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Lignocellulosic and proximate based compositional changes in substrates during cultivation of *Hericium erinaceus* mushroom

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ABSTRACT

The study was carried out to investigate the potential of several kinds of forestry (oak sawdust (OS), poplar sawdust (PS)) and agricultural byproducts (common vessel straw (CV), wheat straw (WS), safflower wastes (SW) and bean straw (BS)) as growing substrate for *Hericium erinaceus* cultivation, but also assessing the chemical and lignocellulosic changes occurred in the growing substrates during *H. erinaceus* cultivation process to understand the needs of the fungus. Moreover, the proximate and lignocellulosic composition of *H. erinaceus* spent mushroom substrate were presented in the study. Among substrates, CV appeared to promote earliness by presenting shorter cropping periods (29 d), whereas maximum yields (115.8 g/kg and 37.3% BE) and heavier basidiomata (54.1 g) were produced by OS substrates. Spawn running time was correlated positively with carbon to nitrogen ratio (C:N) and lignin content of substrates (r^2 = 0.946), while BEs was positively correlated with lignin content (r^2 = 0.846) and inversely correlated to cellulose/ lignin ratio (r^2 =–0.955). The moisture, N, ash, lignocellulosic content of all the substrates were changed greatly during *H. erinaceus* cultivation, but their rates of change varied at different growing stages. The findings indicate that the shortened spawn run period was correlated with the loss of substrate hemicellulose and high lignin concentration in conjunction to their low cellulose/lignin ratio applies a positive effect on yield and BEs of *H.erinaceus*.

1. Introduction

Although straw obtained from different crops has been used as a poultry feed supplement, its high lignin and low protein content limits its use. Agricultural residues also provide potential for bioenergy production, as in bioethanol and biofertilizers (Cantrell et al., 2010; Ogbo, 2010; Sarkar et al., 2012). On the other hand, mushrooom cultivation can present an alternative method for reducing environmental pollution problems and at the same time produce a tasty and healthful food. Plant residues possess high lignin content and are also rich in cellulose and hemicellulose. White rot *Basidiomycetes* have the potential for degradation of various agricultural wastes via their oxidative ligninolytic systems which include enzymes such as cellulase, hemicellulase, lignin peroxidase, laccases and manganese peroxidase (Lim et al., 2013). Thus, it is possible to cultivate these fungi in a wide range of lignocellulosic substrates, including different agriculture and forestry wastes. Agricultural wastes exhibit different compositions of ash, cellulose, hemicellulose, lignin, nitrogen and macro/micro elements. The chemical nature of the substrates used to cultivate mushrooms has a striking influence on their yields. During the development of mushroom mycelia and fruitbody growth, a number of biochemical changes occur as a result of the production of extracellular enzymes in the growing substrate (Kurt and Buyukalaca, 2010; Luz et al., 2012). The degradation capacity of the lignocellulosic components changes depending on genetic factors among species and strains, composition of the growing medium and environmental factors (Blanchette, 1991).

Hericium erinaceus, known as monkey head, lion's mane or pom pom mushroom, is an important edible and medical mushroom which has been used in traditional Chinese medicine for centuries. *H. erinaceus* extract is used in the prevention and treatment of some diseases such as cancer (Mizuno, 1999), dementia (Kawagishi and Zhuang, 2008), hypercholesterolemia (Liang et al., 2013), hyperglycemia (Hiwatashi et al., 2010). It belongs to the *Basidiomycota* and grows on hardwoods in nature. Unlike *Pleurotus* spp. or *Lentinula edodes* (shiitake), *H. erinaceus* is not commonly cultivated in the USA and Europe, although its reputation is steadily growing because of its delightful taste and nutritional value, in addition to medicinal properities.

The fungal degradation of agrowastes using *Pleurotus ostreatus* (Li et al., 2001; Kurt and Buyukalaca, 2010) and *Lentinula edodes* (Philippoussis et al., 2003; Cavallazzi et al., 2004; Gaitán-Hernández et al., 2006; Hiyama et al., 2011; Atila, 2019) and biochemical changes in agroresidues have been discussed in previous studies. Several studies

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have focused on determining the yield and productivity of *H. erinaceus* on various types of substrates (Ko et al., 2005; Hassan, 2007; Figlas et al., 2007) and the effects of substrates on nutritional composition and antioxidant capacity of the *H. erinaceus* fruitbody (Koutrotsios et al., 2016; Atila et al., 2018). However, under mushroom-growing conditions with this species in lignocellulosic substrates, the chemical and lignocellulosic changes are largely unknown. In order to get a better understanding of the lignocellulose degradation mechanism in *H. erinaceus* cultivation, the degradation process needs to be revealed and analyzed in detail. Lastly, no data has yet been provided in the literature on the physicochemical properities of the spent mushroom substrate of *H. erinaceus*.

The aim of this study was to assess the chemical and lignocellulosical changes occurring in the growing substrates during the *H. erinaceus* cultivation process in order to understand the needs of the fungus. Moreover, some physicochemical properities of *H. erinaceus* spent mushroom substrate were presented in the study.

2. Materials and methods

2.1. Strain and substrates

Hericium erinaceus strain He-6 isolated from Izmit, Turkey, was examined in the study. The strain is maintained in the Mushroom Culture Collection of Ahi Evran University (Kirsehir, Turkey) at 4 °C. Grain spawn was prepared as previously described by Atila (2017).

Six different types of agricultural and forestry wastes were used in the study. Wheat straw (WS), common vetch straw (CV), bean straw (BS) and safflower waste (SW) were obtained from local fields in Kırşehir (Turkey) during the 2016–2017 harvest season. Oak sawdust (OS) and poplar sawdust (PS) were obtained from Izmir and Kirsehir, respectively.

2.2. Substrate preparation and mushroom cultivation

The straws and agricultural waste (safflower, common vetch, wheat and bean) were chopped into 2–3 cm-long pieces. No treatment was applied to the 60–80 mesh-sized sawdust (oak and poplar). The sawdusts and straws were soaked for 24 h in water after which excess water was removed. Heat resistant polypropylene bags were then filled with 1 kg of the substrates and autoclaved at 121 °C for 90 min. When the temperature of the sterilized substrates reached 25 °C, the bags were inoculated with spawn of *H. erinaceus* at 3% of the substrate fresh weight. Ten bags were prepared per substrate. The experiment was conducted in a randomized plot design.

