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Research Article

Yield, quality and enzyme activity of shiitake mushroom (*Lentinula edodes*) grown on different agricultural wastes

Gökhan BAKTEMUR[1]*, Ecem KARA[2], Mahmut YARAR[3], Nurten YILMAZ[4], Erdal AĞÇAM[5], Asiye AKYILDIZ[5], Hatıra TAŞKIN[2,3]

[1]*Sivas University of Science and Technology, Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, Sivas, Turkey; gbaktemur@gmail.com (*corresponding author)*
[2]*Cukurova University, Faculty of Agriculture, Department of Horticulture, 01330 Adana, Turkey; ecemkara33@gmail.com; hatirataskin1@gmail.com*
[3]*Cukurova University, Institute of Natural and Applied Sciences, Department of Biotechnology, 01330 Adana, Turkey; mahmuttyarar@gmail.com*
[4]*Çukurova University, Faculty of Agriculture, Department of Animal Science, 01330 Adana, Turkey; ntoy@cu.edu.tr*
[5]*Cukurova University, Faculty of Agriculture, Department of Food Engineering, 01330, Adana, Turkey; erdalagcam@gmail.com; asiye1@cu.edu.tr*

Abstract

In this study, it was aimed to investigate cultivation of *Lentinula edodes* by using different agricultural wastes (oak sawdust, poplar sawdust, wheat stalk, peanut shell, corncob and vine pruning waste) and to determine the most suitable growing mixture/mixtures. For this purpose, 12 growing mixtures were tested. Within the scope of the experiment, besides measurement of yield and quality parameters of mushrooms, properties of agricultural wastes and enzyme activities (laccase and cellulase) of mixtures at different periods were measured. Based on results of the study, the highest and lowest amounts of nitrogen were obtained from after harvest (1.71%) and after sterilization (1.34%) periods, respectively. While the highest amount of carbon was at the after-sterilization period (46.6%), the lowest amount was recorded at the after harvest (45.64%) period. The fastest and slowest mycelia development time was observed in A7 (21.67 days) and A4 (50 days) mixtures, respectively. While the highest yield was determined in A5 (299.59 g kg^{-1}) mixture, A9 (55.99 g kg^{-1}), A6 (65.59 g kg^{-1}) and A11 (75.47 g kg^{-1}) gave the lowest results. While the highest biological activity rate was recorded in A3 (93.65 %) and A5 (92.90%), the lowest was observed in A11 (21.45%), A6 (19.85%) and A9 (19.22%) mixtures. The highest and lowest protein amounts were determined in the A5, A7 and A10, A9 and C mixtures, respectively. The highest cellulase and laccase activities were found in A3 (3.16 IU g^{-1}) and A7 (2164.48 U g^{-1}), respectively.

Keywords: corncob; peanut shell; vine pruning waste; wheat stalk

Introduction

With the increasing human population density, need for food is increasing day by day and mushrooms are important nutrient sources. Edible mushrooms have essential ingredients such as vitamins, minerals,

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essential fatty acids and protein containing essential amino acids that are functional for human health. In the past half century, there has been a rapid increase in world mushroom production. The amount of mushroom production, which was 495 127 tons in 1961, has reached to 8 993 280 tons today (FAO, 2018). Although mushroom cultivation in Turkey, especially in recent years show a very rapid development, it has not yet reached to the desired amount because of the availability of many alternative agricultural crops. However, today, market demand for mushrooms has increased in Turkey.

Lentinula edodes known as "shiitake" is one of the mushroom species that is ignored in Turkey, despite it is commonly produced and consumed in the world. The nutrient content of shiitake mushroom was reported as 2.93% protein (wb), 15.45 mg 100 g^{-1} wb vitamin C, 90.0 µg 100 g^{-1} folic acid, 0.04 mg 100 g^{-1} wb thiamin, 0.10 mg 100 g^{-1} wb riboflavin, 3.23 mg 100 g^{-1} wb niacin, 10.44 mg kg^{-1} wb Zn, 7.22 mg kg^{-1} wb Fe, 986.67 mg kg^{-1} wb P, 116.4 mg kg^{-1} wb Ca, 328.13 mg kg^{-1} wb Mg, 1619.33 mg kg^{-1} wb K, 435.43 mg kg^{-1} wb Na in the first harvest by Çağlarırmak (2007). In addition to its fresh consumption, its products such as tablet and tea have been prepared and sold for medical consumption. Today, rapid human population growth, urbanization, industrialization, limitation of agricultural areas and destruction of the ecological landscape has reduced sources of nutrients, and as a result, people have discovered alternative food sources. In many countries, it is ensured that agricultural wastes such as roots, straw, bran and molasses have been processed by industry during harvest of agricultural products. In Turkey, most of these wastes have been burned or left in the environment, and the remaining part is used as animal feed. Burning these wastes harms nature and the soil. Considering all these, these wastes can be easily utilized in mushroom cultivation. In many countries where agricultural production is intense, many agricultural wastes, which can be provided with abundant and cheap costs, have been used in mushroom cultivation without any pre-treatment (Kara and Sezer, 1992; Akyüz and Kırbağ, 2009). Different substrate materials such as hazelnut husk, wheat straw, wheat-rice-soybean bran, beech sawdust, corncobs, cocoa husk, cotton waste, coir pith, almond bark, walnut shell, olive waste, linter-residue of textile fibre, guar-corn-sunflower seed-cotton-grape-coffee residues, chickpea straw, corn stalk, alfalfa hay, sunflower head residue, vineyard pruning waste, paddy straw, sugar cane bagasse, oak-poplar-teak-sal-eucalyptus sawdust have been tested in *L. edodes* production (Salmones *et al.*, 1999; Fan and Soccol, 2005; Özçelik and Pekşen, 2006, 2007; Gaitán-Hernández *et al.*, 2006; Philippoussis *et al.*, 2007; Escobar *et al.*, 2007; Puri *et al.*, 2011; Casaril *et al.*, 2011; Puri, 2012; Sözbir, 2014; Mata and Savoie, 2018; Atila, 2019; Yu *et al.*, 2021).

The aims of this study: (i) determination of availability of agricultural wastes such as vine pruning waste, corncob and peanut shell, which can be found commonly in Turkey and many part of world in *L. edodes* cultivation (ii) evaluation of the reducibility of the demand for oak sawdust in shiitake production (iii) investigating the laccase and cellulase enzyme activities of growing mixtures including different agricultural wastes and determining the effects of these enzymes on making agricultural wastes more effective.

Materials and Methods

Cultivation of Lentinula edodes in different growing mixtures

In the study, 4320 numbered *L. edodes* strain obtained from Sylvan Cultivating Excellence was used. Wheat stalk, peanut shell, corncob and vine pruning waste were preferred as agricultural wastes due to their common use and availability in Turkey. As wood material, poplar sawdust and oak sawdust used commonly in *L. edodes* cultivation were added to growing mixtures (Stamets, 1993). In addition to the main ingredients of mixture, soy flour (5%) was added (Table 1). During the preparation of the growing mixtures, measurements were performed with a pH meter to adjust the pH and gypsum and lime were supplemented according to the results.

Table 1. Growing mixtures used in *Lentinula edodes* production

Growing mixtures	Number
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 Corncob + 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 Corncob + 1 Wheat bran	A10
1 Oak sawdust + 1 Vine pruning waste + 1 Wheat bran	A11

In order to adjust the moisture content of growing mixtures, soaking was carried out with tap water at regular intervals. At the end of soaking, excess water of the material was drained, followed by adding 1% lime together with wheat bran and soy flour. The substrate mixtures prepared were filled into high temperature resistant polypropylene bags as 1 kg per bag. Cotton plugs were used to close the mouths of the bags, which were then tied with packing tires. Growing bags were sterilized in an autoclave at 121 °C under 1.2 atm pressure for 90 minutes and the bags were removed from the autoclave and waited for cooling. Spawn inoculation was carried out in a sterile bench by mixing 50 g of spawn per bag. Cultivation bags were placed into mushroom growing rooms having 25±2 °C temperature and 80-90% humidity. After development of mycelia, they were kept at 10 °C for two days (shocking) in order to stimulate mushroom formation and then temperature was adjusted to 20±1 °C. Also in this period, fluorescent lamps were used for 12 hours a day, the room humidity was increased to 90-95% and the ventilation was performed 4-7 times per hour to keep CO_2 below 1000 ppm.

Analyses performed in growing mixtures

In the preparation phase of the mixtures; pH, moisture, nitrogen, ash, carbon, hemicellulose, cellulose and lignin analyses were carried out at three different periods: after sterilization, mycelia development and after harvest.

pH analyses

For each application, 10 g of sample was weighed; 100 mL of pure water was added and kept for 1.5 hours. Then, water of the mixture was filtered and measurement was carried out with a pH-meter.

Determination of moisture content

Wet weights of the samples were determined for each application and then they were dried in an oven set at 65 °C until they reached constant weight. After dry weights were determined, % moisture content of the mixtures was found by subtracting the values obtained from 100.

