.54^{e}	10.21^{a}	0.61^{g}	3.26^{ef}	6.97^{c}	8.67^{b}	5.79^{d}	*
V30	Benzyl methyl sulfide	nd	nd	0.14^{b}	nd	0.12^{c}	0.19^{a}	nd	*
V31	1,3,5-trithiane	nd	0.43^{a}	nd	nd	nd	0.35^{b}	nd	*
V32	Dimethyl tetrasulfide	0.08^{d}	2.24^{a}	nd	nd	nd	nd	nd	*
V33	1,2,4-trithiolane	7.75^{a}	7.59^{a}	0.70^{g}	1.22^{fg}	1.53^{f}	2.12^{e}	1.33^{f}	*
V34	2-phenylpropenal	0.35^{cd}	0.10^{ef}	0.39^{cd}	nd	nd	nd	nd	*
V35	Benzyl alcohol	2.20^{e}	2.86^{d}	5.16^{b}	5.83^{a}	4.11^{c}	5.24^{b}	2.95^{d}	*
V36	Phenylethyl alcohol	0.32^{g}	1.29^{c}	0.62^{ef}	1.18^{d}	1.74^{b}	2.17^{a}	1.14^{d}	*
V37	2-phenyl-2-butenal	0.54^{de}	0.36^{ef}	1.62^{c}	4.87^{a}	1.97^{bc}	2.17^{b}	0.42^{def}	*
V38	Methylthio(methylthio)methyl sulfone	0.19^{b}	0.93^{a}	nd	nd	nd	nd	nd	*
V39	2,4,5-trithiahexane 2,2-dioxide	3.68^{c}	12.31^{a}	0.75^{e}	0.39^{e}	6.35^{b}	6.04^{b}	2.92^{cd}	*
V40	1,2,4,5-tetrathiane	1.35^{a}	0.19^{f}	nd	nd	nd	0.24^{f}	nd	*
V41	Methane, tris(methylthio)-	0.56^{d}	3.41^{a}	nd	nd	1.67^{b}	1.08^{c}	1.15^{c}	*

In a study carried out by Mata et al. (2014), similar to our research, different substrate materials were tested in terms of aroma composition of shiitake mushroom. In the substrate mixtures containing barley straw and oak powder; 3-octanone, 1-octen-3-ol, benzeneacetaldehyde and benzaldehyde were found to be main volatiles with 79.72%, 18.93%, 0.76% and 0.57%, respectively. The most important volatiles of sugar cane bagasse substrate were 3-octanone with 79.58%, 1-octen-3-ol with 9.92%, 2-penten-1-ol with 9.32% and 1,2,4-trithiolane with 1.18%. Mushroom samples obtained from substrate mixture prepared from beech tree litter and oak powder (*Platanus mexicana*) had 3-octanone (97.77%) and 2-penten-1-ol (2.22%). 3-octanone (71.40%), 2-pentylfuran (11.78%), limonene (6.89%), benzeneacetaldehyde (5.76%) and benzaldehyde (4.15%) were the major volatiles in the oak wood sawdust. In our analyses, 1-octen-3-ol varied between 0.36% and 48.66% among substrate mixtures tested. It was detected between 9.92% and 18.93% in the research of Mata et al. (2014). Similarly, while amount of 3-octanone, benzaldehyde and benzeneacetaldehyde ranged from 2.10% to 12.05%, from 2.02% to 21.76% and from 0.21% to 10.12% in this study, they changed between 71.40% and 97.77%, 0.57% and 4.14% and 0.76% and 5.76% respectively in the study carried out by Mata et al. (2014). The high amount of 3-octanone in their study may be related to a small number of volatiles (eight) detected.

Many different volatiles come together to create a different flavour and odour in mushrooms. Especially, the presence of 1-octen-3-ol known as mushroom alcohol and its amount is very important (Taşkın, 2013; Taşkın et al., 2013; Bozok et al., 2015; Bozok et al., 2018; Taşkın et al., 2019). Together with alcohols, sulphur containing compounds are known as effective in mushroom odour (Hiraide, 2006). 2,4-trithiolane and 1,2,4,6-tetrathiepane have been reported as major sulphur containing volatiles of shiitake mushroom in literature and also its use as an indicator in estimating odour of dried *L. edodes* has been emphasized (Hiraide, 2006). To increase sulphur content of shiitake mushroom, rice bran detected as rich in terms of sulphur was used in the cultivation of this mushroom species and then measured by Hiraide (2006). Among main volatiles detected in this study for fresh shiitake mushroom; 1-octen-3-ol, 3-octanone, 3-octanol, 1, 2, 4-trithiolane, benzeneacetaldehyde and benzaldehyde are responsible for mushroom-butter-resinous, sweet fruity, cod liver oil, egg-garlic, fruity and bitter almond, respectively (Mata et al., 2014).