The spawn running period was conducted at a temperature of 24 ± 2 °C and relative air humidity of 85%. After mycelial growth was completed, the temperature was lowered to 18 ± 2 °C and relative air humidity increased to 90%, with illumination provided for 8 h daily. Furthermore, CO_2 levels were maintained at less than 1200 ppm. This study was conducted at the Mushroom Production Unit of the Ahi Evran University Faculty of Agriculture in Kirsehir, Turkey.

The following parameters were evaluated for the assessment of the substrates: (i) spawn running period (days), (ii) time to primordia formation, (iii) time to first harvest, (iv) yield as harvested mushroom weight (g)/wet substrate weight (kg), (v) biological efficiency percentage (BE%) and (vi) average mushroom weight (g). Average mushroom weight was calculated as the ratio of the total yield to the total number of fruitbodies obtained. The BE% was calculated as follows: ([weight of fresh mushrooms harvested (g) /weight of dry substrate (g)] × 100).

2.3. Substrate analyses

Using a spatula, samples were taken at approximately 1 cm in depth from five different regions of three of bags selected randomly at each of the three growth stages: (i) mycelium inoculation (Day 0), (ii) after the spawn running period and (iii) at the end of the harvest period. Substrate samples were dried at 60 °C for 48 h before grinding them to 1mm-sieve size using a rotary mill.

Analysis was carried out in the Field Crop Laboratories of Ahi Evran University, Kirsehir, Turkey. Moisture, ash and pH were determined according to AOAC (1995), while hemicellulose, cellulose and lignin contents of the substrates were determined as described by Vansoest et al. (1991). The Kjeldahl method was used to determine the total nitrogen content of the substrates and fruitbodies. The C/N ratio was calculated is follows: C/N = (%C /%N). All analyses were conducted in triplicate.

2.4. Statistical analyses

The effect of substrates on productivity and yield was analyzed using the 16.0 version of SPSS. Differences among mean values of results were assessed using the Tukey *post hoc* test at a 95% confidence level. Pearson's correlation coefficient was used to evaluate relationships between variables such as cultivation parameters and changes of nutritional and lignocellulosic content of substrates during the cultivation process at a significance level of 5%.

3. Results and discussion

3.1. Proximate analysis and lignocellulosic composition of initial substrates

The proximate analysis and lignocellulosic composition of the initial substrates are presented in Table 1 and Table 2, respectively. The substrate differences in moisture, pH, ash, nitrogen and C/N ratios were found to be statistically significant ($p < 0.05$). The pH, moisture and ash content of the substrates varied, with ranges of 4.4–6.5%, 68.5–70.5% and 2.2–7.6%, respectively. The amount of nitrogen was higher in agricultural wastes than in forestry wastes. The substrate with the richest nitrogen content was the CV (1.22%), while the PS had the lowest nitrogen content (0.14%), followed by the OS (0.19%). The PS presented the lowest carbon content (56.8%). The C/N ratio ranged from 44.2 to 393.1. The cellulose, hemicellulose and lignin content of the substrates showed ranges of 32.4–54.4%, 11.6–26.1% and 6.1–23.0%, respectively. The initial amount of lignin was similar for CV, WS, SW and BS and was significantly lower than that of OS and PS.

Table 1
Proximate analysis of growing substrates tested in the study.

	Moisture (%)	pH	Ash (%)	C (%)	N (%)	C/N
OS	69.0 ± 0.39^{ns}	4.37 ± 0.09**e	2.50 ± 0.01**e	56.6 ± 0.00** b	0.19 ± 0.00**e	299.6 ± 6.6** b
PS	69.0 ± 0.79	5.94 ± 0.06 bc	2.16 ± 0.10 f	56.8 ± 0.06 a	0.14 ± 0.01 e	393.1 ± 17.0 a
CV	67.0 ± 1.20	5.48 ± 0.09 d	7.10 ± 0.16 b	53.9 ± 0.02 e	1.22 ± 0.02 a	44.2 ± 0.66 d
WS	68.5 ± 0.86	6.53 ± 0.11 a	4.51 ± 0.05 c	55.4 ± 0.03 d	0.37 ± 0.00 d	151.6 ± 1.63 c
SW	70.5 ± 0.39	5.76 ± 0.06 c	3.17 ± 0.02 d	56.2 ± 0.01 c	0.94 ± 0.02 c	60.1 ± 1.62 d
BS	68.5 ± 2.56	6.19 ± 0.03 b	7.64 ± 0.10 a	53.6 ± 0.06 f	1.17 ± 0.02 b	45.9 ± 0.84 d

OS:oak sawdust; PS: poplar sawdust; CV: common vetch straw; WS: wheat straw; SW:safflower waste; BS:bean straw. Asterisks indicate significance at $*P < 0.05$, $**P < 0.01$, ^{ns} not significant; values within the same column followed by the same letter are not significantly different by Tukey's test.

Table 2
Lignocellulosic composition of growing substrates tested in the study.

	Cellulose (%)	Lignin (%)	Hemicellulose (%)	Cel/Lig
OS	44.8 ± 0.83** d	23.0 ± 0.48** a	14.3 ± 0.11**c	1.95 ± 0.04 e
PS	53.5 ± 0.74 b	20.2 ± 0.54 b	11.6 ± 0.21 d	2.65 ± 0.05 d
CV	32.4 ± 0.56 f	9.5 ± 0.26 c	18.1 ± 1.49 b	3.41 ± 0.04 c
WS	50.6 ± 0.87 c	9.0 ± 0.36 c	26.1 ± 0.21 a	5.25 ± 0.18 b
SW	54.4 ± 0.98 a	6.1 ± 0.23 e	11.8 ± 1.83 d	8.90 ± 0.38 a
BS	40.3 ± 1.82 e	7.3 ± 0.12 d	17.8 ± 2.81 b	5.50 ± 0.29 b

OS:oak sawdust; PS: poplar sawdust; CV: common vetch straw; WS: wheat straw; SW:safflower waste; BS:bean straw. Asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.01$, ^{ns} not significant; values within the same column followed by the same letter are not significantly different by Tukey's test.