Nitrogen analyses

After the samples were dried and ground, % nitrogen determination was carried out using Kjeldahl method.

Ash analyses

It was determined by burning the samples in an ash oven at 525±25 °C and the results were determined as %.

Carbon analyses

50% of the organic matter is obtained by subtracting ash amount from 100 calculated as carbon (Gerrits, 1985; Cormican and Staunton, 1991).

Calculation of C/N ratio

It was found by proportioning calculated carbon amount to nitrogen amount.

Determination of hemicellulose, cellulose, lignin and cellulose/lignin amounts

In order to determine the hemicellulose, cellulose and lignin amounts of growing mixtures; samples were taken at after sterilization, mycelia development and after harvest periods. Analyses were performed on ANKOM 200/220 Fiber Analyzer with Detergent Fiber Analysis method (Van Soest *et al.*, 1991; Kurt, 2008; Kutlu, 2008).

Neutral Detergent Fiber (NDF) analysis

The method used in NDF analysis was as follows: after F57 bag tare is taken, 0.5 g sample passed through a 1 mm sieve, 120 g FND20C + 20 mL triethylene glycol in 1800 mL purified water, 2 L NDF solution + 4 mL alpha-amylase + 20 g sodium sulphite, 75 minutes waiting in Ankom analyser at 100 °C temperature, evacuation, in 2 L 80-90 °C water + 4 mL alpha-amylase waiting for 3 minutes, evacuation, in 2 L 80-90 °C water 2 times waiting for 3 minutes, waiting for 3 minutes in 250 mL acetone, drying process in the oven at 105 °C for 2-4 hours, as the last step, the bags were weighed as gram.

Acid Detergent Fiber (ADF) analysis

The method used for ADF analysis was as follows: after F57 bag tare is taken, 0.5 g sample passed through a 1 mm sieve, in 2 L ADF solution which was prepared in 1 N H_2SO_4, 60 minutes waiting in Ankom analyser at 100 °C temperature, evacuation, 3 times waiting for 5 minutes in 2 L 80-90 °C water, evacuation, waiting for 3 minutes in 250 mL acetone, drying process in the oven at 105°C for 2-4 hours, as the last step, the bags were weighed as gram.

Acid Detergent Lignin (ADL) analysis

It is remaining cell wall component after ADF is treated with an acid that will dissolve the cellulose and contains lignin. The method used for ADL analysis was as follow: shaking in 72% H_2SO_4 for 30 minutes and waiting for 3 hours, washing with tap water until pH neutral, evacuation, waiting for 3 minutes in 250 mL acetone, drying process in the oven at 105 °C for 3 hours, burning the crucibles containing bags, the weight of which is recorded, in an ash oven set at 550±15 °C for 2 hours.

The hemicellulose, cellulose and lignin amounts of the samples were calculated as follows:

Hemicellulose % = NDF - ADF

Cellulose % = ADF - ADL

Lignin % = ADL

Determination of laccase activity

In determining the laccase activity of samples, the methods of Şık and Ünyayar (1998) and Rani *et al.* (2008) were adapted to laboratory conditions. First of all, 50 mL of pure water was added to 25 g of sample and it was homogenized for 2 minutes in a high-speed homogenizer. To increase enzyme extraction, the sample mixture was treated again with Ultraturak equipment for 1 minute at 10000 rpm. Subsequently, the sample mixture was centrifuged (4000 rpm 1 minute, 4 °C, 20 minutes) to separate liquid part containing enzyme from solid part. 100 µL from the supernatant fraction was transferred to a glass tube and 1 mL of 1% guaiacol solution (prepared in 50% ethyl alcohol solution, v/v) and 3.9 mL of buffer solution (0.1M NaH_2PO_4, 30 °C, pH=7, w/v) were supplemented into it, and then stirred. The first absorbance measurement against the control was

taken in a spectrophotometer (Perkin Elmer-Lambda 25, USA) set at 465 nm without delay. After the first absorbance measurement, the tubes reacted were kept in a stirred water bath at 30 °C and the measurements were repeated at 2-minute intervals.

The graphical method was used to calculate the laccase activity. Using linear part of graphic obtained, the slope was calculated as abs min^{-1}. After the slope of the curve was determined as abs/min, the laccase activity was expressed as "Unit". The term of the "Unit" in spectrophotometric enzyme activity is equal to each change of 0.001 units per min in absorbance. Laccase activity was initially expressed as Unit per mL extract, and then it was converted to the Unit per gram of fresh weight mushroom tissue. Accordingly, the formula used in calculating laccase activity is given below:

$$Laccase\,activty\ \left(U\ g^{-1}\right)=\left(\frac{E}{0.001}\right).\left(\frac{H_r}{H_e}\right).\left(\frac{V_T}{W}\right)$$

E: slope of the linear part of the absorbance-time curve (abs min^{-1})
He: the volume of the enzyme extract in the reaction mixture (mL)
Hr: total volume of the reaction mixture (mL)
VT: obtained total enzyme extract (mL)
W: sample amount (g)

Determination of cellulase activity

Method of König *et al.* (2002) was adapted to laboratory conditions for cellulase activity of the samples. For this, 50 mL of distilled water was added to 25 g of sample and it was homogenized for 2 minutes in a high-speed homogenizer. To increase enzyme extraction, the sample mixture was treated again with Ultraturax equipment at 10000 rpm for 1 minute. Then, 1 g was taken from the homogenized sample mixture into a 15 mL centrifuge tube and 9 mL of buffer solution (100 mmol L^{-1} acetic acid, pH=5.0 and 20 mmol L^{-1} $CaCl_2$ containing solution) was added on it and mixed using a vortex. Tubes were centrifuged (4000 rpm, 4 °C, 20 minutes) to obtain the enzyme extract used in the analysis. 100 µL from the clear part was transferred to a glass tube and 300 µL of 1% carboxy-methyl-cellulose solution was added to it and incubated in a shaking water bath for 20 minutes at 40 °C. After incubation, the tubes were cooled rapidly to room temperature (~24 °C) and 150 µL reaction stopper solution [DNSA (1% w/v, dinitrosalicylic acid) + sodium potassium hydroxide solution (10% v/v, 4 mol L^{-1} NaOH and 4 mol L^{-1} KOH solution) + potassium sodium tartrate tetrahydrate (30% w/v)] was injected into tubes and mixed using vortex. Following this, it was incubated for 10 minutes in a water bath set at 95-100 °C. After the process, the tubes were cooled to room temperature and 2000 µL of distilled water was added. Absorbance measurement was performed using spectrophotometer (Perkin Elmer-Lambda 25, USA) set at 530 nm.

In the calculation of cellulase activity, the relationship between absorbance values and glucose concentrations obtained after applying the analysis steps to the glucose solution in the range of 5-100 mmol L^{-1} was used. For this purpose, absorbance values and glucose concentrations were respectively processed on y-axis and x-axis, and the slope of the obtained graph (m) was used in the calculations. Cellulase activity was expressed as IU. This activity value defines amount of enzyme that has the potential to produce 1 µmol of reducing sugar per minute from cellulose media. The following formula was used to calculate the cellulase activity of mushroom samples:

$$Cellulase\,activty\ \left(IU\,g^{-1}\right)=\frac{\Delta OD_{530}.D.V}{m.W.t}$$

ΔOD530: absorbance value (abs)
D: the dilution factor during obtaining mushroom solution
V: volume obtained after the addition of buffer (mL)

M: slope of the standard curve (abs.mL μmol^{-1})
W: sample amount (g)
t: incubation time (minute)

Analyses performed in mushrooms

Determining of mycelia development time

After mycelia inoculation, the time it takes until the mycelia development all over the bag was calculated as days.

Calculation of biological efficiency rates

The biological efficiency rate was calculated as presented below (Royse, 1985).

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh mushroom weight}}{\text{Dry substrate weight}} \times 100$$

Calculating of total yield

Separate daily harvests were performed from all applications and repetitions in the experiment and the products obtained were weighed on a scale. Following end of the harvest period, total yield amount obtained for each application was revealed by assessing all yield values obtained.

Calculating of mushroom weight

The weight of the mushrooms harvested was determined as g by weighing stipe and cap together in a scale.

Determination of cap-stipe diameter and length

Cap diameter, stipe diameter and stipe length measurements were carried out in five mushroom samples randomly selected with a caliper as mm and their averages were calculated. Cap diameter was measured in the widest and narrowest part of the cap. Stipe diameter was obtained from the middle of cap and stipe. Stipe length was measured in place where stipe is connected with cap.

Determination of mushroom firmness

The firmness was measured from two different points on the surface of the mushroom samples with a penetrometer (lb $inch^{2-1}$) in five mushrooms randomly selected and the averages were determined.

Determination of dry matter

Fresh samples obtained from the first harvest were first weighed on a scale and then dried in food driers adjusted to 65 °C until their weight became constant. The dried samples were weighed again and the dry matter amounts were determined as %.

Protein analysis

After fresh samples obtained from the first harvest were dried and ground, then nitrogen determination was performed based on modified Kjeldahl method. The protein content was determined by multiplying of nitrogen value by the factor of 6.25 as %.

