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REGULAR ARTICLE

Influence of cultivation methods on the chemical and nutritional characteristics of *Lentinula edodes*

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ABSTRACT

Lentinula edodes (Shiitake) is an edible mushroom with excellent nutritional potential, aroma and flavor. It's traditionally produced on wood logs, but this practice has been replaced by cultivation on axenic substrates (AS) made from different materials that are stored in plastic bags. This papper aimed to evaluate the nutritional composition of *L. edodes* grown on *Quercus acutíssima* (QA) and AS by correlating their chemical composition with the media on which they were grown. Culture media were analyzed for their density, moisture content, ash, extractives, lignin (soluble and insoluble) and holocelulose before *L. edodes* inoculation, as well as after the second consecutive harvest this mushrooms. The mushrooms from the second harvest of each culture media were characterized regarding their moisture content, protein, ash, lipids, dietary fiber (soluble and insoluble), α, β and glucanas total, carbohydrates and minerals. Mushrooms cultivated on AS showed higher protein content, macrominerals and lipids, when compared to mushrooms cultivated on QA. AS initially contained lower lignin content, less holocelulose and a reduced C:N ratio when compared to QA. The results showed that the composition of the culture medium influenced the nutritional composition of *L. edodes* mushrooms.

Keywords: C:N ratio; Glucan; Lignocellulosic substrates; Mineral content; Mushroom production

INTRODUCTION

Edible mushrooms provide high-quality protein and present a higher productivity and biological efficiency than animal protein. They are rich in dietary fiber, minerals and vitamins and have a low lipid content, as well as a high proportion of polyunsaturated fatty acids (Jonathan et al., 2013). As they have a high protein •and a low fat content, mushrooms are excellent foods to be used in low-calorie diets (Ribeiro et al., 2009).

Edible mushroom production in Brazil is still restricted to the southeast and southern regions due to favorable weather conditions. The most cultivated and consumed species are of European and Asian origin, such as *Agaricus bisporus* (Champignon), *Lentinula edodes* (Shiitake) and mushrooms of the *Pleurotus* (Shimeji) genus. Among edible mushrooms species produced worldwide, *L. edodes* (of Asian origin) ranks second, behind Champignon (Gaitán-Hernández et al., 2011).

Commercial interest in *L. edodes* mushrooms has increased in recent years, mainly due to its high value in the international market, which occurred not only because of its considerable nutritional potential and excellent aroma and taste, but also due to its medicinal properties (Gaitán-Hernández, et al., 2011). Like many other mushrooms, *L. edodes* has a high nutritional value and contributes to a healthy diet.

L. edodes is a lignocellulolitic fungus, which provides an enzyme system that allows it to use complex carbon sources such as cellulose, hemicellulose and lignin (Andrade et al., 2013). The chemical composition of mushrooms is directly influenced by the chemical composition of the substrate on which the fungus is grown, which allows the

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accumulation of certain macro- and micronutrients (Bento; Casaril, 2012).

Traditionally, *L. edodes* is grown on tree trunks, of species such as eucalyptus, oak, mango and avocado. *Quercus acutissima* (sawtooth oak or Japanese oak) is native to East Asia, and can be found throughout China. It has a significant ecological and commercial importance, and is commonly grown in North America. *Q. acutissima* provides excellent material for the construction industry and charcoal production, and it can also be used in the cultivation of edible fungi cultivation (Zhang et al., 2013).

Mushroom cultivation utilizing tree logs has recently been replaced by cultivation on substrates prepared under controlled conditions and stored in plastic bags (bag-log cultivation) (Piccinin et al., 2010). The substrates can be made from various forest and/or agro-industrial residues available in an area where mushrooms are grown (Andrade et al., 2013).

In Brazil, the most widely used forest residue is made from the *Eucalyptus* genus, although such tree is not native to Brazil (Andrade et al., 2013). Sugarcane bagasse, corn straw and corncobs are among types of agro-industrial waste that have been gaining ground as substrate material. Furthermore, supplementation of the substrate with wheat bran, rice, soya, oats or corn, improves nutrient availability, leading to better mycelial growth (Gaitán-Hernández et al., 2011; Junior; Paccola-Meirelles, 2010; Regina et al., 2009). A balanced mix of these types of waste is arranged in sterile plastics bags (axenic substrate) to prevent contamination of the culture medium. The main advantages of this method include a shorter duration period for the mushroom cultivation cycle and higher yields (Sánchez, 2004).

The aim of this study was to evaluate the chemical and biochemical composition of *L. edodes* mushrooms grown on *Q. acutíssima* and axenic substrate and to correlate mushroom composition with its growth media.