The maximum extent of initial lignin content (23.0%) was observed in OS. The highest concentration of cellulose was determined in SW, while the highest hemicellulose content was exhibited in WS.

3.2. Effect of substrates on yield performance of H. Erinaceus

In this study, different agricultural and forestry by-products were evaluated as growing substrates in *H. erinaceus* cultivation. The yield performance of the substrates was characterized by defined spawn running time, duration required for pinhead formation and time required for the first harvest. *Hericium erinaceus* demonstrated mycelium growth ability on all substrates. As shown in Table 3, *H. erinaceus* completed mycelial growth between 17.0 and 23.6 d depending on the substrates. There were no significant differences among incubation times of CV, WS, SW or BS, but mycelial growth was accomplished significantly more slowly on OS and PS. However, the resulting times obtained were shorter than those of some previous studies (Ko et al., 2005; Hassan, 2007). Reduction in the duration of the spawn running period could help to reduce the fungal and bacterial contamination risk during the colonization stage.

Hericium erinaceus completed the production process within 60 d in all substrates tested in the study. Although mycelial growth on OS was completed considerably later (by nearly 6–7 d) than on the agricultural wastes, it exhibited the shortest time to primordia initiation, with an average 5.2 d. Primordia formation was noted after 24.0–30.0 d from substrate inoculation, whereas 29.0–35.8 d were required for starting the harvest in the substrates tested. Atila et al. (2018) reported that primordia initiation and first harvest had taken place around 30–46 and 34–54 d, respectively, on substrates supplemented with additive materials like wheat bran, cotton seed hulls and olive press cake. Similarly, Koutrotsios et al. (2016) determined the formation of *H. erinaceus* primordia to be after 39–45 d from substrate inoculation.

The differences among the substrates in total yield, BE% and average mushroom weight were significant ($p < 0.05$). The mushroom yields were presented as fresh weight of the mushrooms harvested in 60 d per unit wet weight of the substrates. Total fresh mushroom yield varied from 36.4 to 115.8 g/kg. The comparison of *H. erinaceus* growth on the six different substrates indicated that the sawdust substrates OS and PS ensured higher yields of the fungus. The BE differed significantly, ranging from 32.4 to 37.3% in the softwood and hardwood types of sawdust, and from 12.3 to 28.2% in the agricultural wastes. Ko et al. (2005) reported that the BE of *H. erinaceus* grown on oak sawdust substrate was 31%; however, in that case, the sawdust was added to rice bran at a ratio of 4:1. Figlas et al. (2007) found a BE of 30.4% for *H. erinaceus* grown on sunflower seed hull substrate. The use of CV and BS as substrates for *H. erinaceus* cultivation hastened the process of mushroom production, but did not increase the yield as that of the OS and PS. Nevertheless, the CV and BS supported satisfactory yields compared to previous studies. On the other hand, a previous study using wheat straw reported a yield of 140–157 g/kg and 36.5–40.4% BE in *H. erinaceus* (Hassan, 2007), which were considerably higher than those of the WS substrate in the present study. The number of harvests obtained in the tested substrates were two, except for the SW substrate. The substrates influenced the yield distribution as well as the yield and BE of *H. erinaceus*.

Differences were noted in the mushroom yields among the flushes produced on various substrates. Of the total yields, 66.2, 71.7 and 62.4% were obtained from the first flushes on OS, PS and CV, while BS presented equal yield distributions from two flushes. Yield distribution displayed a different pattern in the WS substrate, with 57.9% of the total obtained in the second flush. Regarding average weight of the harvested fruitbodies, similar to the yield potential, OS (54.1 g) appeared to support the production of heavier mushrooms, while the lightest fruitbodies were recorded on SW (19.1 g).

No correlation was found between N content and spawn running time. Moreover, substrate nitrogen concentration had no direct effect on mushroom yield. Total N content was lower on OS and PS, where the maximum yield mean value was obtained. Although Kurt and Buyukalaca (2010) determined higher enzyme activities in media with high N content, they reported that high N content could be related to the decrease in yields of *P. ostreatus*. Spawn running time was correlated positively with the C/N ratio ($r^2 = 0.857$) and lignin content ($r^2 = 0.946$) of the substrates, while BE was positively correlated with the lignin content ($r^2 = 0.846$) and inversely correlated with the cellulose/lignin ratio ($r^2 = -0.955$). Faster mycelial growth was observed inthe growing substrates, which had the lower C/N ratio (between 44.2 and 151.6). The physical properties of substrates can influence their yield and BE as well as their nutritional composition and lignocellulosic content. The SW provided relatively low values during the cultivation of *H. erinaceus*, and results showed that SW substrate possessed a higher moisture content. This could have caused low air availability in the substrate, potentially leading to the anerobic conditions which are indispensable for enzymatic degradation activity, thus resulting in low mushroom yields.

Table 3
Effect of different substrates on production of *Hericium erinaceus* mushroom.

Substrate	Spawn running time (days)	Day to primordial initial (days)	Days to first harvest (days)	Yield distrubution		Total yield (g/kg)	BE (%)	AMW (g)
				Flush I (g)	Flush II (g)			
OS	23.6 ± 1.20** a	28.8 ± 0.40**b	33.8 ± 0.40**b	76.7	39.1	115.8 ± 4.08** a	37.3 ± 1.31** a	54.1 ± 4.01** a
PS	21.2 ± 0.75 b	26.8 ± 0.75 c	31.0 ± 0.63 c	72.1	28.4	100.5 ± 2.74 b	32.4 ± 0.88 b	46.2 ± 2.97 b
CV	16.8 ± 0.40 c	24.0 ± 0.63 e	29.0 ± 0.63 d	54.6	32.9	87.5 ± 2.70 c	28.2 ± 0.87 c	43.8 ± 2.25 b
WS	17.6 ± 0.49 c	25.2 ± 0.40 d	30.2 ± 0.40 cd	25.2	34.6	59.8 ± 2.27 e	19.5 ± 0.72 d	22.7 ± 1.85 c
SS	18.0 ± 0.00 c	30.0 ± 0.00 a	35.8 ± 1.17 a	36.4	–	36.4 ± 2.24 f	12.3 ± 0.77 e	19.1 ± 2.11 d
BS	17.0 ± 0.00 c	24.6 ± 0.49 de	30.6 ± 1.36 cd	38.9	41.4	80.3 ± 0.63 d	25.5 ± 0.64 c	33.4 ± 1.69 c

OS:oak sawdust; PS: poplar sawdust; CV: common vetch straw; WS: wheat straw; SW:safflower waste; BS:bean straw. BE: Biological efficiency; AMW:Average mushroom weight. Asterisks indicate significance at $^{*}P < 0.05$. $^{**}P < 0.01$; values within the same column followed by the same letter are not significantly different by Tukey 's test.