Colour analyses

The measurements were carried out in five mushroom samples chosen randomly from the cap part (as two readings) with a colour meter as L, a and b values. Differences in the colour tone were expressed as ho. The colour meter device was calibrated with a white ceramic plate (L=96.96, a=0.08 and b=1.83) before starting

the measurement. L, a and b indicates darkness-lightness, green-red and blue-yellowness respectively in the colour meter.

Statistical evaluation

The experiment was conducted with three replications and three bags in each repetition according to the randomized plot design. The data obtained were analysed in the JMP statistical package program. Percentage values were converted to angle values and statistical analysis was applied. LSD test was applied to data for which difference was statistically significant. In addition, JMP correlation analysis was applied to the parameters that are thought to be related.

Results

Results of analyses performed in growing mixtures

pH value of growing mixtures

Based on different periods, the highest pH value was obtained from after sterilization (6.46) period. The average pH value of the growing mixtures was determined to be the highest in A5 (5.33). The lowest values were observed in A1 (4.85), C (4.85), A9 (4.84), A6 (4.83), A7 (4.82) and A8 (4.72). In mixture x period interaction, pH values varied between 3.65 and 6.80. Accordingly, the highest interaction occurred in A11 (6.80) at the after-sterilization period. The lowest pH value in the interaction was obtained in A8 (3.65) at after the mycelia development period (Table 2).

Table 2. pH values of different growing mixtures at different periods

Periods				
Mixtures	After sterilization	After mycelia development	After harvest	Mean
C	6.30 c	4.19 hıj	4.06 ı-l	4.85
A1	6.61 ab	4.10 h-k	3.85 lm	4.85
A2	6.43 bc	4.29 ghı	4.65 ef	5.12
A3	6.44 bc	4.60 ef	4.05 ı-l	5.03
A4	6.62 ab	4.19 hıj	4.00 jkl	4.94
A5	6.26 c	4.99 d	4.74 e	5.33
A6	6.26 c	3.92 kl	4.32 gh	4.83
A7	6.35 c	3.91 kl	4.19 hıj	4.82
A8	6.62 ab	3.65 m	3.90 kl	4.72
A9	6.49 bc	4.03 jkl	3.99 jkl	4.84
A10	6.39 bc	4.29 ghı	4.12 h-k	4.93
A11	6.80 a	4.45 fg	3.89 kl	5.05
Mean	6.46 *A*	4.22 *B*	4.15 *B*	
LSD periods***= 0.07; LSD mixtures= Ö.D.; LSD periods x mixtures***= 0.24				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Moisture content of growing mixtures

The highest moisture content was recorded in A2 (72.10%). The lowest values were determined in A8 (65.54%), A10 (65.37%) and A11 (65.16%). Among periods, the highest moisture content was obtained at the after harvest (68.88%) (Table 3). With the primordium stage, the growing bags were opened. Due to the differences in the content of growing mixtures, their water keeping capacity also varied. Following the opening of the bags, increases and decreases among periods have been determined in terms of the moisture content. The highest moisture content was determined in A2 (75.47%) at after harvest period. The lowest contents were

recorded in A6-after sterilization (64.72%), A10-after mycelia development (64.62%), A1(64.4%)-A8 (64.23%)-A11 (62.54%)-after sterilization in interactions (Table 3).

Table 3. Moisture content of growing mixtures at different periods (%)

	Periods			
Mixtures	After sterilization	After mycelia development	After harvest	Mean
C	70.21 cde	68.80 d-h	68.07 g-l	69.02 BC
A1	64.41 q	66.82 k-n	65.79 m-q	65.67 DE
A2	71.08 bc	69.76 c-f	75.47 a	72.10 A
A3	71.24 bc	69.63 c-g	72.49 b	71.12 AB
A4	67.08 ı-m	71.21 bc	70.57 c	69.62 BC
A5	68.74 d-ı	70.72 c	70.30 cd	69.92 ABC
A6	64.72 q	66.66 k-o	67.94 h-l	66.44 DE
A7	67.03 j-n	68.54 e-j	68.24 f-k	67.94 CD
A8	64.23 q	67.03 j-n	65.38 n-q	65.54 E
A9	65.06 opq	66.55 l-p	67.67 h-l	66.43 DE
A10	64.89 p-q	64.62 q	66.60 k-o	65.37 E
A11	62.54 r	64.91 pq	68.03 g-l	65.16 E
Mean	66.77 *B*	67.94 *AB*	68.88 *A*	
LSD periods**= 0.50; LSD mixtures***= 1.00; LSD periods x mixtures***= 1.67				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Nitrogen amounts of growing mixtures

The highest nitrogen amount was observed at the after-harvest period (1.71%). In terms of growing mixtures, while A4 (1.67%) giving the highest result was followed by A11 (1.64%), A6 (1.64%) and A9 (1.62%), the lowest nitrogen amount was determined in C (1.26%) and A3 (1.26%). We have realized that some of the mixtures having high nitrogen content show low Biological Efficiency (BE) ratios. Considering interactions, the highest nitrogen amount was detected in A4-after harvest (2.05%). The lowest interaction was obtained from C-after sterilization (1.08%) (Table 4).

Table 4. Nitrogen amount of growing mixtures at different periods (%)

Mixtures	Periods			
	After sterilization	After mycelia development	After harvest	Mean
C	1.08 r	1.23 pq	1.47 ı-l	1.26 F
A1	1.28 opq	1.47 ı-l	1.67 de	1.47 CD
A2	1.31 n-q	1.54 f-ı	1.94 ab	1.59 B
A3	1.21 q	1.21 q	1.36 l-o	1.26 F
A4	1.34 m-p	1.62 d-g	2.05 a	1.67 A
A5	1.38 k-o	1.21 q	1.61 efg	1.40 E
A6	1.49 h-k	1.60 e-h	1.83 bc	1.64 AB
A7	1.27 opq	1.28 opq	1.70 de	1.41 DE
A8	1.45 ı-m	1.49 h-k	1.60 e-h	1.51 C
A9	1.51 g-j	1.63 def	1.73 cd	1.62 AB
A10	1.32 n-q	1.33 nop	1.70 de	1.45 CDE
A11	1.42 j-n	1.61 efg	1.91 b	1.64 AB
Mean	1.34 *C*	1.43 *B*	1.71 *A*	
LSD periods***= 0.03; LSD mixtures***= 0.07; LSD periods x mixtures***= 0.11				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Ash contents of growing mixtures

Average ash amount of the growing mixtures varied between 6.63% and 9.21%. Accordingly, the highest ash amount was determined in A5 (9.21%). The lowest values were recorded in A7 (6.85%) and A10 (6.84%). Among periods, while the highest ash amount was observed at after harvest (8.72%), the lowest value was at after sterilization (6.69%). Interactions have shown that ash values vary between 5.52% and 10.50%. The highest interaction occurred in A9-after harvest (10.50%). The lowest ash amount in interactions was found in A4 (5.74%), A10 (5.69%), A2 (5.52%)-after sterilization (Table 5).

Table 5. Ash contents of different growing mixtures at different periods (%)

Mixtures	Periods			
	After sterilization	After mycelia development	After harvest	Mean
C	6.33 k-o	7.49 g-k	8.09 e-ı	7.30 DE
A1	6.95 h-m	8.36 d-g	7.84 ghı	7.72 D
A2	5.52 o	6.09 l-o	8.27 d-g	6.63 E
A3	6.07 l-o	9.42 a-d	9.79 ab	8.43 BC
A4	5.74 mno	6.57 j-o	9.45 a-d	7.25 DE
A5	8.13 e-h	9.79 ab	9.72 abc	9.21 A
A6	8.25 d-g	8.57 c-g	9.22 b-e	8.68 AB
A7	7.62 g-j	6.87 ı-n	6.06 l-o	6.85 E
A8	6.99 h-l	8.35 d-g	8.00 f-ı	7.78 CD
A9	6.47 j-o	8.56 c-g	10.50 a	8.51 B
A10	5.69 no	6.22 l-o	8.62 b-g	6.84 E
A11	6.54 j-o	7.68 g-j	9.14 b-f	7.79 CD
Mean	6.69 *C*	7.83 *B*	8.72 *A*	
LSD periods***= 0.35; LSD mixtures***= 0.70; LSD periods x mixtures***= 1.22				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Carbon contents of growing mixtures

Average carbon amount of the growing mixtures varied between 45.39% and 46.69%. The highest carbon contents were in A2 (46.69%), A10 (46.58%) and A7 (46.57%), while the lowest values were found in A9 (45.75%) together with A6 (45.66%) and A5 (45.39%) assessed in the same group. Among periods, while the highest carbon amount was obtained from after sterilization (46.65%), the lowest value was observed at the after harvest (45.64%) period. Carbon amounts of interactions changed between 44.75% and 47.24%. The highest interaction occurred in A2-after sterilization (47.24%). The lowest carbon amounts were determined in A3-after harvest (45.11%), A5-after mycelia development (45.11%) and A9-after harvest (44.75%) (Table 6).

