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Changes in lignocellulosic fractions of growing substrates during the cultivation of *Hypsizygus ulmarius* mushroom and its effects on mushroom productivity

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ABSTRACT

The study focuses on determining the relationship between the degradation of lignocellulosic fractions of substrates used in *Hypsizygus ulmarius* cultivation and mushroom yield in order to present a wider range of data on the needs of the fungus. In the study, *H. ulmarius* was cultivated on bean straw (BS), corn silage (CS) and wheat straw (WS), poplar sawdust (PPS), pine sawdust (PS) and spent mushroom substrate (SMS). The degradation process of lignocellulosic wastes was characterized during 90 days of cultivation period and the results were correlated by comparing the mycelial growth and yield. BS was the best substrate for *H. ulmarius* due to its shorter crop cycle (28 days), high yield and BE% (340.0 g/kg and 93.1%). Consumption of hemicellulose was observed predominantly, during the spawn running period of cultivation. The decrease in lignin level during *H. ulmarius* cultivation is significantly lower compared to hemicellulose and cellulose. Cellulose was degraded mainly during the fruiting period, and it was observed that high cellulose degradation during the fruiting stage are positively correlated with the yield and biological efficiency of *H. ulmarius*. High lignin content of the substrate is a limiting factor for *H ulmarius* yield. The chemical analysis confirmed the decrease in pH, C:N ratio, lignocellulosic content and increase in EC, nitrogen and the ash content of substrates during cultivation of *H. ulmarius*. The study revealed that the key factor dominating a successful *H. ulmarius* cultivation to be substrate having moderate amount of N and low lignin and high cellulose content.

1. Introduction

Mushroom cultivation is an excellent method to turn lignocellulosic wastes into protein-rich food (Mandeel et al., 2005). Moreover, this bioconversion process helps the reduction of environmental pollution by avoiding waste accumulation (Chang and Wasser, 2017). Sawdust, woodchips, the logs of appropriate tree species; agricultural waste materials such as cereal straw, soybean straw, corn stalks, waste hulls, cotton gin trash, olive mill residue, and coffee ground spent etc. have been used successfully for cultivation of numerous mushroom species in previous studies (Zhang et al., 2002; Koutrotsios et al., 2014; Carrasco-Cabrera et al., 2019; Yamauchi et al., 2019; Atila, 2020).

Basidiomycetes mushrooms that produce hydrolytic and oxidative extracellular enzymes have an important role in the degradation of organic matter. The enzymatic system attacks cellulose, hemicellulose and lignin and decomposes them into a form that the fungus can utilize (Peralta et al., 2017). The composition of lignocellulosic substrates is one of the most important factors affecting mycelium growth, mushroom yield and the quality (Rezaeian et al., 2021). The substrate could also influence the nutritional value and bioactive content of cultivated mushrooms (Ruiz-Rodriguez et al., 2010; Atila et al., 2017; Carrasco et al., 2018).

Hypsizygus ulmarius (Bull.Fr) Red Head, also known as elm oyster mushroom, is a *Basidiomycetous* fungus and belongs to the family *Lyophyllaceae* of order *Agaricales*. *H. ulmarius* often grows in clusters on living elm trees or elm logs in nature (Greeshma et al., 2019). This edible mushroom is commercially cultivated especially in Asia and Europe, and it has been gaining popularity due to low-cost production technology and higher biological efficiency (Kumar et al., 2019a). It has an excellent taste and an attractive shape with a large and light white fruiting body (Baghel et al., 2019). *H. ulmarius* is similar to oyster mushroom in appearance, but differs in cultivation cycle and biological efficiency. In

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addition to its culinary value, it is rich in bioactive compounds such as phenol, tannins, antioxidants, water-soluble polysaccharides, alkaloids and proteins (Krasnopolskaya et al., 2008; Greeshma et al., 2016; Al-Faqeeh et al., 2020).

It is important to know biochemical changes occurring in the growing substrates during the mushroom cultivation process in order to understand the needs of the fungus. In our previous studies, biochemical changes of substrates during cultivation of *Lentinula edodes* Atila (2019a) and *Hericium erinaceus* Atila (2019b) were documented. However, to our knowledge, no information is available regarding the biochemical changes in the lignocellulosic constituent of substrates during the cultivation of the *H. ulmarius*. Besides, it has not yet fully known that how the changes in chemical and lignocellulosic composition of substrates may affect the mycelial growth and the yield of the mushroom as well as the properties of spent *H ulmarius* mushroom substrate. The main objective of the present work is to determine degradation pattern of lignocellulosic fractions of the agricultural and forest industry wastes during *H. ulmarius* cultivation and to correlate the results with the mushroom yield obtained from the wastes. A secondary objective is to describe properties of spent mushroom substrate generated after the cultivation of the *H ulmarius* mushroom.