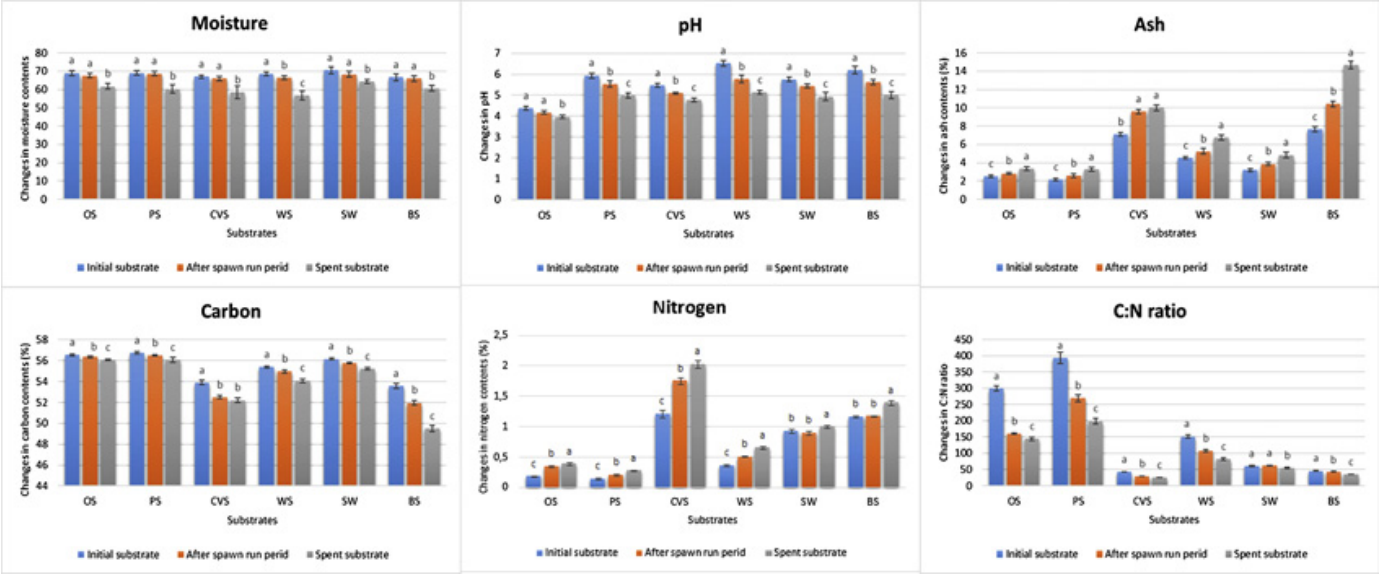

Fig. 1. Changes of proximate composition of substrates tested during *Hericium erinaceus* cultivation period.

3.3. Chemical changes in substrates

To investigate the changes that occured in the substrates during the cultivation process, the moisture, pH, C, N and C/N ratio were monitored in the initial substrate, after mycelial growth and after *H. erinaceus* cultivation. As shown on Fig. 1, the proximal composition of the substrates varied significantly among the three different stages ($p < 0.01$).

The substrate moisture content decreased slightly in the spawn running period with differences of 0.41–3.2%; however, decreases in the moisture content of the substrates reached 8.7–17.3% by the end of cultivation, as shown in Table 4. The decrease in the substrate moisture content could be ascribed to transfer of water to the atmosphere and the fruiting bodies. *Hericium erinaceus* mycelium caused a rapid decrease in the pH of the substrates during the cultivation period with the pH dropping from 4.4 to 6.5 to 4.2–5.8 after the spawn running period and to 4.0–5.1 by the ending of cultivation. The decrease of pH detected during the cultivation period in all three substrates is in agreement with previous results on *Lentinula edodes* (Philippoussis et al., 2003) and *Ganoderma lucidum* (Peksen et al., 2011). The pH may have been influenced by the presence of metabolic waste products in the substrate (Jonathan et al., 2008) or by substances such as organic acid that were formed during the cultivation process.

Significant differences were observed in the percentage of ash in the substrates for the three sampling periods ($p < 0.01$), with a tendency for it to increase (33.7–91.9%). This finding is in agreement with Koutrotsios et al. (2014). The OS substrates showed lower increases of ash content than those of other substrates, although the aforementioned substrate exhibited the highest yield. The increase in ash content of the substrates during the cultivation period could have been due to bioconversion and loss of lignocellulosic compounds (Singh, 2000).

After the spawn running period, the concentration of N in OS, PS, CV and WS increased relative to that of the initial substrate, while it decreased in SW and did not change in BS. The concentration of N in OS increased from 0.19 to 0.35%, an increase of 86.4%, after full mycelial colonization. However, the nitrogen content of the substrate increased during the entire cultivation period and reached 0.39% after 60 d of cultivation, an increase of 106.7% from the initial substrate. A similar result was presented by Adenipekun and Dada (2013) with cotton waste, rice straw and cocoa pod husk decayed by *Pleurotus pulmonarius*. The N content was highest in the spent substrate of CV and lowest in the spent substrate of PS. Substrates that supported a successful mushroom

Table 4
The percentage rates of change in proximate and lignocellulosic composition of growing substrates at different stages of *Hericium erinaceus* cultivation.