Maximum number of volatiles were obtained from A2 (3 poplar sawdust + 1 wheat bran), A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) and A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) substrate mixtures with 36 compounds and they were followed by A1 (3 oak sawdust + 1 wheat bran) with 33, A3 (3 wheat stalk + 1 wheat bran) with 30, A6 (3 peanut shell + 1 wheat bran) with 26, A7 (3 corn cob +1 wheat bran) and C (oak sawdust) with 25, A10 (1 oak sawdust + 1 corn cob + 1 wheat bran) with 23, A8 (3 vine pruning waste+ 1 wheat bran), A9 (1 oak sawdust + 1 peanut shell + 1 wheat bran) and A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran) with 20. Although there is significant difference between the substrate mixtures in terms of number of compounds, when content of the substrate mixtures is considered, there is no evidence to say that the substrate mixtures containing only agricultural wastes or only wood sawdust produce more compounds.

When we evaluated substrate mixtures in terms of eight-carbon components, the highest percentage was recorded in C (oak sawdust) with 64.79%. Eight-carbon compounds are reported to be unstable during the drying process in literature (Li et al., 2019). The rate for other substrates was as follows: A1 (3 oak sawdust + 1 wheat bran) with 27.53%, A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 26.74%, A2 (3 poplar sawdust + 1 wheat bran) with 25.87%, A3 (3 wheat stalk + 1 wheat bran) with 22.44%, A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) with 21.27%, A7 (3 corn cob +1 wheat bran) with 21.05%, A8 (3 vine pruning waste+ 1 wheat bran) with 10.42%, A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran) with 6.4%, A9 (1 oak sawdust + 1 peanut shell + 1 wheat bran) with 5.82%, A10 (1 oak sawdust + 1 corn cob + 1 wheat bran) with 5.11% and A6 (3 peanut shell + 1 wheat bran) with 3.51%. As we emphasized before, it is clear that substrate mixtures containing wood straw are richer for eight-carbon components. The fact that having of the substrate mixture including only oak sawdust almost twice eight-carbon components than the substrate mixture nearest can be considered as an important result.

In contrast to the eight-carbon volatiles, agricultural wastes were richer than sulphur containing substrate mixtures such as A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran) with 71.33%, A9 (1 oak sawdust + 1 peanut shell + 1 wheat bran) with 65.91%, A10 (1 oak sawdust + 1 corn cob + 1 wheat bran) with 64.98%, A6 (3 peanut shell + 1 wheat bran) with 64.70% and A8 (3 vine pruning waste+ 1 wheat bran) with 52.71%. Ranking of the other substrates was as follows: A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) with 45.75%, A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 44.99%, A2 (3 poplar sawdust + 1 wheat bran) with 43.47%, A3 (3 wheat stalk + 1 wheat bran) with 39.62%, A7 (3 corn cob +1 wheat bran) with 37.60%, A1 (3 oak sawdust + 1 wheat bran) with 37.53% and C (oak sawdust) with 25.27%.

The substrate mixtures including agricultural wastes seem again rich in terms of alcohols like this sulphur containing substrates: A10 (1 oak sawdust + 1 corn cob + 1 wheat bran) with 7.54%, A8 (3 vine pruning waste+ 1 wheat bran) with 7.01%, A9 (1 oak sawdust + 1 peanut shell + 1 wheat bran) with 5.85%, A7 (3 corn cob +1 wheat bran) with 5.78%, A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran) with 4.34%, A6 (3 peanut shell + 1 wheat bran) with 4.15%, A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) with 3.92%, A3 (3 wheat stalk + 1 wheat bran) with 3.80%, A2 (3 poplar sawdust + 1 wheat bran) with 3.19%, A1 (3 oak sawdust + 1 wheat bran) with 2.90%, A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 2.81% and C (oak sawdust) with 0.32%.

Substrate mixture A7 (3 corn cob +1 wheat bran) was the most successful in containing aldehydes with 33.25% and followed by A8 (3 vine pruning waste + 1 wheat bran) with 27.85%, A3 (3 wheat stalk + 1 wheat bran) with 27.57%, A1 (3 oak sawdust + 1 wheat bran) with 25.73%, A6 (3 peanut shell + 1 wheat bran) with 25.46%, A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) with 22.32%, A2 (3 poplar sawdust + 1 wheat bran) with 20.80%, A9 (1 oak sawdust + 1 peanut shell + 1 wheat bran) with 20.67%, A10 (1 oak sawdust + 1 corn cob + 1 wheat bran) with 20.28%, A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 18.17%, A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran) with 14.96% and C (oak sawdust) with 4.1%.

The substrate mixtures including wood sawdust were better in terms of hydrocarbons. Even no data was obtained from A8, A9 and A10 substrate mixtures supplemented agricultural wastes, the other mixtures could produce hydrocarbons [C (oak sawdust) with 1.57%, A2 (3 poplar sawdust + 1 wheat bran) with 0.65%, A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 0.57%, A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) with 0.52%, A3 (3 wheat stalk + 1 wheat bran) with 0.44%, A1 (3 oak sawdust + 1 wheat bran) with 0.26%, A6 (3 peanut shell + 1 wheat bran) with 0.25%, A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran) with 0.23%, A7 (3 corn cob +1 wheat bran) with 0.15%].