2. Materials and methods

2.1. Strain and substrates

The pure culture of a commercial strain of *H. ulmarius* was obtained from the Homegreen Spawn Company (Netherlands) and stored on Potato dextrose agar (PDA, Merck) at 4 °C. The spawn used in the study was prepared as previously described by Atila (2017b)

The agricultural wastes (bean straw (BS), corn silage (CS) and wheat straw (WS)) was gathered from local farms, whereas poplar sawdust (PPS) and pine sawdust (PS) were purchased from a lumber mill, in Kırşehir, Turkey. SMS was the residual material produced after the cultivation of *Pleurotus ostreatus* mushroom and was obtained from Özbostancı Mushroom Farm, in Kırşehir, Turkey.

2.2. Substrate preparation and mushroom cultivation

The study was carried out at Ahi Evran University Agriculture Faculty Mushroom Production Unit in Kırşehir, Turkey. The WS and BS were chopped into small pieces (2–3 cm). No treatment was applied to the PPS and PS (60–80 mesh size), CS (1–2 cm) and SMS. The moisture of substrates was adjusted to 70% of humidity by using tap water, and polypropylene bags (25 × 40 cm) were filled with 1 kg of substrates, then the bags were sterilized at 121 °C, 1 atm for 1.5 h. When the sterilized bags reached at 20–25 °C temperature, they were inoculated with 30 g of *H. ulmarius* spawn in laminar flow. Then, the bags were moved into the incubation room and maintained at 25 °C in dark during incubation period. After complete colonization of the substrate, they were transferred to growing room and the cotton plugs at the top of the plastic bags were removed. The temperature (15–18 °C), humidity levels (80–90%), ventilation (CO_2<1000 ppm) and the photoperiod (12 h light/12 h dark, using a 20 W fluorescent light) of the growing room were adjusted to induce fruiting Stamets (1993). The mushrooms were harvested when the in-rolled margins of the fruit-body began to flatten. The experiment which lasted for 90 days, was carried out by a completely randomised plot design, with 10 replications.

Cultivation parameters such as effects of spawn running time (day), days to first primordia initiation (day), days to first harvest (day), yield (g/kg), biological efficiency (BE%) and average mushroom weight (g) were evaluated during the cultivation period of *H. ulmarius* on different substrates. The total yield obtained from substrates was expressed as grams of fresh mushrooms harvested at maturity per kg of a wet substrate (w/w). The biological efficiency (BE%) was calculated by using the formula; BE (%) = (fresh weight of harvested fruit-body per bag/dry weight of growing medium per bag) x100. The average mushroom weight (AMW) was defined as the rate of the total weight of fresh fruit-bodies harvested by their number.

2.3. Substrate analyses

The proximate content and lignocellulosic composition of substrates were analysed by using the samples taken in different stages; (a) initial substrate (after sterilization), b) after spawn running period and c) the spent substrate (after mushroom cultivation). The samples, weighing approximately 100 g, were taken from 3 different bags at different cultivation stages of each substrate. After the samples were dried in an oven at 60 °C, they stored at 4 °C till the analysis. The ash was measured as it was described previously AOAC (1995). pH and electrolyte conductivity of solutions were measured by a pH meter (Ohaus, Starter3100) and a conductivity meter (Mettler Toledo 7100) according to the methods of Cavins et al., (2000). The contents of cellulose, hemicellulose, and lignin were measured by using the method of Van Soest et al. (1991). The cellulose, lignin and hemicellulose content of samples were calculated as it is shown in the Eqs. (1) (2) (3) below;

(1) Cellulose = ADF-ADL;(2) Lignin = ADL; (3) Hemicellulose=NDF-ADF (Zadrazil and Brunnert, 1982). The total nitrogen was determined by The Kjeldahl method. The total carbon was estimated from the ash content according to the formula of Tiquia and Tam (2000). All analyses were conducted in triplicate.