	Moisture	ph	Ash	C	N	C:N	Cellulose	Lignin	Hemicellulose	cel:lig
OS										
After spawn run	(-) 2.1	(-) 4.58	(+) 12.6	(-) 0.32	(+) 86.4	(-) 46.5	(+) 0.2	(-) 10.0	(-) 14.4	(+) 11.3
After harvest	(-) 10.6	(-) 9.38	(+) 33.7	(-) 0.86	(+) 106.7	(-) 52.0	(-) 15.1	(-) 35.9	(-) 28,4	(+) 32,5
PS										
After spawn run	(-) 0.41	(-) 6.9	(+) 20.0	(-) 0.44	(+) 45.2	(-) 31.45	(-) 4.8	(-) 11.2	(-) 13.0	(+) 7.3
After harvest	(-) 12.5	(-) 16.0	(+) 52.5	(-) 1.16	(+) 95.9	(-) 49.53	(-) 17,5	(-) 30.4	(-) 30.6	(+) 18.6
CV										
After spawn run	(-) 1.5	(-) 6.9	(+) 35.0	(-) 0.5	(+) 44.2	(-) 30.3	(-) 10.3	(+) 18,5	(-) 35.0	(-) 24.3
After harvest	(-) 12.6	(-) 13.0	(+) 41.2	(-) 1.0	(+) 66.7	(-) 39.4	(-) 21.9	(-) 24.9	(-) 38.1	(+) 4.1
WS										
After spawn run	(-) 3.2	(-) 11.4	(+) 16.0	(-) 0.8	(+) 40.1	(-) 29.2	(-) 6.3	(+) 14.4	(-) 29.4	(-) 6.6
After harvest	(-) 17.3	(-) 21.5	(+) 49.3	(-) 2.3	(+) 80.1	(-) 45.8	(-) 29.2	(-) 10.3	(-) 33.9	(-) 15.8
SH										
After spawn run	(-) 3.21	(-) 5.6	(+) 21.6	0.71	(-) 3.3	(+) 2.66	(-) 11.3	(+) 9.3	(-) 20.6	(-) 18.8
After harvest	(-) 8.7	(-) 14.4	(+) 52.0	1.70	(+) 7.6	(-) 8.5	(-) 15.0	(-) 6.8	(-) 27.7	(-) 8.8
BS										
After spawn run	(-) 1.1	(-) 9.1	(+) 36.4	3.0	(+) 1.14	(-) 4.14	(+) 9.5	(+) 15.2	(-) 38,7	(-) 5.0
After harvest	(-) 9.1	(-) 19.1	(+) 91.9	7.6	(+) 19.8	(-) 22.9	(-) 24.4	(-) 22.7	(-) 44.3	(-) 2.1

OS:oak sawdust; PS: poplar sawdust; CV: common vetch straw; WS: wheat straw; SW:safflower waste; BS:bean straw.

production reached a higher N content compared to those substrates that failed in yield. The protein content of the spent substrate of SW was only 1.01% higher than the initial 7.6%. Belewu and Belewu (2005) speculated that spent mushroom substrate contained higher protein than initial substrate because of the addition of fungal proteins during solubilization and degradation. However, the final N concentration in the spent mushroom substrates was dependent on the initial nitrogen content of the pertinent substrate as well as the degree of degradation. According to Leifa et al. (2001), the increase of protein content in the substrates could be attributed to the weight loss during mushroom cultivation, degradation of lignocellulose and liberation of CO_2. Although some researchers (Kurtzman, 1976; Singh, 2000) have suggested that *Pleurotus* species have the ability to fix nitrogen from air, this increase in N content of the spent substrates was ascribed to the mycelial and fruitbody residues left in the media.

The results indicated that there was a continuous decrease in the C/N ratio of the substrates during cultivation. In general, the C/N ratio in the colonized substrates was lower than those of the initial substrates. In the spawn running period, the C/N ratio in OS, PS, CV, WS and BS decreased, while this did not happen in the SW. However, regarding the C/N, significantly lower levels were found in all spent substrates at differences of 8.5–52.0% compared to the pertinent initial substrates, mainly due to the decomposition of carbon components and increase of N content by the *H. erinaceus* mycelium.

3.4. Degradation of lignocellulosic material

The lignocellulosic composition of the substrates varied significantly among the three different stages ($p < 0.01$). The results demonstrated that substantially high amounts of hemicellulose were consumed during the spawn running period of *H. erinaceus*. Average hemicellulose loss was between 13.0 and 38.7% during mycelial growth depending on the substrates. Hemicellulose can be removed more easily than lignin and cellulose because of its structure and physical and physicochemical properties (Sanchez, 2009). For this reason, hemicellulose may be preferred by *H. erinaceus* as an energy source for mycelial growth. Substrates were seen to be divided into two distinct groups in terms of hemicellulose degradation. The first group, which included the agricultural waste substrates, exhibited a considerable degradation of hemicellulose and a fast but sparse growth. In general, the rate of degradation of hemicellulose in the agricultural wastes slowed down after the spawn running period. The most pronounced biodegradation of hemicellulose was observed in BS (38.7%) for the spawn running period and a ratio of only 6.4% for the fructification stage in the same substrate. The second group, which included the PS and OS, was decreased 13.0% and 14.4% in the spawn running period and 17.6% and 14.0% in the fructification period, respectively. *Hericium erinaceus* caused hardly any decrease in the hemicellulose content of the sawdusts in the spawn running period and mycelial growth was slower and denser in the sawdusts.

Although there was no correlation between the spawn running period and hemicellulose content of the substrates, a positive correlation was determined between the loss of substrate hemicellulose and a shortened spawn running period ($r^2 = 0.852$). The results showed that the fast mycelial growth of *H. erinaceus* may have been be reflected in the fast degradation of hemicellulose. High loss of hemicellulose on CV, WS and BS during mycelial growth, explained the faster growth and early fruiting of *H. erinaceus* on these substrates. The changes in cellulose content was in accordance with the initial cellulose value. The maximum decrease in cellulose content during mycelial growth was obtained in the SW substrate (11.3%). Considering the results obtained after the spawn running period (Fig. 2), it was assumed that no significant cellulose degradation occurred in the OS and PS substrates. However, the cellulose content of BS increased by 9.5%. The loss of cellulose in the post harvest period (13.8–29.5%) was significantly higher than that in the spawn running period. Li et al. (2001) reported that the cellulose degradation ratio in the first flush period was faster than in the spawn running and primordia periods in *P. ostreatus* cultivation. In general, degradation of cellulose was lower than that of lignin and hemicellulose in the substrates tested.