Table 6. Carbon contents of different growing mixtures at different periods (%)

	Periods			
Mixtures	After sterilization	After mycelia development	After harvest	Mean
C	46.83 a-e	46.25 e-ı	45.96 g-k	46.35 AB
A1	46.52 c-h	45.82 ı-l	46.08 ghı	46.14 B
A2	47.24 a	46.95 a-d	45.86 ı-l	46.69 A
A3	46.96 a-d	45.29 l-o	45.11 no	45.79 CD
A4	47.13 abc	46.71 a-f	45.28 l-o	46.37 AB
A5	45.94 h-k	45.11 no	45.14 mno	45.39 E
A6	45.88 ı-l	45.72 ı-m	45.39 k-n	45.66 DE
A7	46.19 f-ı	46.56 b-g	46.97 a-d	46.57 A

A8	46.51 d-h	45.83 ı-l	46.00 g-j	46.11 BC
A9	46.77 a-f	45.72 ı-m	44.75 o	45.75 D
A10	47.16 ab	46.89 a-d	45.69 ı-n	46.58 A
A11	46.73 a-f	46.16 f-ı	45.43 j-n	46.11 BC
Mean	46.65 *A*	46.08 *B*	45.64 *C*	
LSD periods***= 0.18; LSD mixtures***= 0.35; LSD periods x mixtures***= 0.61				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Carbon/Nitrogen ratios of growing mixtures

The highest carbon/nitrogen ratio was recorded at the after sterilization (35.18) period. It is expected that the carbon/nitrogen ratio decreases in the later stages of mushroom cultivation. This ratio ranged from 27.48 to 37.63. The highest carbon/nitrogen ratio was obtained from C (37.63) and A3 (36.57). The lowest values were obtained in A11 (28.51), A9 (27.70) and A6 (27.48). Regarded with interactions, the highest interaction occurred in C-after sterilization (43.71). The lowest ratios were observed in A9 (24.03), A11 (23.85), A2 (23.70) and A4 (22.26)-after the harvest period (Table 7).

Table 7. Carbon/Nitrogen ratio of different growing mixtures at different periods

	Periods			
Mixtures	After sterilization	After mycelia development	After harvest	Mean
C	43.71 a	37.62 bc	31.55 ghı	37.63 A
A1	36.45 bc	31.17 g-j	27.65 m	31.76 BC
A2	36.07 cd	30.56 h-l	23.70 o	30.11 DE
A3	38.92 b	37.54 bc	33.25 e-h	36.57 A
A4	35.21 c-f	28.84 j-m	22.26 o	28.77 EF
A5	33.41 d-g	37.39 bc	28.38 klm	33.06 B
A6	30.86 g-k	26.76 mn	24.82 no	27.48 F
A7	35.69 cde	36.48 bc	27.69 m	33.29 B
A8	32.15 gh	30.76 g-l	28.92 ı-m	30.61 CD
A9	30.97 g-k	28.11 lm	24.03 o	27.70 F
A10	35.72 cde	35.27 c-f	26.94 mn	32.65 B
A11	32.99 fgh	28.69 j-m	23.85 o	28.51 F
Mean	35.18 *A*	32.43 *B*	26.92 *C*	
LSD periods***= 0.78; LSD mixtures***= 1.56; LSD periods x mixtures***= 2.70				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Hemicellulose contents of growing mixtures

The highest hemicellulose amounts were obtained at the after sterilization (19.71%) and after harvest (18.94%) periods. The lowest amount occurred at after mycelia development (13.83%). Among mixtures, while A10 (22.86%) and A7 (22.76%) gave the highest values, the lowest amounts observed in C (13.08%), A6 (12.99%) and A11 (12.75%). Interactions showed that the highest amount of hemicellulose was obtained in A3-after harvest (31.25%). The lowest amount of hemicellulose occurred in A3-after mycelia development (5.07%) (Table 8).

Table 8. Hemicellulose contents of different growing mixtures at different periods (%)

	Periods			
Mixtures	After sterilization	After mycelia development	After harvest	Mean
C	13.97 no	6.28 rs	18.99 g-l	13.08
A1	20.63 e-h	10.40 p	20.87 e-h	17.30
A2	16.66 lmn	9.04 pq	23.69 bcd	16.46
A3	24.44 bc	5.07 s	31.25 a	20.25
A4	19.78 f-k	8.61 pqr	17.12 klm	15.17
A5	21.02 d-h	20.08 e-j	15.43 mno	18.84
A6	13.72 o	8.68 pqr	16.56 lmn	12.99
A7	25.50 b	21.44 d-g	21.34 d-h	22.76
A8	18.66 h-l	21.11 d-h	17.04 lm	18.93
A9	17.43 j-m	20.33 e-ı	17.77 ı-m	18.51
A10	22.78 b-e	25.09 b	20.71 e-h	22.86
A11	21.95 c-f	9.86 p	6.44 qrs	12.75
Mean	19.71 *A*	13.83 *B*	18.94 *A*	
LSD periods*= 0.82; LSD mixtures = N.I.; LSD periods x mixtures***= 2.74				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Cellulose contents of growing mixtures

While the highest amount of cellulose was found at the after sterilization (36.91%), the lowest value was observed at the after harvest (33.01%) period. Regarded with mixtures, the highest result was obtained from C (44.39%). The lowest cellulose amount was determined in A5 (29.76%). Among interactions, the highest amount of cellulose was found in C-after mycelia development (49.09%). The lowest amount of cellulose was determined in A10-after mycelia development (25.59%) and A2-after harvest (23.98%) (Table 9).

Table 9. Cellulose contents of different growing mixtures at different periods (%)

	Periods			
Mixtures	After sterilization	After mycelia development	After harvest	Mean
C	41.04 def	49.09 a	43.04 cd	44.39
A1	32.70 k-o	44.40 bc	37.76 gh	38.29
A2	46.37 ab	32.23 m-q	23.98 u	34.19
A3	35.26 h-m	35.67 hk	31.00 n-s	33.97
A4	34.08 ı-n	32.91 j-o	31.20 n-s	32.73
A5	29.08 rs	29.42 p-s	30.78 o-s	29.76
A6	41.71 cde	38.01 fgh	35.45 h-l	38.39
A7	32.42 l-p	30.17 o-s	28.76 s	30.45
A8	37.60 gh	28.66 st	28.60 st	31.62
A9	39.90 efg	29.16 qrs	42.54 cde	37.20
A10	35.80 hıj	25.59 tu	30.92 o-s	30.77
A11	36.90 ghı	29.39 p-s	32.08 n-r	32.79
Mean	36.91	33.72	33.01	
LSD periods = N.I.; LSD mixtures = N.I.; LSD periods x mixtures***= 3.10				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Lignin contents of growing mixtures

The highest amount of lignin was detected at the after-harvest period (15.83%). Based on data obtained, it has been observed that addition of oak sawdust does not have a positive or negative effect on the lignin concentration of mixtures. Among mixtures, the highest value was found in A4 (16.85%). The lowest amounts

of lignin were recorded in A7 (13.56%), A1 (13.23%) and A10 (13.20%). When interactions were considered, the highest data was determined in A2-after harvest (19.95%). The lowest data were observed in A5-after mycelia development (10.88%), A3-after sterilization (10.15%) and A3-after mycelia development (9.64%) (Table 10).

Table 10. Lignin contents of different growing mixtures at different periods (%)

	Periods			
Mixtures	After sterilization	After mycelia development	After harvest	Mean
C	17.90 bcd	15.91 efg	15.73 fg	16.51 AB
A1	13.01 klm	14.05 h-k	12.64 lm	13.23 CD
A2	13.28 klm	14.85 ghı	19.95 a	16.03 ABC
A3	10.15 o	9.64 o	13.15 klm	10.98 D
A4	17.21 cde	16.63 def	16.72 c-f	16.85 A
A5	13.53 jkl	10.88 no	17.35 cd	13.92 A-D
A6	15.73 fg	15.26 gh	17.42 cd	16.14 ABC
A7	14.78 g-j	12.99 klm	12.92 klm	13.56 BCD
A8	17.93 bc	12.60 lm	15.60 fg	15.37 ABC
A9	15.22 gh	12.16 mn	16.03 efg	14.47 ABC
A10	13.60 ı-l	12.56 lm	13.43 klm	13.20 CD
A11	12.19 m	13.02 klm	19.00 ab	14.73 ABC
Mean	14.54 *AB*	13.38 *B*	15.83 *A*	
LSD periods* = 0.39; LSD mixtures* = 0.78; LSD periods x mixtures***= 1.30				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Cellulose/Lignin ratio of growing mixtures

While the highest cellulose/lignin ratios were obtained at the after sterilization (2.60) and after mycelia development (2.55) periods, the lowest value was determined at the after harvest (2.05) period. Among the mixtures, the highest cellulose/lignin ratio was recorded in A3 (3.18). The lowest results were found in A8 (2.07) and A4 (1.94). In terms of interactions, the highest cellulose/lignin ratio was observed in A3-after mycelia development (3.71). The lowest values were detected in A11-after harvest (1.69) and A2 (1.21)-after harvest (Table 11).