MATERIAL AND METHODS

Samples

Lentinula edodes mushrooms produced on *Quercus acutissima* (QA) (sawtooth oak) and the logs of this wood, were provided by producers in the city of Campina Grande do Sul, Paraná State, Brazil. Samples of *L. edodes* produced on axenic substrate (AS) were provided by producers in the town of Cornélio Procópio, Paraná State, Brazil. AS was prepared with sawdust (40%), eucalyptus sawdust (43%), wheat bran (12%), maize germ (4%) and limestone (1%). QA and AS were analyzed in two stages: in the absence of fungal inoculum (starting time) and after the second fruiting and harvesting of *L. edodes* (end time). The mushrooms from the second fruiting of the culture media studied were analyzed. All analyzes were performed at the Brazilian Agricultural Research Corporation (Embrapa Forestry) laboratories, located in Colombo, Paraná State, Brazil.

Characterization of growing material of l. Edodes

QA: the basic density was determined according to NBR 11941-02 (ABNT, 2003); the apparent density is obtained from the basic density, considering the moisture content of the wood. Moisture was determined by kiln-drying at 105°C until a constant weight. The fixed mineral residue (ash) was obtained by burning it in a wood oven at 550°C until it reached a constant weight. Wood material extractives were quantified according to NBR 14853 (ABNT, 2010a). Insoluble lignin content was determined according to NBR 7989 (ABNT, 2010b) and the percentage of acid-soluble lignin was obtained from the absorbance measurement in the ultraviolet (205 nm) in a spectrophotometer, according to Dence (1992). The fraction of holocellulose (hemicellulose pulp) was calculated by difference, with 100 g (dm), minus the sum of the remaining constituents measured in dm. Elemental carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) levels were determined using an elementary analyzer equipment CHNS (CHNS Elementar, model Vario MACRO Cube, Langenselbold, Hesse, Germany). The analysis was performed with three replications. The oxygen content was obtained by difference, considering 100 g minus the sum of the other elements (CHNS).

AS: The apparent density, both at baseline and at the end-point, was determined according to Equation 1. The mass of a portion of the AS was obtained on a precision scale and the volume of that portion was measured by an adaptation of the method described by Machado and Pereira (2010).

$$\rho_s = \frac{m_s}{v_s} \quad (1)$$

Where ρ_s is the density, m_s is the mass and v_s is the AS volume. The other analyses in QA were also applied to AS (start and end time).

Calculations for determining the nutrients consumption present in qa and the as by l. Edodes

To determine the biomass loss of both the wood and substrate after 2 cycles of *L. edodes* mushroom fruiting, Equation 2 (Global Mass Balance) was used:

$$M_s = M_e + M_c \quad (2)$$

Where M_s and M_e are the masses, start and end times, the QA or AS, respectively; and M_c is the mass consumed by the mushroom.

The determination of individual consumption of macro and micro components of QA and AS by *L. edodes* mushrooms was performed using Equation 3 (Partial Balance Mass):

$$M_s * x_{ns} = M_e * x_{ne} + M_c * x_{nc} \quad (3)$$

Where x_{ns} and x_{ne} are the fractions "x" nutrient "n" at start and end times, respectively, present in mushroom cultivation materials; x_{nc} is the fraction of the nutrient "n" consumed by *L. edodes* at the end of the second crop cycle in the same QA log or AS.

Characterization of I. Edodes fruiting body cultivated in QA and AS

Fresh, crushed and homogenized mushrooms were used in the analyses, except for determining their lipid content. The samples for lipid determination were lyophilized (at -50°C and 150 mm Hg for 96 hours) due to the high moisture content present in the original product. The moisture content was determined using AOAC, method No. 925.09 (AOAC, 2005). The ash content was quantified using the AOAC method No. 923.03 (AOAC, 2005). The lipid content was determined by the Soxhlet extraction method using ethyl ether as the extraction solvent. The protein content was evaluated by AOAC method No. 920.87 (AOAC, 2005) and the nitrogen converted into protein by multiplying nitrogen by 4.38, as mushrooms have a high proportion of non-protein nitrogen (NPN) compounds, such as chitin (Rashidi; Yang, 2016; Reis et al., 2012). Total dietary fibre was determined as soluble and insoluble fractions according to the enzymatic-gravimetric method using method No. 991.43 (AOAC, 2005). The total carbohydrate content (including dietary fibres), was calculated by difference (i.e., 100 g of the product minus the sum of ash, moisture, crude protein and lipid). All analyses were performed in triplicate and the results expressed in dry matter (dm).