The lignin content of CV, WS, SW and BS substrates increased drastically at the time of mycelial growth, but the lignin content of these substrates was much lower in the spent substrates compared to the initial substrates. Van Kuijk et al. (2015) also reported increased lignin content during the incubation period. A high ratio of hemicellulose and cellulose degradation might be linked with the increase of lignin content in the dry matter of these substrates. On the other hand, fully colonized OS and PS substrates had a tendency to exhibit decreased lignin compared to the initial substrates. The rate of degradation of lignin was slow during spawn running in OS and PS, but increased suddenly during fructification. The results obtained clearly suggest that lignin had been consumed in the later stages of cultivation. It is evident from the observations made during the experiments that *H. erinaceus* requires different energy sources depending on its stage of growth. Li et al (2001) reported that, in *P. ostreatus* cultivation, lignin was highly degraded during spawn running and especially during the primordia formation period and that its degradation decreased after the primordia stages.

Although shorter spawn running time was correlated negatively with the lignin content of the substrates ($r^2 = 0.946$), the BE was positively correlated with the lignin content ($r^2 = 0.846$) and inversely correlated with the cellulose/lignin ratio ($r^2 = -0.955$). Although Obodai et al. (2003) determined a positive correlation between *P. ostreatus* yield and the lignin content of substrates, Philippoussis et al. (2003) reported that the BE of *P. ostreatus, P. pulmonarius, P. eryngii* and *Agrocybe aegerita* was negatively correlated with the lignin content of the substrates. Morever, in contrast to findings in the present study, they also reported a positive correlation between the BE of the mushroom species and the cellulose/lignin ratio.

Hericium erinaceus presented the highest yield with the highest lignin degradation rate in the OS substrate. The lignin content of OS was decreased from an initial value of 23.0% to a final value of 14.7%, with a decrease 35.9% by the end of cultivation. However, the degradation of lignin by *H. erinaceus* was not satisfactory on the SW substrate (6.8%). According to results obtained in the study, the yield and BE of *H. erinaceus* was strongly correlated with the lignin loss of the substrates ($r^2 = 0.985$ and $r^2 = 0.990$, respectively). Zhai and Han (2018) also reported a positive correlation between lignin degradation rates and the corresponding yields of *Pleurotus* spp.

From these findings it may be concluded that using sawdust as a growing media for *H. erinaceus* cultivation seems to be a practical option to increase both the yield and the biodegradation of lignin. Another finding of the study was that bioconversion of lignocellulosic materials was related to substrate composition. During the spawn running period, the percentage of cellulose and hemicellulose degradation in the three types of straws was generally greater than that of the sawdusts, while lignin degradation of the sawdusts was higher than that of the other substrates. The lignin and hemicellulose degradation patterns were similar between OS and PS and among CV, WS and BS. Oriaran et al. (1989) reported that the differences in the rate of degradation among wood species may be related the differences in lignin composition in the structure of the cell walls. On the other hand, lignocellulosic enzyme activity is affected by substrate composition (Blanchette, 1991).Consequently, the similarity of enzymes for sawdust and agricultural wastes could cause similar degradation patterns in the substrates. The low C/N ratio of OS and PS could have favored biodegradation (Dorado et al., 2001). Moreover, low nitrogen content in substrates stimulates lignin degradation (Blanchette, 1991). Kirk et al. (1978) also reported that high N content hindered fungal ability to degrade lignin. On the other hand, lignocellulose degradation may be affected by particle size and was found to be higher in substrates having smaller particles (Cullis et al., 2004).

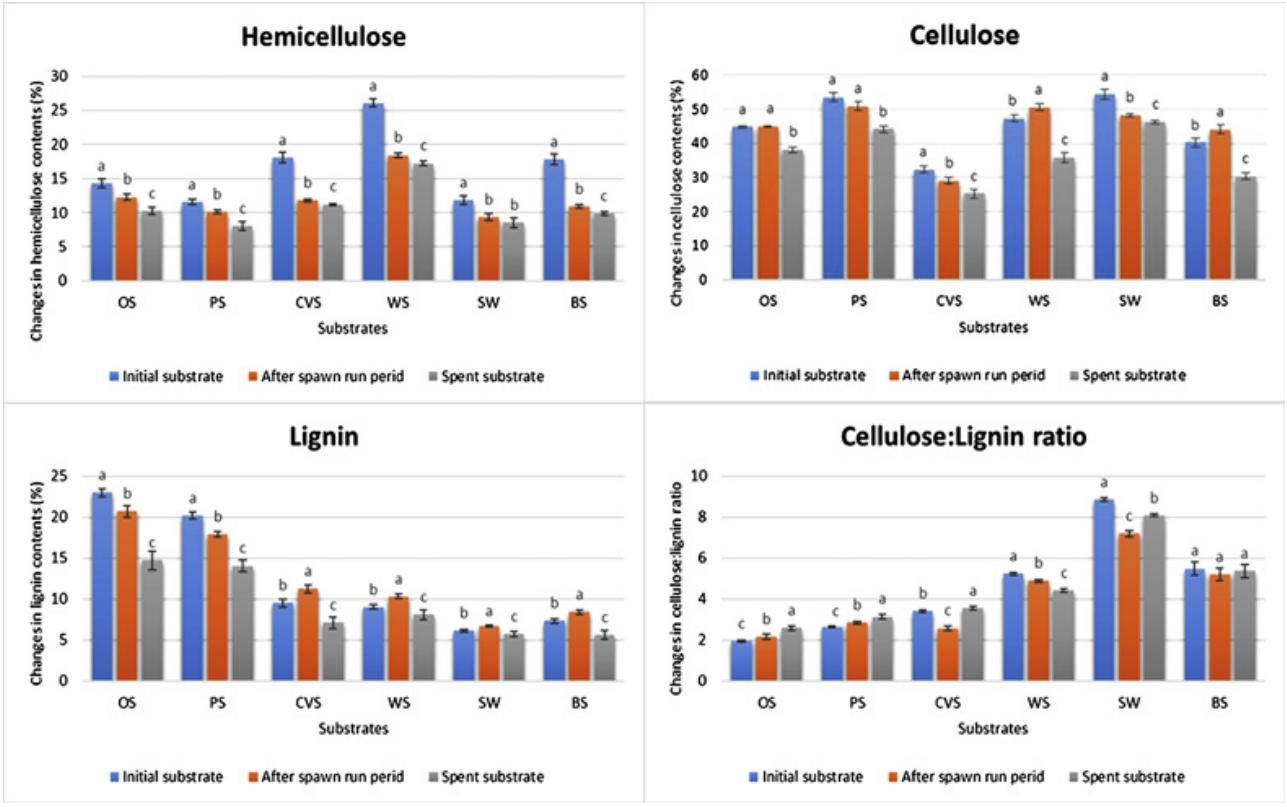

Fig. 2. Changes of lignocellulosic composition of substrates tested during *Hericium erinaceus* cultivation period.