Table 11. Cellulose/Lignin ratio of different growing mixtures at different periods

	Periods			
Mixtures	After sterilization	After mycelia development	After harvest	Mean
C	2.29 h-o	3.09 c	2.74 de	2.71
A1	2.51 e-j	3.16 bc	3.01 cd	2.90
A2	3.49 ab	2.17 j-q	1.21 t	2.29
A3	3.48 ab	3.71 a	2.36 f-m	3.18
A4	1.98 n-s	1.98 o-s	1.87 p-s	1.94
A5	2.15 k-q	2.71 def	1.78 rs	2.21
A6	2.65 efg	2.49 e-k	2.03 m-s	2.39
A7	2.19 j-p	2.33 g-n	2.22 j-o	2.25
A8	2.10 l-r	2.28 ı-o	1.83 qrs	2.07
A9	2.63 e-ı	2.40 e-l	2.70 def	2.58
A10	2.63 e-h	2.05 l-r	2.37 f-m	2.35
A11	3.04 cd	2.26 j-o	1.69 s	2.33
Mean	2.60	2.55	2.15	
LSD periods = N.I.; LSD mixtures = N.I.; LSD periods x mixtures***= 0.35				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Laccase enzyme activity of different growing mixtures

The highest laccase activity (2873.82 U g^{-1}) was recorded on the 25th day. When the average laccase activity of the mixtures was examined, no statistically significant difference was found. While the highest laccase activity was observed in A7 (2164.48 U g^{-1}), the lowest was obtained from C (294.12 U g^{-1}). Among interactions, the highest laccase activity was observed in A11 (11484.50 U g^{-1}) on the 25th day. The lowest laccase activities were recorded in A11-15th day (17.25 U g^{-1}), A3-15th day (16.88 U g^{-1}), A7-10th day (16.35 U g^{-1}) A10-15th day (7.5 U g-^{1}) and A2-15th day (5.25 U g^{-1}) (Table 12).

Table 12. Laccase enzyme activity of different growing mixtures at different periods (U g^{-1})

Mixture	5th day	10th day	15th day	20th day	25th day	AH	Mean
C	1418.50 f-ı	41.10 l	46.75 l	131.05 kl	98.88 l	28.45 l	294.12
A1	1458.50 fgh	543.33 h-l	179.25 kl	377.45 h-l	3939.15 de	135.50 kl	1105.53
A2	80.50 l	1379.95 g-j	5.25 l	282.85 jkl	750.28 h-l	21.95 l	420.13
A3	461.25 h-l	167.18 kl	16.88 l	22.50 l	641.48 h-l	2103.30 fg	568.76
A4	353.00 h-l	37.30 l	528.60 h-l	4088.55 de	3850.75 e	208.40 kl	1511.10
A5	410.50 h-l	122.50 kl	2491.20 f	819.95 h-l	239.75 kl	120.40 kl	700.72
A6	343.00 ı-l	65.75 l	75.85 l	133.3 kl	246.25 kl	5021.65 cd	980.97
A7	115.00 kl	16.35 l	126.25 kl	4556.00 de	922.13 h-l	7251.15 b	2164.48
A8	281.50 jkl	98.55 l	94.75 l	178.70 kl	5822.85 c	591.40 h-l	1177.96
A9	648.50 h-l	292.65 jkl	57.00 l	1373.95 g-j	608.00 h-l	1217.50 g-k	699.60
A10	344.00 ı-l	28.00 l	7.50 l	872.35 h-l	5881.88 c	204.15 kl	1222.98
A11	342.00 ı-l	258.95 kl	17.25 l	281.20 jkl	11484.50 a	46.90 l	2071.80
Mean	521.35 *B*	254.30 *B*	303.88 *B*	1093.15 *B*	2873.82 *A*	1412.56 *AB*	
LSD periods* = 323.85; LSD mixtures = N.I.; LSD periods x mixtures***= 1106.47							

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Cellulase enzyme activity of different growing mixtures

The highest cellulase activity was observed on the 10th day (2.55 IU g^{-1}). In terms of mixtures, the highest cellulase activity was found in A3 (3.16 IU g^{-1}). The lowest values were recorded in the A5 (0.95 IU g^{-1}), A1 (0.89 IU g^{-1}), A8 (0.81 IU g^{-1}), A4 (0.69 IU g^{-1}) and A6 (0.69 IU g^{-1}). Among interactions, the highest cellulase activity was obtained in C-10th day (6.26 IU g^{-1}). The lowest cellulase activities were detected in A8-20th day (0.23 IU g^{-1}) and A6-15th day (0.18 IU g^{-1}) (Table 13).

Table 13. Cellulase enzyme activity of different growing mixtures at different periods (IU g^{-1})

Mixture	5th day	10th day	15th day	20th day	25th day	AH	Mean
C	0.84 o-w	6.26 a	2.06 e-h	0.67 q-x	0.81 o-w	1.30 ı-p	1.99 B
A1	0.91 o-v	1.57 h-n	0.97 m-v	0.76 p-x	0.64 r-x	0.50 t-x	0.89 C
A2	1.11 k-t	4.15 c	0.55 t-x	0.85 o-w	0.95 n-v	1.71 g-l	1.55 BC
A3	2.38 ef	4.97 b	4.31 c	2.27 efg	3.17 d	1.85 e-l	3.16 A
A4	0.89 o-v	1.10 l-t	0.42 u-x	0.78 o-x	0.56 t-x	0.42 u-x	0.69 C
A5	1.24 ı-r	0.81 o-w	0.54 t-x	0.56 t-x	0.83 o-w	1.73 g-k	0.95 C
A6	1.29 ı-q	1.22 j-s	0.18 x	0.50 t-x	0.42 u-x	0.51 t-x	0.69 C
A7	0.62 s-x	4.38 bc	1.79 f-j	0.86 o-w	0.50 t-x	1.02 m-u	1.53 BC
A8	0.73 p-x	1.22 j-s	1.41 ı-o	0.23 wx	0.75 p-x	0.55 t-x	0.81 C
A9	1.33 ı-p	1.00 m-u	1.28 ı-q	2.16 e-h	0.77 p-x	0.80 o-x	1.22 BC
A10	0.46 u-x	1.59 h-m	2.43 e	3.25 d	1.26 ı-r	0.97 m-v	1.66 BC
A11	0.75 p-x	2.30 efg	2.08 e-h	0.81 o-w	0.34 vwx	0.72 p-x	1.17 BC
Mean	1.05 *B*	2.55 *A*	1.50 *B*	1.14 *B*	0.92 *B*	1.01 *B*	
LSD periods*** = 0.18; LSD mixtures*** = 0.26; LSD periods x mixtures***= 0.63							

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Results of analyses performed in the mushrooms

Mycelia development, yield and biological efficiency

Mycelia development time ranged from 21.66 to 50.00 days. The fastest mycelia development was obtained from A7 (21.67 days). The slowest development was observed in A4 (50 days) (Table 14). Average yield values varied between 55.9 and 299.59 g kg^{-1}. The highest yield was found in A5 (299.59 g kg^{-1}). The lowest values were determined in A9 (55.99 g kg^{-1}), A6 (65.59 g kg^{-1}) and A11 (75.47 g kg^{-1}) (Table 14). It has been determined that biological efficiency rates varied between 19.22% and 93.65%. While the highest biological efficiency rates were found in A3 (93.65%) and A5 (92.90%), the lowest rates were obtained from A11 (21.45%), A6 (19.85%) and A9 (19.22%) (Figure 1, Table 14).

Table 14. Mycelia development time, yield and biological efficiency obtained from different growing mixtures

Mixtures	Mycelia development (days)	Biological efficiency (%)	Yield (g kg^{-1})
C	23.33 cd	53.65 d	162.55 d
A1	23.67 cd	45.04 d	151.53 de
A2	44.00 b	83.10 b	218.45 c
A3	41.00 b	93.65 a	259.27 b
A4	50.00 a	71.74 c	233.64 bc
A5	24.67 cd	92.90 ab	299.59 a
A6	22.33 cd	19.85 f	65.59 g
A7	21.67 d	34.66 e	116.01 f
A8	22.67 cd	65.69 c	227.08 bc
A9	25.00 c	19.22 f	55.99 g
A10	22.67 cd	33.73 e	128.04 ef
A11	25.00 c	21.45 f	75.47 g
	LSD mix***= 3.13	LSD mix***= 10.32	LSD mix***= 33.65

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Figure 1. A *L. edodes* mushrooms obtained from the study

Weight, cap-stipe diameter and stipe length

Average mushroom weight changed between 14.98 and 33.52 g. While the highest mushroom weight was obtained from C (33.52 g), the lowest value was observed in A5 (14.98 g) (Table 15). The average cap diameter varied between 45.36 and 61.33 mm and the highest cap diameter was recorded in A8 (61.33 mm). The lowest value was recorded in A3 (45.36 mm) (Table 15). Stipe length ranged from 21.31 to 49.11 mm. The highest stipe length was detected in A10 (49.11 mm). The lowest values were obtained from A1 (27.01 mm), A3 (25.75 mm) and A4 (21.31 mm) (Table 15). Stipe diameter varied between 8.89 and 27.24 mm. The highest stipe diameter value was observed in A6 (61.33 mm). The lowest values were obtained from A11 (9.71 mm) and A2 (8.89 mm) (Table 15).