The glucans in the mushrooms were quantified by an enzymatic colorimetric method using an Enzymatic Yeast β-Glucan Assay Kit® (Megazyme® - IDA Business Park, Bray, Co. Wicklow, A98 YV29 Ireland). Total glucans and α-glucans were quantified in triplicate, and the difference was considered as a β-glucan fraction. The results were expressed as milligrams of β-glucan per gram of dm.

Mineral content was quantified following instructions set out by Silva (1999), Sarruge and Haag (1974) and Nogueira and Souza (2005). After nitro-perchloric digestion, macronutrients sodium (Na) and potassium (K) were determined in a spectrophotometer flame. Calcium (Ca) and magnesium (Mg) were quantified with the aid of atomic absorption spectroscopy. Phosphorus (P) was determined in a spectrophotometer UV/VIS, with a wavelength equal to 660 nm. Micronutrients, iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were determined by atomic absorption spectroscopy. Two replicates were performed for each mineral analysis.

Statistical analysis

Analysis of variance (ANOVA), Student's T-test and Tukey's test were performed using the software Statistica version 8.0 with a significance level of $p \leq 0.05$. These statistical tools were used for separating the mean values of the analysed parameters.

RESULTS AND DISCUSSION

The physicochemical characterization results of QA wood and AS at initial (without inoculum *L. edodes*) and final (after the second harvest of *L. edodes*) times, used in the mushroom production, are shown in Tables 1-2 and Figure 1. Table 1 presents the results of analyses carried out in wood, bark and AS. Table 2 was prepared by performing global and partial mass balance (Equations 2 and 3) for all compounds analyzed in 1.00 m³ of material.

The apparent density of the wood, bark and AS decreased significantly after two mushroom production cycles of

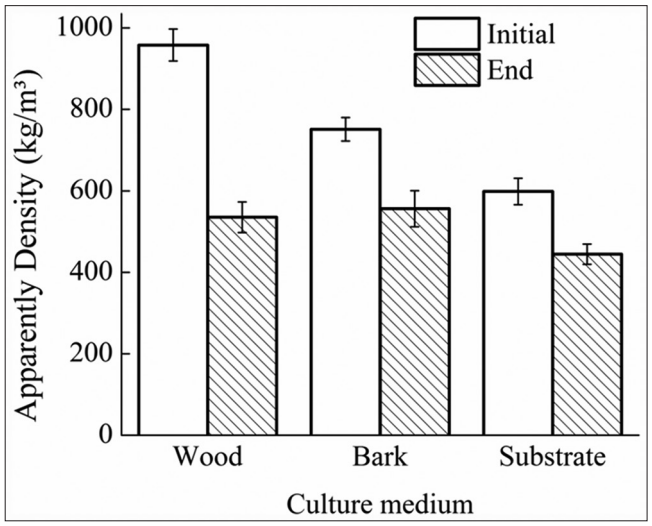

Fig 1. Apparent density of the wood and *Quercus acutíssima* bark and axenic substrate at initial (without inoculum of *L. edodes*) and final time (after second crop of *L. edodes*).

Table 1: Physical–chemical and elementary characterization of *Quercus acutíssima* (QA) and axenic substrate (AS)