2.4. Statistical analysis

The results were statistically analysed and interpreted using the analysis of variance (ANOVA). The means of the individual groups were compared via Tukey's test at a significance level of 5% by using SPSS 16.0 software program. Pearson's correlation coefficients were calculated by using the correlation function of Microsoft Excel program (2010).

3. Results and discussion

3.1. Proximal and lignocellulosic content of the substrates tested in the study

Significant differences ($p < 0.01$) were found among the substrates for pH, EC, total N, ash, C:N, hemicellulose, cellulose and lignin (Table 1).

The highest pH was determined in SMS (7.73). Ec value of WS, BS, CS and SMS varied between 1.50 and 3.47 dS m^{-1}. On the other hand, EC value in PPS (0.73 dS m^{-1}) and PS (0.53 dS m^{-1}) was dramatically lower than that in the agricultural wastes. The ash differed among substrates, the highest and the lowest values corresponding to SMS (27.03%) and PS (0.50%). The nitrogen content and C:N ratio of the substrate are the essential factors for a successful mushroom cultivation (Philippoussis et al., 2003; Gaitan-Hernandez et al., 2011). The highest N content (1.35%) and the lowest C:N ratio were determined in CS substrate (35.6). The lowest initial N content (0.29% and 0.28%) and highest C:N ratio (199.85:1 and 210.50:1) were found in PPS and PS, respectively.

Hemicellulose, cellulose, and lignin contents of substrates were significantly different ($p < 0.01$); generally, hemicellulose (3.33%), cellulose (13.56%) and lignin (5.89%) content of SMS were lower than that of the other wastes. The cellulose and hemicellulose contents were similar to PPS and BS. While the highest cellulose (49.01%) and lignin (25.25%) contents were obtained from PPS, the highest hemicellulose content was in WS (28.39%). The lignin was significantly lower in WS, BS and CS compared to sawdust types (8.80%, 13.88% and 13.89%, respectively).

3.2. Effects of agricultural and forest industry wastes on crop cycle and yield performance of H. ulmarius

The number of days taken for the spawn run period (SRP),

Table 1
Initial chemical and lignocellulosic content of growing substrates tested in the study.

Substrates	pH	EC (dS m^{-1})	Ash (%)	N (%)	C (%)	C:N	Hemicellulose (%)	Cellulose (%)	Lignin (%)
PPS	7.26**bc	0.73**e	2.05** e	0.29**e	56.81** b	195.89** a	13.76** d	49.01** a	25.25** a
PS	6.87 d	0.53 e	0.50 f	0.28 e	57.71 a	206.10 a	20.52 b	41.07 b	25.54 a
BS	7.20 c	1.50 d	6.70 d	0.86 b	54.11 c	62.92 c	13.22 d	48.23 a	13.88 b
CS	7.46 b	2.90 c	17.44 b	1.35 a	47.88 e	35.47 d	17.52 c	35.62 d	13.89 b
WS	7.08cd	2.03 b	8.60 c	0.52 d	53.01 d	101,94 b	28.39 a	40.41 c	8.80 c
SMS	7.73a	3.47 a	27.03 a	0.74 c	42.32 f	57.19 c	3.33 e	13.56 e	5.88 d

PPS: poplar sawdust; PS: pine sawdust; BS: bean straw; CS: corn silage; WS: wheat straw; SMS: spent mushroom substrate. 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. (n=3)

primordium initiation (DPI) and the first harvest (DFH) differed significantly ($p < 0.01$) among the substrates. The differences between yield parameters were also significant statistically ($p < 0.01$) (Table 2).

With the exception of CS (31.1 day), SRP of other substrates (19.1–22.5 days) was similar, which were completed in a short time. The fastest colonization was exhibited on SMS substrate, followed by PPS and BS substrates. During the mycelium growth period, expansion of mycelium was very quick, but quite very weak in the SMS substrate. It may be related to the fact that the lignocellulosic content of SMS is much lower than other substrates. Zervakis et al. (2001) interprets fast mycelial extension as an indicator of hyphal progression in a nutritionally poor or unfavourable substrate. SRP on the substrates tested in the study is between acceptable limits when compared to those cited by Sethi et al. (2012), Munna et al. (2019), Kumar et al. (2019b) for *H ulmarius* grown on different growing media.