Table 15. Weight, cap-stipe diameter and stipe length of mushrooms grown on different growing mixtures

Mixtures	Weight (g kg^{-1})	Cap diameter (mm)	Stipe diameter (mm)	Stipe length (mm)
C	33.52 a	54.81	13.85 bcd	40.51 a-d
A1	19.25 ef	51.02	12.95 bcd	27.01 fg
A2	28.58 abc	50.50	8.89 d	29.61 efg
A3	25.76 cde	45.36	13.81 bcd	25.75 fg
A4	30.05 abc	58.87	16.77 b	21.31 g
A5	14.98 f	53.92	10.46 cd	34.10 c-f
A6	21.68 de	57.93	27.24 a	32.36 def
A7	24.89 cde	55.48	12.81 bcd	38.09 b-e
A8	29.51 abc	61.33	11.76 bcd	40.93 a-d
A9	31.74 ab	54.41	15.01 bc	44.87 ab
A10	29.49 abc	58.64	14.09 bcd	49.11 a
A11	27.61 a-d	56.14	9.71 cd	44.07 abc
	LSD mix***=5.99	LSD mix= N.I.	LSD mix***= 5.58	LSD mix***= 9.98

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Firmness, dry matter and protein contents

The average firmness ranged from 1.77 to 2.34 lb inch$^{2-1}$. The highest value was detected in A9 (2.34 lb inch$^{2-1}$). The lowest values were obtained from A2 (1.81 inch$^{2-1}$) and A3 (1.77 inch$^{2-1}$) (Table 16). The average dry matter amount varied between 8.59% and 11.65%. The highest amount of dry matter was recorded in A6 (11.65%) The lowest value was obtained from C (8.59%) (Table 16). The average protein content changed between 21.25% and 35.62%. While the highest protein contents were detected in A5 (35.62 %) and A7 (35.39%), the lowest values were obtained from A10 (26.95%), A9 (26.69%) and C (21.25%) (Table 16).

Colour values

Lightness (L*) value varied between 30.07 and 44.37. While the highest lightness values were recorded in A11 (44.37), A7 (43.12) and A6 (42.96), the lowest values were obtained from A9 (30.29) and A5 (30.07) (Table 17). Accordingly, it was determined that the average chroma (C*) value ranged from 10.96 to 22.50. Based on the chart, while the highest C* value was recorded in A2 (22.50), the lowest values were obtained from A5 (11.35), A9 (11.04) and A1 (10.96) (Table 17). The average hue (h^0) value changed between 50.28 and 64.14. While the highest h^o values were observed in A2 (64.14) and A11 (61.70), the lowest values were obtained from A3 (53.20), A8 (53.06), A9 (52.89), C (52.87), A10 (51.63), A4 (50.33) and A5 (50.28) (Table 17).

Table 16. Firmness, dry matter and protein contents of mushrooms obtained from different mixtures

Mixtures	Firmness (lb inch$^{2-1}$)	Dry matter (%)	Protein amount (%)
C	1.98 b-e	8.59 f	21.25 f
A1	1.94 cde	9.61e	33.06 bc
A2	1.81 de	9.99 de	32.39 c
A3	1.77 e	9.69 de	33.19 bc
A4	1.89 cde	10.15 cde	34.54 ab
A5	1.89 cde	10.14 cde	35.62 a
A6	2.07 a-d	11.65 a	32.73 bc
A7	2.03 b-e	10.26 b-e	35.39 a
A8	2.16 abc	10.65 bcd	29.93 d
A9	2.34 a	9.61 e	26.69 e
A10	2.25 ab	11.21 ab	26.95 e
A11	1.93 cde	10.99 abc	31.41 cd
	LSD mix**= 0.30	LSD mix***= 0.98	LSD mix***= 2.01

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Table 17. Lightness (L*), chroma (C*) and hue (h^0) values of mushrooms grown on different growing mixtures

Mixtures	Lightness (L*) value	Chroma (C*) value	Hue (h^0) value
C	33.44 cd	13.38	52.87 c
A1	38.47 abc	16.46	54.26 bc
A2	41.21 ab	22.50	64.14 a
A3	34.18 bcd	13.81	53.20 c
A4	32.81 cd	14.70	50.33 c
A5	30.07 d	11.35	50.28 c
A6	42.96 a	19.74	60.35 ab
A7	43.12 a	17.08	60.89 ab
A8	32.27 cd	10.96	53.06 c
A9	30.29 d	11.04	52.89 c
A10	33.50 cd	14.49	51.63 c
A11	44.37 a	17.43	61.70 a
	LSD mix***= 7.07	LSD mix= N.I.	LSD mix***= 7.13

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant (2): N.I. Not important; *P<0.05. **p ≤ 0.01. ***p ≤ 0.001

Discussion

In the study, properties of the growing mixtures were determined at different stages of production. The aim of determination at different periods was to answer question of whether mixtures can maintain ideal conditions, to determine what changes at what stage and to observe effect of the substrates used preparing growing mixtures on the properties of the mixtures at different periods.

The pH value of the mixtures generally tended to decrease in the later stages of cultivation and mean of the periods showed a decrease from 6.46 to 4.15. Philippoussis *et al.* (2002) reported that the pH values decreased steadily and changed between 6.37 and 7.31 at the beginning, then decreased between 4.49 and 5.01 at the after mycelia development period in shiitake production. This finding was supported by a different study of the same researchers (Philippoussis *et al.*, 2003). Adenipekun and Okunlade (2012) revealed that change in pH may be associated with the presence of metabolic waste products in the growing mixtures and the increase in nitrogen content.

In this study, moisture content of the different periods varied between 66.77 % and 68.88 %. The moisture content of the mixtures was between 65.16% and 72.10%. Morais *et al.* (2000) reported that mixtures preserve their moisture contents during mushroom development. Sözbir (2014) determined that the moisture content was between 33.88% and 74.50% depending on the mixtures in the shiitake production. It was stated that the mixtures including hard wastes have lower moisture content than others. Similarly, in our study, moisture content of A8 and A11 mixtures were found to be lower than the others.

The highest amount of nitrogen was detected in the A4 (1 oak sawdust + 1 poplar sawdust + 1 bran) and a general increasing trend was observed in the amount of nitrogen in the later stages of cultivation. Philippoussis *et al.* (2003) found that nitrogen amounts were 1.20%, 1.33% and 0.96% in oak sawdust (OS), wheat straw (WS) and corncob (CC) mixtures, respectively in the shiitake cultivation. Kurt (2008) reported that bran contains about 1.83% nitrogen and the nitrogen amount of the mixtures including bran was higher than other mixtures in *P. ostreatus*. In addition, it was recorded that the highest and the lowest nitrogen amounts were obtained at the after harvest and after sterilization periods, respectively. In another study carried out on *L. edodes* by Atila (2019), nitrogen content increased at the after-harvest period when compared with initial composition and after spawn run periods. The highest ash amount was recorded in the A5 (1 oak sawdust + 1 wheat stalk + 1 bran) and a general trend of increase in ash amount was observed in the later stages of cultivation. Kurt (2008) found that amount of ash increased based on sampling periods of mixtures in *Pleurotus*. Gaitán-Hernández *et al.* (2011) reported that the ash content of the three mixtures tested increased during the primordium stage compared to control in shiitake mushroom. Atila (2019) reported that the initial ash content varied between 4.8% and 6.7%, then it ranged from 5.7% to 7.8% and from 5.8% to 8.5% at the after mycelia development and after harvest periods, respectively in the shiitake cultivation.

The highest carbon amount was determined in A7 (3 corncob + 1 bran). In a study conducted by Kurt (2008) on the *Pleurotus* genus, it was determined that among the growing mixtures containing bran, C content of wheat stalk and bran mixture was lower than other mixtures and this finding was explained by the low specific gravity of wheat stalk. In our study, a similar situation was observed in A3 (3 wheat stalks: 1 bran). Sözbir (2014) reported that average amount of carbon varied at the sterilization, mycelia development and the after-harvest periods depending on the content of the mixtures in shiitake mushroom. Kibar *et al.* (2016) found close values in carbon amounts of different mixtures (45.27-45.71%) in *P. eryngii*. Similar results were obtained in our study.