The cultivation of *H. erinaceus* resulted in 6.8–35.9% of lignin, 28.4–44.3% of hemicellulose and 15.0–29.0% of cellulose being degraded in the spent substrates of the agricultural and sawdust wastes. Moreover, the cellulose/lignin ratio increased in OS, PS and CV, decreased in WS and SW and did not change in BS. *Hericium erinaceus* grown on wheat straw used 27.5% of the cellulose, 55.0% of the hemicellulose and 28.4% of the lignin after 60 d. The same fungus degraded 21.6% of the cellulose, 45.8% of the hemicellulose and 41.0% of the lignin of the sawdusts at the end of the cycle. Analysis of the lignocellulosic composition of the SW spent substrate revealed that 69.8% of the original hemicellulose, 86.2% of the original cellulose and 86.8% of the original lignin remained and were not consumed during production. Koutrotsios et al (2014) also reported different lignin, cellulose and hemicellulose losses in different substrates in the cultivation of *P. ostreatus* and *Agrocybe aegerita*.

4. Conclusion

The sawdust expecially hardwood sawdust is most suitable substrate efficient production of *Hericium erinaceus*. However, the using of common vessel straw and bean straw on *H. erinaceus* cultivation as a substrate hastened the process of mushroom production and these substrates may supported satisfactory yields. The results of the present study demostrated the high lignin concentration of kinds of sawdust in conjunction to their low cellulose: lignin ratio applies a positive effect on yield performance of *H. erinaceus*. However, it appeared that yield performance of *H. erinaceus* was not effected by N, hemicellulose and cellulose contents of substrates. In addition, shortened spawn run period was correlated with the loss in hemicellulose content of substrate whereas yield performance was related to loss in lignin content. Using of agro–forestry wastes on *H. erinaceus* cultivation induce the protein enrichment of these wastes in a short period of time, while the amount of lignocellulosic content in the substrates was reduced, making it more digestible. Apart from producing the nutritional and medicinal food, *H. erinaceus* cultivation would represent an economical and environmentalist strategy for dispose of agricultural and forestry wastes.

References

Adenipekun, C.O., Dada, O.J., 2013. Biodegradation of three agricultural wastes bya white-rotfungus, Pleurotus pulmonarius (Fries) Quelet. J. Nat. Sci. 11 (2), 19–25.

A.O.A.C, 1995. In: Williams, S. (Ed.), Association of Official Analytical Chemists, 16th edition. Washington, USA.

Atila, F., 2017. Evaluation of suitability of various agro-wastes for productivity of *pleurotus djamor*, *pleurotus citrinopileatus* and *pleurotus eryngii* mushrooms. J. Exp. Agric. Int. 17 (5), 1–11.

Atila, F., Tuzel, Y., Fernandez, J.A., Cano, A.F., 2018. The effect of some agro– industrial wastes on yield, nutritional characteristics and antioxidant activities of *Hericium erinaceus* isolates. Sci. Hort. 238, 246–254.

Atila, F., 2019. Compositional changes in lignocellulosic content of some agro-wastes during the production cycle of shiitake mushroom. Sci. Hort. 245, 263–268.

Belewu, M.A., Belewu, K.Y., 2005. Cultivation of mushrooms (*Volvariella volvacea)* on banana leaves. Afr. J. Biotechnol. 4 (12), 1401–1403.

Blanchette, R.A., 1991. Delignification by wood–decay fungi. Annu. Rev. Phytopathol. 29, 381–398.

Cantrell, K.B., Ducey, T., Ro, K.S., Hunt, P.G., 2010. Livestock waste–to–bioenergy generation opportunities. Bioresour. Technol. 99, 7941–7953.

Cavallazzi, J.R.P., Brito, M.D.S., Oliveira, M.G.D.A., Villas–Boas, S.G., Kasuya, M.C.M., 2004. Lignocellulolytic enzymes profile of three *Lentinula edodes* (Berk.) Pegler strains during cultivation on eucalyptus bark–based medium. J. Food Agric. Environ 2, 291–297.

Cullis, I.F., Saddler, J.N., Mansfield, S.D., 2004. Effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. Biotechnol. Bioeng. 85 (4), 413–421.

Dorado, J., Field, J., Almendros, G., Sierra–Alvarez, R., 2001. Nitrogen–removal with protease as a method to improve the selective delignification of hemp stemwood by the white–rot fungus Bjerkandera sp. Strain BOS55. Appl. Microbiol. Biotechnol. 57 (1–2), 205–211.

Figlas, D., Matute, R.G., Curvetto, N.R., 2007. Cultivation of culinary–medicinal lion's mane mushroom *Hericium erinaceus* (Bull.: Fr.) Pers. (*Aphyllophoromycetideae*) on substrate containing sunflower seed hulls. Int. J. Med. Mushroom 9, 67–73.

Gaitán-Hernández, R., Esqueda, M., Gutiérrez, A., Sánchez, A., Beltrán-García, M., 2006. Bioconversion of agrowastes by *Lentinula edodes*: the high potential of viticulture residues. Appl. Microbiol. Biotechnol. 71 (4), 432–439.

Hassan, F.R.H., 2007. Cultivation of the monkey head mushroom (*Hericium erinaceus*) in Egypt. J. Appl. Sci. Res. 3 (10), 1229–1233.