The periods required to *H. ulmarius* for primordium initiation (DPI) of PPS, PS, WS, BS and CS substrates varied between 32.6 and 50.6 days. The SMS substrate failed to induce primordia after the incubation, therefore it is unsuitable for the cultivation of *H. ulmarius*. This may be associated to the fact that the cellulose content of the substrate is too as well as after the spawn run period the hemicellulose content of substrate drops to 0. The longest time for primordium formation was on CS substrate while primordial initiation took considerably shorter time (32.6 d and 38.6 d, respectively) on BS and WS substrates. The sawdust substrates, PPS and PS, showed similar a behaviour on days for pinning initiation (43.5 d and 45.1 d, respectively) and days for the first harvest (51.4 d and 53.1 d, respectively).

The BS substrate also took shorter time to start fruiting, with an average of 39.8 d, while the average time fluctuated between 46.0 (WS) and 62.0 d (CS) for the other four substrates. Although our results are similar to the findings of Malayil et al., (2017) who reported that DPI ranged between 45–47 days, the times presented in the study are longer when compared to other authors who reported that primordial initiation appeared after 20.33–33.0 d of spawning (Sethi et al., 2012; Munna et al., 2019; Kumar et al., 2019b; Khade et al., 2019).

DFH has been reported as 24–31 d and 27–34 d for *H. ulmarius* by Munna et al., (2019) and Khade et al. (2019), respectively, whereas in the present experiment the DFH was varied between 39.8–62.0 d. The CS substrate showed an extremely longer crop duration than the previous studies. This difference between the results obtained in different studies can be attributed to the physical and chemical properties of the substrate as well as the strain used.

Determination of correlations between substrate contents and cultivation process may offer good parameters for evaluating the needs of *H. ulmarius* mushroom. There was a significant positive relation between spawn running period and ash (r^2 = +0.868) and N (r^2 = +0.795) contents of substrates. Conversely, a negative correlation was found between spawn running period and cellulose content of substrates (r^2 = -0.883). Our findings are compatible with Philippoissis et al (2001) who reported a relatively higher N content of substrate has a negative effect on mycelial growth. The data from this experiment demonstrates that the substrates, with lower N, and ash and a higher cellulose content support shortened spawn running period of *H. ulmarius*.

Total fresh mushroom production varied from 102.4 g/kg (PS) to 340.0 g/kg (BS). The number of flush obtained from the tested substrates fluctuated from two to four. The flush percentages that obtained from each substrate were different. Yield was very similar to both types of sawdust (PPS and PS), with an average of 108.8 and 102.4 g/kg, respectively. 65.7% and 64.0% of the total were obtained in the first flush in the sawdust substrates (Table 2). Agricultural wastes came in three flushes with the exception of BS which produced four flushes. First flush represented 40.0%, 56.5% and 49.2% of production on BS, CS and WS, respectively.

BE (%) on different substrates varied between 36.0% and 93.1% and no statistical difference among two of the sawdusts was evaluated. The highest BE (%) was accomplished on BS, with a difference from the rest of the substrates. Although colonization of PPS and PS substrates was considerably fast, these substrates were the least efficient fruitbody producer (36.0% and 41.0%, respectively) and significantly different from the BE obtained on BS (93.1%), CS (61.8%) and WS (67.4%). The obtained yield in the study appeared to be extremely lower than the findings of Malayil et al (2017), who cultivated *H. ulmarius* both on WS and biogas digester liquid sprayed WS, registered a BE of 231.7% and 437.5%, respectively. On the other hand, the BE values on substrates tested in the study were located within the range as cited in the previous studies (32.0% to 125%) where supplemented wheat straw, rice straw and alternative substrates such as seaweed, banana leaves, several cakes and flours were used (Kumar et al., 2019b; Khade et al., 2019; Munna

Table 2
Effect of different substrates on cultivation of *Hypsizygus ulmarius* mushroom.