The highest carbon/nitrogen ratio was recorded in C (oak sawdust) and difference among periods was striking. Philippoussis *et al.* (2003) determined that the C/N ratios ranged from 25 to 50/1 in *L. edodes*. Gaitán-Hernández *et al.* (2011) reported that the C/N ratio decreased in the vine pruning waste (VP) and barley straw (BS) mixtures at the primordium formation phase, although this decrease was not seen in the wheat straw (WS) mixture in the shiitake cultivation. Philippoussis *et al.* (2011) realized that the C/N ratio varied between 57.07 and 72.02 before the additives were supplemented to the mixture, then this amount decreased between 33.48 and 36.94 after additives in *L. edodes*. Kibar *et al.* (2016) found that C/N ratios were lower in mixtures including bran than without bran due to the high nitrogen content of bran. In a study carried out by Atila (2019) on shiitake mushroom, C/N amounts were determined based on sampling period and while C/N amounts varied between 25.8 and 160.9 at the beginning of cultivation, these amounts varied between 20.9-118.5 and 19.8-80.6 at the mycelia development and after harvest periods, respectively.

The highest amount of hemicellulose was found in A10 (1 oak sawdust + 1 corncob + 1 bran) and A7 (3 corncob + 1 bran) mixtures and a decrease was observed at the later stages of cultivation. Philippoussis *et al.* (2003) reported that hemicellulose amount varied between 12.6% and 37.0 % and wheat straw (WS) and corncob (CC) mixtures had two and three times more hemicellulose content than oak sawdust (OS) mixture, respectively in shiitake cultivation. Gaitán-Hernández *et al.* (2006) determined that amount of hemicellulose in all mixtures especially vine pruning waste (VP) decreased at after mycelia development period. However, this decrease created a positive correlation in terms of BE value in VP, whereas this correlation was not observed in BS and WS mixtures. Similarly, in our study, BE ratio were also low in A11 (1 oak sawdust + 1 vine pruning

waste + 1 bran) having decreasing in hemicellulose amount. Adenipekun and Okunlade (2012) found that in the period of 0-90 days, hemicellulose amount decreased in both mixtures tested in *P. ostreatus*. The amount of hemicellulose decreased from 11.60% to 3.64% and from 8.11% to 2.85% in mixtures prepared with wood sawdust and corn waste, respectively. Atila (2019) determined that the highest and the lowest amounts of hemicellulose were in corn stalk (CS) with 30.4% and OS (oak sawdust) with 6.3% respectively at initial period. With the development of the mycelia, the hemicellulose amount of the mixtures has decreased significantly.

In this study, the highest cellulose amount was obtained from C (oak sawdust). In a study conducted by Philippoussis *et al.* (2003) on shiitake mushroom, cellulose amounts varied between 37.5% and 47.7% and the lowest and highest amounts of cellulose content were recorded in wheat straw (WS) (37.5%) and oak sawdust (OS) (47.7%), respectively. Kurt (2008) found that the amount of cellulose in different mixtures increased and decreased during different periods in the genus *Pleurotus*. It was reported that the amount of cellulose increased during the mycelia development and after harvest periods due to presence of nutrients needed for mushroom growth in the mixtures. As a result, it was found that amount of cellulose both increased and decreased at the after-harvest period. In some mixtures tested in our study, amount of cellulose increased at the after-harvest period like Kurt (2008)' finding, while it decreased in the others. Atila (2019) reported that cellulose amounts varied between 26.4% and 41.9% at the initial period of shiitake production. While the cellulose content of the mixtures at after mycelia development varied between 26.4% and 44.6%, it changed between 20.4% and 34.5% at after harvest.

In this study, the highest lignin amount was recorded in A4 (1 oak sawdust + 1 poplar sawdust + 1 bran) and C (oak sawdust) mixtures. Philippoussis *et al.* (2003) found that lignin amounts varied between 6.7% and 16.0% in shiitake production and while the lowest lignin amount was found in barley straw (WS) (6.7%), the highest value was recorded in oak sawdust (OS) (16.0%). Kurt (2008) recorded that lignin in different mixtures used hemicellulose effectively, whereas the consumption of cellulose and lignin differed according to the mixtures and periods. In a study carried out by Sözbir (2014) on shiitake mushroom, it was determined that the percentage increase in lignin amount at after mycelia development was due to the decrease in the amount of holocellulose.

The highest cellulose/lignin ratio was determined in A3 (3 wheat stalk + 1 bran) and there was a decrease at different periods during mushroom cultivation. Philippoussis *et al.* (2003) reported that the cellulose/lignin content varied between 3.0% and 5.6% and the lowest and the highest lignin contents were detected in oak sawdust (OS) (3.0%) and wheat straw (WS) (5.6%) in shiitake cultivation. Atila (2019) emphasized that the ratio of cellulose/lignin increased in the mixtures depending on decreasing in hemicellulose content.

During development of mycelia, biochemical changes occur because of production of extracellular enzymes. These enzymes convert/degrade insoluble and large components of lignocellulosic materials into soluble and low molecular weight compounds that can be taken up by the intracellular enzymes of the mushroom for nutrition. Therefore, enzymes have a significant role in mushroom development (Kuforiji and Fasidi, 2008). It was reported by Ohga and Royse (2001) that laccase and cellulase activities are important in mycelial growth and mushroom development during *L. edodes* cultivation. The highest laccase activity was recorded in A7 (3 corncob + 1 bran) and on the 25th day among periods. In a study carried out by Lechner and Papinutti (2006) on *L. tigrinus*, laccase activity was examined at different periods and it was observed that laccase activity increased during mycelia development, while it decreased rapidly in the following stages and became stable during mushroom formation. Kurt and Büyükalaca (2010) determined that the laccase activity of *P. ostreatus* reached to the highest value on the 10th day of mycelia development and then a gradual decrease occurred until the first harvest. While they obtained the highest laccase activity from wheat stalk-bran (2: 1) mixture as 5.48 U mg^{-1}, they found the lowest value in vine pruning residues as 0.30 U mg^{-1}. The highest laccase activity of *P. sajor-caju* occurred on the 10th day of mycelial development and in sesame stem-bran mixture (2: 1) as 3.85 U mg^{-1}, while the lowest value was determined as 0.30 U mg^{-1} in vine pruning residue. Elisashvili *et al.* (2015) examined the laccase activities of *L. edodes* 3715 and 3721 strains in different growing mixtures. It was found that laccase activity increased with mycelia development in both strains, then started to decrease in

the primordium stage, then a rapid decrease was observed during mushroom development, finally it increased again at the after-harvest period. While they observed that the laccase activity of *L. edodes* 3715 strain at after mycelia development was 9.9 U g^{-1}, it decreased to 8.3 U g^{-1} and 0.7 U g^{-1} at the primordium stage and during mushroom harvest, respectively. In the analysis performed six days after harvest, they found that the laccase activities increased again and reached 8.6 U g^{-1}. The laccase activity of *L. edodes* 3721 strain was 5.3 U g^{-1} at after mycelia development period, then decreased during primordium stage, then ended during harvest and finally increased to 13.6 U g^{-1} in analysis performed 7 days after harvest.

Among growing mixtures, the highest cellulase activity was recorded in A3 (3 wheat stalk + 1 bran) and on the 10th day periods. Ohga and Royse (2001) reported that laccase activity of *L. edodes* cultivated in sawdust-based mixture reached maximum during the mycelia development stage, while it rapidly decreased during the fruiting body stage. In contrast to laccase activity, it was determined that cellulase activity showed a rapid increase during the fruiting body stage period. While a decrease was observed in laccase activity in primordium stage, it was found that cellulase activity increased in the same period. Lechner and Papinutti (2006) examined the cellulase activity of *L. tigrinus* and reported that the cellulase activity showed maximum performance approximately 90 days after inoculation. Kurt and Büyükalaca (2010) recorded the highest cellulase activity in growing mixtures containing bran. They also found that cellulase activity resulted in higher biological efficiency and total yield. When the cellulase activity of A3 mixture having the highest biological efficiency value was examined, similar results were observed in our study. Elisashvili *et al.* (2015) reported that cellulase activity of *L. edodes* decreased with mycelial development in both strains studied, then started to increase in the primordium stage, then a rapid increase was observed during mushroom development, finally decreased at after harvest period.

The fastest mycelia development was observed in A7 (3 corncob + 1 bran) and mycelia development ranged between 22 and 50 days. In the study conducted by Morais *et al.* (2000) and Özçelik and Pekşen (2007) on shiitake mushroom, it was determined that the mycelia development varied between 80 and 90 days and 83 and 59 days, respectively. Sözbir (2014) found that while the fastest mycelia development was obtained with 50 days in 3CK (walnut shell) + M (oak) and CK (walnut shell) + M (oak) mixtures, the slowest mycelia development was detected in T + 3M (oak sawdust) mixture with 95.5 days in *L. edodes*. Elisashvili *et al.* (2015) reported that the completion of mycelia development varies between 24 and 29 days depending on the mushroom strain and growing mixtures. Atila (2019) emphasized that mycelia development in shiitake mushroom ranged between 32.4 and 46.0 days. It was observed that while mixture including alfalfa (AH) was the fastest (32.4 days) and corn stalk (CS) was the slowest (46 days) in terms of mycelia development among five different mixtures tested. Our study results show that the mycelia development time is within the limits specified in the literature or faster. This situation can be explained by the different growing mixture content, mycelia strains used and the controlled climate conditions of the mushroom growing rooms.