Hiwatashi, K., Kosaka, Y., Suzuki, N., Hata, K., Mukaiyama, T., Sakamoto, K., Shirakawa,

H., Komai, M., 2010. Yamabushitake mushroom (Hericium erinaceus) improved lipid metabolism in mice fed a high-fat diet. Biosci. Biotechnol. Biochem. 74 (7), 1447–1451.
Hiyama, R., Gisusi, S., Harada, A., 2011. Evaluation of waste mushroom medium from cultivation of shiitake mushroom (*Lentinula edodes*) as feedstock of enzymic saccharification. J. Wood Sci. 57 (5), 429–435.
Jonathan, S.G., Fasidi, I.O., Ajayi, A.O., Adegeye, O., 2008. Biodegradation of Nigerian wood wastes by *Pleurotus tuber-regium* (Fries) Singer. Bioresour. Technol. 99 (4), 807–811.
Kawagishi, H., Zhuang, C., 2008. Compounds for dementia from Hericium erinaceum. Drugs Future 33 (2), 149–155.
Kirk, T.K., Schultz, E., Connors, W.J., Lorenz, L.F., Zeikus, J.G., 1978. Influence of culture parameters on lignin metabolism by *P. Chrysosporium*. Arch. Microbiol. 117 (3), 277–285.
Ko, H.G., Park, H.G., Park, S.H., Choi, C.W., Kim, S.H., Park, W.M., 2005. Comparative study of mycelial growth and basidiomata formation in seven different species of the edible mushroom genus Hericium. Bioresour. Technol. 96, 1439–1444.
Koutrotsios, G., Mountzouris, K.C., Chatzipavlidis, I., Zervakis, G.I., 2014. Bioconversion of lignocellulosic residues by *Agrocybe cylindracea* and *Pleurotus ostreatus* mushroom fungi – assessment of their effect on the final product and spent substrate properties. Food Chem. 161, 127–135.
Koutrotsios, G., Larou, E., Mountzouris, K.C., Zervakis, G., 2016. Detoxification of olive millwastewater and bioconversion of olive crop residues into high–value–added biomass by the choice edible mushroom Hericium erinaceus. Appl. Biochem. Biotechnol. 180 (2), 195–209.
Kurt, S., Buyukalaca, S., 2010. Yield performances and changes in enzyme activities of *Pleurotus* spp. (*P. Ostreatus* and *P. sajor–caju*) cultivated on different agricultural wastes. Bioresour. Technol. 101, 3164–3169.
Kurtzman, R.H., 1976. Nitration of *Pleurotus sapidus* effects of lipid. Mycologia. 68, 268–295.
Leifa, F., Pandey, A., Soccol, C.R., 2001. Production of *Flammulina velutipes* on coffee husk and coffee spent-ground. Braz. Arch. Biol. Technol. 44 (2), 205–212.
Li, X., Pang, Y., Zhang, R., 2001. Compositional changes of cottonseed hull substrate during *P. Ostreatus* growth and the effects on the feding value of the spent substrate. Bioresour. Tecnology 80, 157–161.
Liang, B., Guo, Z., Xie, F., Zhao, A., 2013. Antihyperglycemic and antihyperlipidemic activities of aqueous extract of Hericium erinaceus in experimental diabetic rats. BMC Complement. Altern. Med. 13, 253.
Lim, S.H., Lee, Y.H., Kang, H.W., 2013. Efficient recovery of lignocellulolytic enzymes of spent mushroom compost from oyster mushrooms, *Pleurotus* spp., and potential use in dye decolorization. Mycobiol 41 (4), 214–220.
Luz, J.M.R., Nunes, M.D., Paes, S.A., Torres, D.P., da Silva, M.C.S., Kasuya, M.C.M., 2012. Lignocellulolytic enzyme production of *Pleurotus ostreatus* growth in agroindustrial wastes. Braz. J. Microbiol. 43 (4), 1508–1515.
Mizuno, T., 1999. Bioactive substances in Hericium erinaceus (Bull.: Fr.) Pers. And its medicinal utilization. Int. J. Med. Mushrooms 1 (2), 105–119.
Obodai, M., Cleland–Okine, J., Vowotor, K.A., 2003. Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by–products. J. Indian Microbiol. Biotechnol 30 (3), 146–149.
Ogbo, F.C., 2010. Conversion of cassava wastes for biofertilizer production using phosphate solubilizing fungi. Bioresour. Technol. 101, 4120–4124.
Oriaran, T.P., Labosky Jr., P., Royse, D.J., 1989. Lignin degradation capabilities of Pleurotus ostreatus, Lentinula edodes and Phanerochaete chrysosporium. Wood Fiber Sci. 21, 183–192.
Peksen, A., Yakupoglu, G., Yakupoglu, T., Gulser, C., Ozturk, E., Ozdemir, N., 2011. Changes in chemical compositions of substrates before and after Ganoderma lucidum cultivation. World J. Microbiol. Biotechnol. 27 (3), 637–642.
Philippoussis, A., Diamantopoulou, P., Zervakis, G., 2003. Correlation of the properties of several lignocellulosic substrates to the crop performance of the shiitake mushroom Lentinus edodes. World J. Microbiol. Biotechnol. 19 (6), 551–557.
Sanchez, C., 2009. Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol. Adv. 27 (2), 185–194.
Sarkar, N., Ghosh, S.K., Bannerjee, S., Aikat, K., 2012. Bioethanol production from agricultural wastes: an overview. Renew. Energ. 37 (1), 19–27.
Singh, M.P., 2000. Biodegradation of lignocellulosic wastes through cultivation of *pleurotus sajor–caju*. Proceedings of the 15th International Congress on the Science and Cultivation of Edible Fungi 517–520.
Vansoest, P.J., Robertson, J.B., Lewis, B.A., 1991. Method for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74 (10), 3583–3597.
van Kuijk, S.J.A., Sonnenberg, A.S.M., Baars, J.J.P., Hendriks, W.H.I., Cone, J.W., 2015. Fungal treated lignocellulosic biomass as ruminant feed ingredient: a review. Biotechnol. Adv. 33 (1), 191–202.
Zhai, F.H., Han, J.R., 2018. Decomposition of asparagus old stalks by Pleurotus spp. Under mushroom growing conditions. Sci. Hort. 231, 11–14.