Substrates	Spawn run period (days)	Days to pinhead formation (days)	Days to first harvest (days)	Yield (g/kg)				Total yield (g/kg)	Biological efficiency (%)	Average mushroom weight (g)
				Flush I	Flush II	Flush III	Flush IV			
PPS	19.3**c	43.5**b	51.4** b	71.5	37.31	0.00	0.00	108.78**d	35.97**d	50.5**a
PS	22.0 b	45.1 b	53.1 b	65.55	36.83	0.00	0.00	102.38 d	41.00 d	43.5 a
BS	20.0 c	32.6 d	39.8 d	135.97	118.88	59.04	26.09	339.98 a	93.14 a	28.7 b
CS	31.1 a	50.6 a	62.0 a	119.70	59.17	33.01	0.00	211.88 c	61.83 c	22.3 b
WS	22.5 b	38.6 d	46.0 d	112.62	70.37	46.11	0.00	229.10 b	67.38 b	24.9 b
SMS	19.1 c	No data	No data	No data	No data	No data	No data	No data	No data	No data

PPS: poplar sawdust; PS: pine sawdust; BS: bean straw; CS: corn silage; WS: wheat straw; SMS: spent mushroom substrate. 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.(n=10)

et al., 2019; Hausiku and Mupambwa, 2018; Sethi et al., 2012).

BS was the best substrate for *H. ulmarius* due to its shorter crop cycle (28 days), high yield and BE% (340.0 g/kg and 93.1%). Although the production on CS substrate started considerably later than PPS and PS, the yield was higher than those of these substrates.

The yield and BE(%) are significantly negatively correlated with lignin content of the substrates (r^2 = -0.778 and -0.769) and with their C: N ratio (r^2 = -0.822 and -0.799), but not significantly correlated with their hemicellulose content and cellulose ratio. Philippoussis et al. (2001) reported that there is a negative correlation between BE of *Pleurotus* spp. and *Agrocybe aegerita* and the C:N ratio of substrates, while a negative correlation between the BE of *Volvarialla volvacea* and the C: N ratio of the substrate was reported in the same study. Moreover, a positive relation of yield was found with nitrogen content of the substrates (r^2 = +0.581 and +0.555). However, there was a stronger correlation of initial N content of the substrates with mycelium growth rather than with the yield and BE(%).

According to these results, low lignin content and C:N ratio in initial substrates coincides with the high mushroom production. Each mushroom species needs a different optimum C:N ratio for mycelium growth and the highest yield (Zied et al., 2011).Chang and Miles (1984) reported that C:N ratios were ranged between 32–150 for primordial induction in *Pleurotus* spp., whereas Atila (2019b) reported that optimum C:N ratio was between 44.2 and 151.6 for a faster mycelial growth of *Hericium erinaceus*. However, BS, which is C:N ratio in the range of 63:1, and the moderate N content (0.86%) favour mycelium growth, shorten fruiting time, and increase BE of *H ulmarius*. This may also be related to the suitability of the physical structure of BS for gas exchange, which is very effective in enzymatic degradation activity.

When *H. ulmarius* has grown on lignocellulose substrates, the lignin degradation is lower than that of hemicellulose and cellulose. Less lignin could enhance an enzyme activity and thus ensures a higher mushroom yield and BE (Sivaprakasam, 1980). Wang et al. (2001) also determined a negative correlation between BE and the degradation of lignin. The poor yield observed on PS and PPS substrate could be attributed to the rich lignin content in them and poor ability of *H. ulmarius* to degrade lignin as substances.

The highest AMWs obtained were 50.5 g (PPS) and 43.5 g (PS); they were varied between 22.3 g and 28.7 g on agricultural waste, with no significant differences between them. The represented biggest size group was sawdust on average. The reason for this may be that the small spaces among particles in the sawdust are not favourable on gas exchange, which is indispensable for pinning. For that reason, fewer pinheads may be created on the sawdust substrates and the fewer pinheads that were produced created a larger fruit-body.

3.3. Degradation pattern of substrates

Proximal and lignocellulosic content of the spent *H. ulmarius* substrates was presented in Table 3.

Significant differences were found between the courses of cultivation cycle analysed substrates ($p < 0.01$). pH values significantly decreased in spawn running period and the decrease in pH continued during the fruiting stage in the substrates (Fig 1). Ash and N contents increased significantly in all substrates after the spawn run period. This increase continued during the fruiting period. After the mushroom cultivation, concentration of ash and nitrogen in the spent substrates was higher; between 32.4–158% and 7.6–81.2%, than that of the initial substrates, respectively.

The increase in the ash content in the substrates at the end of the harvest can be explained by the increase in the concentration of inorganic elements as a result of the loss of organic matter in the substrates (Singh, 2000; Escalona et al., 2001).Although Rao and Naik (1990) mentioned that the presence of some of nitrogen fixers microorganisms in substrate may lead to an increase in the N content of the substrates, this increase in N level can be attributed to the mycelium and mushroom residue remaining in the spent substrate. Under normal conditions, since mycelium uses nitrogen in the substrate during spawn run period and fruitbody formation, the amount of N in the substrate should tend to decrease during the mushroom cultivation process. However, the N in the spent substrate is the total of the substrate, mushroom residue and mycelium proteins, and it is not possible to determine the loss of nitrogen from the substrate, as no analysis has been made to distinguish between N contents of these constituents.