For the two compounds described as ketone, A5 (1 oak sawdust + 1 wheat stalk + 1 wheat bran) with 4.30%, A4 (1 oak sawdust + 1 poplar sawdust + 1 wheat bran) with 4.24%, A3 (3 wheat stalk + 1 wheat bran) with 4.01%, A1 (3 oak sawdust + 1 wheat bran) with 3.44% and A2 (3 poplar sawdust + 1 wheat bran) with 2.93% were found to be better than A11 (oak sawdust + 1 vine pruning waste + 1 wheat bran) with 1.20%, C (oak sawdust) with 1.12%, A10 (1 oak sawdust + 1 corn cob + 1 wheat bran) with 0.83%, A6 (3 peanut shell + 1 wheat bran) with 0.74%, A9 (1 oak sawdust + 1 peanut shell + 1 wheat bran) with 0.63%, A7 (3 corn cob +1 wheat bran) with 0.64% and A8 (3 vine pruning waste+ 1 wheat bran) with 0.59%.

In a study carried out by Li et al. (2019) on effect of different agricultural wastes on the volatile profile of shiitake mushroom; sawdust, wheat bran, rice bran, soy bean pulp and corn meal were used and 82 volatiles were obtained including 7 sulphur containing components (49.55%), 8 eight-carbon components (46.47%), 19 aldehydes (35.11%), 10 alcohols (22.82%), 7 esters (46.26%), 5 ketones (23.85%), 10 acid components

(33.71%) and 16 other compounds (58.18%). In their study, sulphur containing compounds and aldehydes were dominant except CS6 (sawdust, wheat bran and gypsum). While sulphur containing compounds were the highest in CS1 (sawdust, wheat bran and gypsum) and CS4 (sawdust, wheat bran, soy bean pulp and gypsum), maximum aldehydes were obtained from CS2 (sawdust, wheat bran, corn meal and gypsum). Agricultural wastes seem quite successful in obtaining sulphur containing compounds similar to our finding. However, while soy bean pulp, cereal bran, wheat bran and rice bran increased the amount of sulphur containing compounds, corn meal had positive effect on aldehyde concentration. Also, the highest data for aldehydes was recorded in A7 (3 corn cob + 1 wheat bran). Both our A7 mixture and Li et al. (2019)' CS2 and CS7 mixtures contained corn wastes. This finding may mean that corn wastes have an important role on aldehydes content of shiitake mushroom.

In a very early study carried out by Chen and Ho in 1986, 18 noncyclic and cyclic sulphur containing volatiles (13 of them were assessed as new) were reported in fresh shiitake mushroom samples. Hiraide et al. (2004) emphasized that sulphur containing compounds have very important role in shiitake mushroom smell, especially in dried mushroom samples. 1,2,4-trithiolane and 1,2,4,6-tetrathiepane were found as main volatiles, especially 1,2,4- trithiolane was suggested as indicator. Volatile profile of shiitake mushroom was determined at the young, immature, mature and old growing stages by Cho et al. (2003) and 129, 129, 111 and 120 volatiles were detected, respectively. 1-octen-3-ol, 3-octanol, 3-octanone and 4-octen-3-one were identified as major components. They also realized that while 1-octen-3-ol content decreased with maturation, 3-octanone increased. The effect of pH on formation and concentration of volatiles was studied by Chen et al. (1984). It was found that while pH 5.0–5.5 was better for eight-carbon compounds, pH 7.0 was suitable for sulphurous compounds in shiitake mushroom. The researchers considered that two different enzyme systems were active for these different volatile groups.

Conclusion

In this study, the changes on the aroma profile with the use of different agricultural materials in shiitake mushroom cultivation were assessed. Oak sawdust, poplar sawdust, wheat bran, wheat stalk, peanut shell, corn cob and vine pruning waste were used as combination with each other at different ratios. Important results were obtained when experiments were completed: (i) eight-carbon components and hydrocarbons were detected at higher amount in the substrate mixtures including wood straw than agricultural wastes (ii) sulphur containing substrates and alcohols were found rather than in the substrate mixtures including agricultural wastes (iii) corn wastes seem to have considerable effect on aldehydes content (iii) dimethyl trisulfide, benzaldehyde, dimethyl disulfide, 1-octen-3-ol and 3-octanone were identified as major volatile components. Overall, the substrate material has significantly affected the volatile aroma composition of the mushroom. Mushrooms are consumed because of their unique taste and aroma. Therefore, the results obtained from this study are important in terms of practice. For example, sulphur containing component are very important in the formation of odour in the shiitake mushroom and therefore, it is very valuable to have knowledge which substrate materials can lead to increase in the amount of the sulphur containing volatiles. The availability and efficiency of agricultural wastes in mushroom cultivation will reduce the need for wood sawdust. There are two important reasons for wanting to reduce the use of wood sawdust in mushroom production. The first is the difficulty of the availability of wood sawdust. The second is that while wood may be used in different production areas, agricultural wastes do not have different uses.

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