The highest biological efficiency (BE) rate was recorded in A3 (3 wheat stalk + 1 bran) and A5 (1 oak sawdust + 1 wheat stalk + 1 bran) and values varied between 19.22% and 93.65%. Diehle and Royse (1986) reported that BE in shiitake mushroom ranged from 6.1% to 124 %. In a study carried out by Philippoussis *et al.* (2003) on *L. edodes*, BE changed among growing mixtures and the highest and lowest BE values were recorded in wheat straw (WS) (54.17%) and oak sawdust (OS) (41.07%)-corncob (CC) (80.64%) mixtures, respectively. Philippoussis *et al.* (2007) obtained similar results. Ashrafuzzaman *et al.* (2009) found that the BE values changed between 79.48% and 101.84%. The highest BE value was obtained from jack fruit chips mixture. Moonmoon *et al.* (2011) reported that the highest BE in shiitake mushroom was recorded in sawdust (SD) (76.6%) mixture including 25% wheat bran (WB). Sharma et al. (2013) determined that the BE ratios of OE-329 and OE-388 strains prepared with 10% wheat bran were higher (46.2% and 66.8%, respectively) than other mixtures. Ranjbar and Olfati (2017) found that BE rate of wheat straw growing mixture (59.32%) was lower in shiitake mushroom compared with oak, maple and fir sawdust. After the additives were supplemented, they found the highest BE (92.35%) in oak mixture.

The highest yield was obtained from A5 (1 oak sawdust + 1 wheat stalk + 1 bran) and yield varied between 55.99 and 299.59 g kg^{-1}. Özçelik and Pekşen (2007) determined that the yield among growing mixtures in shiitake mushroom ranged between 150.77 and 233.92 g kg^{-1}. Ashrafuzzaman *et al.* (2009) reported that the highest yield of shiitake mushroom occurred in jack fruit chips mixture with 332 g, while the lowest yield was obtained from cotton tree mixture with 212.2 g. Annepu *et al.* (2019) observed that the total yield values in shiitake mushroom ranged between 79.55 and 325.40 g. Atila (2019) observed that the highest yield of shiitake mushroom was in sunflower waste (SFH) (233.7 g kg^{-1}) and it was followed by chickpea straw (CPS) (228.1 g kg^{-1}). The lowest yield was determined in corn stalk (CS) (87.9 g kg^{-1}) mixture. In our study, it was determined that yield increased in growing mixtures tested except A9 and A11 which are mixtures including oak sawdust. The reason of low yield obtained from A9 and A11 despite the addition of oak sawdust could be attributed to the C/N amounts. As a result, it has been determined that better results are obtained by mixing oak sawdust, which are commonly used in shiitake mushroom, with other substrates.

The highest mushroom weight was recorded in C (oak sawdust) and values varied between 14.98 and 33.52 g. In a study conducted by Philippoussis *et al.* (2003) on shiitake mushroom, the highest mean mushroom weight was recorded in wheat straw (WS) (22.96 g) and oak sawdust (OS) (23.93 g) mixtures. In another study carried by Philippoussis *et al.* (2007) in *L. edodes*, they determined that the average mushroom weight ranged between 19.01 and 21.40 g. Martínez-Guerrero *et al.* (2012) reported that the average mushroom weight in shiitake mushroom changed between 41 and 70 g. Sözbir (2014) recorded the highest mushroom weight from BK (almond shell) + 3M (oak sawdust) mixture (39.47 g) in *L. edodes*, while the lowest weight was in 3KT + M mixture (10.14 g). Ranjbar and Olfati (2017) found that the highest mushroom weight occurred in mixtures including rice bran with 33.51 g in *L. edodes*. In a study carried out by Annepu *et al.* (2019) on shiitake mushroom, it was determined that the average mushroom weight ranged between 18.29 and 36.58 g. Atila (2019) reported that average mushroom weight varied between 12.9 and 19.4 in *L. edodes*.

The highest cap diameter, stipe diameter and stipe length were obtained from A8 (3 vine pruning waste + 1 bran), A6 (3 peanut shell + 1 bran) and A10 (1 oak sawdust + 1 corncob + 1 bran) mixtures, respectively. Cap diameter, stipe diameter and stipe length values ranged from 45.36 to 61.33 mm, from 8.89 to 27.24 mm and from 21.31 to 49.11 mm, respectively. In a study carried out by Philippoussis *et al.* (2003) on shiitake mushroom, it was determined that mean cap diameter values of the mixtures were in the same statistic group and changed between 6.33 and 6.83 cm. In another study conducted by Philippoussis *et al.* (2007) on shiitake mushroom, average cap diameter values varied between 5.44-5.50 cm. Ashrafuzzaman *et al.* (2009) reported that average cap diameter value changed between 58.4 and 71.1 mm in different mixtures and the highest and lowest stipe diameters were in Jak fruit chips (13.5 mm) and in lead tree (11.2 mm) mixtures, respectively in shiitake mushroom. They also found that while the highest stipe length was recorded in the teak tree (49.8 mm), the lowest stipe length was observed in the magnolia tree (40.5 mm). Moonmoon et al. (2011) found that the average cap diameter value of the mixtures in *L. edodes* varied between 3.4 and 7.9 cm. In their study, while the stipe diameter values ranged from 0.7 to 1.3 cm, the average stipe length value varied between 3.3 and 6.0 cm.

The highest mushroom firmness was determined in A9 (1 oak sawdust + 1 peanut shell + 1 bran) and values varied between 1.77 and 2.34 lb $inch^{2-1}$. Sözbir (2014) obtained the highest mushroom firmess from 3BK (almond shell) + M (oak sawdust) (2.13) mixture in shiitake mushroom, while the lowest firmness value was recorded in BK (almond shell) + M (oak sawdust) and Z (olive pulp) + 3M (oak sawdust) (1.33 kg) mixtures.

The highest dry matter amount was determined in A6 (3 peanut shell + 1 bran) and values changed between 8.59% and 11.65%. Özçelik and Pekşen (2006) reported that the dry matter amounts of shiitake mushroom ranged from 8.25% to 16.66%. Sözbir (2014) found that the dry matter amounts of shiitake mushroom changed between 11.86% and 43.76%. Ranjbar *et al.* (2017) recorded that the dry matter amounts of shiitake mushroom varied between 23.6% and 28.6%.

The highest amount of protein was determined in A5 (1 oak sawdust + 1 wheat stalk + 1 bran) and values varied between 21.25% and 35.39%. Özçelik and Pekşen (2006) determined that the average amount of

protein in shiitake mushroom ranged from 15.60% to 25.72%. Philippoussis *et al.* (2007) reported that the average protein amount of the mixtures in *L. edodes* varied between 11.03% and 18.97%. Ranjbar *et al.* (2017) determined that the average protein amount of shiitake mushroom changed between 12.29% and 15.84%.

Conclusions

Positive results have been obtained in terms of using of agricultural wastes commonly found in Turkey and many regions of the world in the cultivation of *L. edodes*. Many parameters were examined in the study and they were evaluated. Each of these parameters has importance in both practical and scientific terms. However, when the parameters that may be important for the producers are examined, the results can be evaluated as follows: in terms of yield, while the highest result (299.59 g kg^{-1}) was observed in A5 (1 oak sawdust + 1 wheat stalk + 1 bran), the lowest value was recorded in A9 (55.99 g kg^{-1}) (1 oak sawdust + 1 peanut shell + 1 bran), A6 (65.59 g kg^{-1}) (3 peanut shell + 1 bran) and A11 (75.47 g kg^{-1}) (1 oak sawdust + 1 vine pruning waste + 1 bran). In general, wheat stalk and peanut shell seem advantageous and disadvantageous in yield, respectively. The highest results in biological efficiency related to yield are in A3 (93.65%) (3 wheat stalk + 1 bran) and A5 (92.90%) mixtures. The lowest values were recorded in A11 (21.45%), A6 (19.85%) and A9 (19.22%). The mycelia development time, which is a parameter for obtaining earlier product and may also be important for producers, is the fastest in A7 (21.67 days) (3 corncob + 1 bran) and is the slowest in A4 (50 days) (1 oak sawdust + 1 poplar sawdust + 1 bran). Corncob seems to be advantageous in terms of earliness. Considering that mushrooms are prominent in terms of protein content in diet, while the highest results in terms of protein content were recorded in A5 (35.62%) and A7 (35.39%), the lowest values were observed in A10 (26.95%) (1 oak sawdust + 1 corncob + 1 bran), A9 (26.69%) and C (21.25%) (oak sawdust). When these parameters, which will be important for producers, are evaluated together, A5, A7 and A3 mixtures seem advantageous. These mixtures can be recommended to producers.

Authors' Contributions

GB and HT designed and managed experiments. GB, EK and MY cultured shiitake mushroom and carried out most of analyses. GB, NY, EA and AA performed enzyme analysis. GB and HT wrote the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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