C:N ratio decreased by 25.4% on PPS, 18.6% on PS, 38.13%WS, 45.14% BS and 14.7% CS after the *H. ulmarius* cultivation, although this decrease occurred more in substrates with higher BE such as BS and WS. Sánchez and Royse (2002) also reported the C:N ratio decreases in the substrates after the mushroom cultivation. During the mushroom cultivation, the amount of carbon in the substrates decreases as a result of the breakdown of lignocellulosic material by the fungal extracellular enzymes. On the other hand, developing mycelium contribute to the increase of N content of the substrate. While the C ratio decreases in the growing media, it is an expected result that the C: N ratio will decrease as the N ratio increases.

The hemicellulose concentration in the substrates decreased gradually from 3.33–28.39% (before spawn running period) to 0–15.58% (after the mushroom cultivation) (Fig 2). Hemicellulose was the most consumed lignocellulosic content by the *H. ulmarius* mycelium on SPR. However, the degradation rates of hemicellulose in the cultivation periods differed concerning the substrates. In general, a high hemicellulose degradation was observed during the spawn running period on substrates, except WS, whereas it was lower during the fruiting stage.

Our results are in accordance with the previous studies which reported that hemicellulose is an energy source for mushroom in initial stage before the break down of lignin and cellulose (Gaitan-Hernandez et al.,2006; Philippoussis et al., 2003; Philippoussis et al., 2011; Atila, 2019b). Although cellulose content decreased in CS and BS substrates after SRP, it did not changed in PS, PPS, WS substrates.

H. ulmarius exhibited higher degradation rates on agricultural wastes (22.4%, 25.4% and 37% loss on WS, BS and CS, respectively), while keeping most of the cellulose of sawdust intact (8.5 and 2.7% loss on PPS and PS, respectively). The higher yield and BE(%) of *H. ulmarius* grown on CS, WS and BS substrates may related to high degradation of cellulose during the fruiting stage on these substrates. Our view is corroborated by the results of Wang et al (2001) who reported a positive relation between degradation ratio of hemicellulose and cellulose and BE(%). Higher yields and biological efficiencies are related to higher cellulase

Table 3
Chemical and lignocellulosic content of spent mushroom substrate generated after the cultivation of the *Hypsizygus ulmarius*

	pH	EC (dS m^{-1})	Ash (%)	N (%)	C (%)	C:N	Hemicellulose (%)	Cellulose (%)	Lignin (%)
PPS	4.94**c	1.23** d	1.64** d	0.38** d	57.04** a	149.17** b	9.41** b	44.85** a	19.83 a
PS	4.52 c	1.07 d	1.29 d	0.33 d	57.25 a	171.26 a	9.72 b	39.95 b	20.61 a
BS	5.45 b	2.67 b	12.53 b	1.56 a	50.73 a	34.49 d	5.34 c	35.98 c	11.23 c
CS	6.18 a	3.17 a	22.47 a	1.48 b	44.97 b	30.37 e	5.15 c	22.44 e	13.51 b
WS	4.80 c	2.03 c	11.35 c	0.81 c	53.03 b	63.73 c	15.58 a	31.32 d	7.38 d

PPS: poplar sawdust; PS: pine sawdust; BS: bean straw; CS: corn silage; WS: wheat straw; SMS: spent mushroom substrate. 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.($n = 3$)

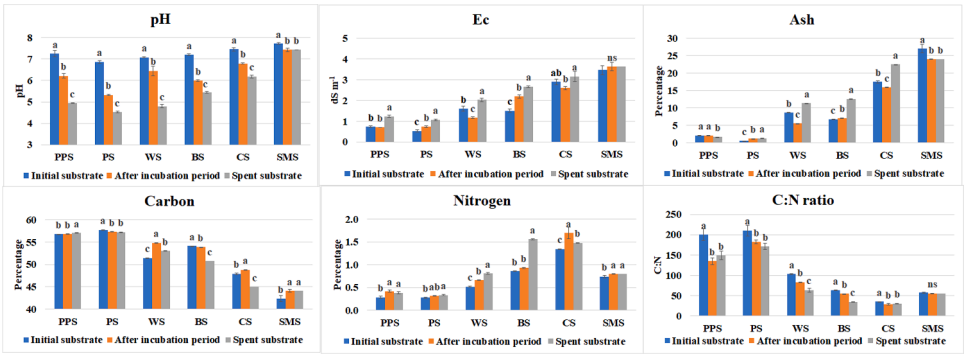

Fig. 1. Changes of proximate composition of substrates tested during *Hypsizygus ulmarius* cultivation period.

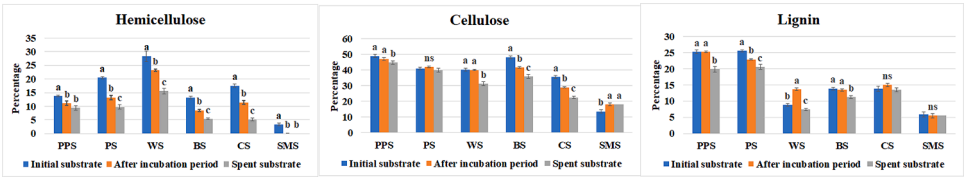

Fig. 2. Changes of lignocellulosic composition of substrates tested during *Hypsizygus ulmarius* cultivation period.

activity (Kurt and Buyukalaca, 2010). Rapid degradation of cellulose in agricultural substrates can lead more efficient use of cellulose, which is essential for fruitbody yield.

After incubation period, the lignin content increased on WS and did not vary in the PPS, BS, CS substrates, whereas the lignin content of PPS slightly declined during the mycelial growth. Although the lignin content decreased only 2.73% in the fruiting period in CS, the decrease in the lignin content of PS and PPS substrates was drastic; from 25.25% to 19.83% and, from 25.54% to 20.61, respectively. There are different views on the effects of the nitrogen on lignolytic enzyme production in the literature. Kachlishvili et al. (2005) mentioned that higher laccase activity of *L. edodes* was obtained in substrates rich in nitrogen in contrast with our results that indicate the low nitrogen content of substrates could increase biodegradation of lignin. On the other hand, Blanchette (1991) reported that, similar to our findings, low N substrate content promotes lignin degradation in some fungal species.

Cellulose consumption was very low in sawdust substrates with high lignin content during the cultivation period. According to the results, it is clear that although the kinds of sawdusts have also high cellulose content, *H. ulmarius* mycelium grown on sawdust prefers lignin consumption rather than cellulose. The chemical composition of the substrate affects the activity of extracellular enzymes that is capable of degrading lignocellulosic content (Morais et al. 2000). Due to the fact that the lignin is difficult to biodegrade, the utilization of mushroom mycelium from cellulose and hemicellulose is reduced (Eriksson et al., 1990).

4. Conclusion

The present results raise that *H. ulmarius* has a preference for substrates containing low lignin and high cellulose and not having low or extreme amount of N. It is clearly seen that BS substrate increases the potential yield of *H. ulmarius* as well as shortens the fruiting period of the mushroom. Moreover, using wheat straw and corn silage on *H. ulmarius* cultivation as a substrate may supported– provide satisfactory yields.

The yield performance of *H. ulmarius* is related to degradation of cellulose content of substrates. Moreover, the high lignin concentration of sawdust kinds causes a negative effect on the mushroom yield. *H. ulmarius* mycelium mostly uses hemicellulose as an energy source during the spawn run period, whereas the use of lignin is limited throughout the cultivation process.

The change rates of proximate content and lignocellulosic composition during the mushroom cultivation differ, depending on the substrates. In general, EC, nitrogen, the ash of the spent substrate increase, while the pH, C: N ratio, cellulose, hemicelluloses and lignin content decrease compared to the initial substrate. Reducing the concentration of lignocellulosic content in agricultural and forest wastes converted them into more nutritive and easily digestible animal feed, they can also be used as a soil conditioner thanks to their high nitrogen content and organic substance. *H. ulmarius* cultivation could provide an efficient utilization and biotransformation of agricultural and forest wastes into the value–added products. In addition, large amounts of wastes could be eliminated without harming the environment by this method.

CRediT authorship contribution statement

Ceren Öztürk: Investigation, Resources, Writing – original draft, Visualization, Project administration. **Funda Atila:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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