Compounds	Wood (QA)		Bark (QA)		AS	
	Start time	End time	Start time	End time	Start time	End time
Moisture (% fm)	28.87±0.29^{c}	26.73±0.46^{c}	18.93±0.14^{e}	22.29±0.15^{d}	63.14±0.72^{a}	60.31±3.24^{b}
Ashes (% dm)	0.71^{d}±0.01	0.63^{d}±0.03	2.42^{c}±0.03	2.20^{c}±0.03	4.93^{a}±0.01	4.11^{b}±0.11
Extractives (%dm)	1.53^{de}±0.11	1.04^{e}±0.01	2.63^{c}±0.09	1.87^{cd}±0.09	5.13^{a}±0.24	3.98^{b}±0.32
Lignin (% dm)						
Insoluble	31.35^{c}±0.36	28.52^{d}±1.07	38.57^{c}±0.01	33.76^{b}±1.40	28.74^{d}±0.17	25.83^{e}±0.15
Soluble	1.88^{c}±0.04	1.87^{c}±0.02	2.05^{b}±0.21	1.72^{d}±0.09	1.89^{c}±0.01	2.23^{a}±0.03
Total	33.23^{c}±0.40	30.39^{e}±1.09	40.62^{a}±0.22	35.48^{b}±1.49	30.63^{d}±0.18	28.06^{f}±0.18
Holocelulose (% dm)	64.53^{b}±0.52	67.94^{a}±1.13	54.70^{f}±0.34	60.45^{d}±1.59	59.31^{e}±0.43	63.85^{c}±0.61
Carbon (C) (% dm)	45.92^{c}±0.09	45.91^{c}±0.10	47.91^{a}±0.04	43.02^{e}±0.01	47.11^{b}±0.09	45.38^{d}±0.06
Oxygen (O) (% dm)	46.55^{c}±0.13	46.88^{b}±0.13	45.08^{d}±0.10	50.38^{a}±0.10	44.67^{e}±0.08	46.81^{b}±0.12
Hydrogen (H) (% dm)	7.24^{a}±0.14	6.89^{b}±0.04	6.52^{c}±0.05	6.27^{d}±0.08	7.41^{a}±0.01	7.00^{b}±0.05
Nitrogen (N) (% dm)	0.24^{d}±0.01	0.32^{c}±0.01	0.48^{b}±0.02	0.32^{c}±0.01	0.76^{a}±0.03	0.75^{a}±0.01
Sulfur (S) (% dm)	0.05^{a}±0.01	N.D.	0.01^{b}±0.00	0.01^{b}±0.01	0.05^{a}±0.01	0.06^{a}±0.00
Ratio C/N (% dm)	191.33	143.47	99.81	134.44	61.99	60.51

Results expressed as mean±standard deviation. Means followed by the same letter in the line are not differ significantly by Tukey test ($p \leq 0.05$). fm = fresh matter. dm – dry matter. N.D. - Not detected.

Table 2: Global and partial mass balance of *Quercus acutíssima* (QA) constituents and axenic substrate (AS): start (without inoculum *L. edodes* materials) and end time (resulting material after the second consecutive production of *L. edodes*)

Compounds	QA Wood (kg/m^3)			QA Bark (kg/m^3)			AS (kg/m^3)		
	Start time	End time	Consumed	Start time	End time	Consumed	Start time	End time	Consumed
Water	276.56±3.30	143.03±1.12	133.53±2.52	142.22±1.72	123.97±0.95	18.25±1.33	377.85±2.55	268.08±2.09	109.77±2.42
Dry matter	681.40±12.22	392.06±10.61	289.34±10.83	609.07±11.79	432.20±10.41	176.87±10.20	220.58±9.53	176.43±7.84	44.15±7.11
Ashes	4.87±0.07	2.48±0.12	2.38±0.19	12.48±0.17	9.50±0.21	2.98±0.38	10.87±0.03	7.24±0.02	3.63±0.05
Extractives	10.42±0.75	4.06±0.06	6.36±0.81	15.99±0.52	8.06±0.20	7.92±0.72	11.31±0.53	7.02±0.56	4.29±1.09
Lignin									
Insoluble	313.59±2.44	111.81±4.18	101.78±6.62	234.93±0.05	145.91±6.05	89.02±6.10	63.40±0.37	45.57±0.26	17.83±0.63
Soluble	12.78±0.30	7.34±0.06	5.44±0.36	12.52±1.27	7.43±0.37	5.08±1.64	4.17±0.02	3.94±0.05	0.23±0.07
Total	226.37±2.74	119.15±4.24	107.22±6.98	247.45±1.32	153.35±6.42	94.10±7.74	67.56±0.39	49.50±0.31	18.06±0.70
Holocelulose	439.71±3.56	392.06±4.42	173.34±7.98	333.16±2.01	261.26±6.83	71.90±8.84	130.83±0.97	112.65±0.89	18.18±1.86
Carbon (C)	312.90±0.58	179.99±0.41	132.90±0.99	291.77±0.22	185.93±0.04	105.84±0.26	103.92±0.20	80.06±0.10	23.82±0.30
Oxygen (O)	317.16±0.62	183.79±0.50	133.37±1.12	274.64±0.62	217.74±0.39	56.85±1.01	98.54±0.17	82.59±0.08	15.95±0.25
Hydrogen (H)	49.33±0.94	27.03±0.14	22.30±1.08	39.74±0.31	27.09±0.35	12.65±0.66	16.34±0.02	12.35±0.09	3.99±0.11
Nitrogen (N)	1.66±0.08	1.24±0.05	0.42±0.13	2.92±0.12	1.38±0.04	1.54±0.16	1.67±0.06	1.32±0.02	0.35±0.08
Sulfur (S)	0.36±0.06	N.D.	0.36±0.06	0.05±0.01	N.D.	0.05±0.01	0.12±0.02	0.10±0.01	0.02±0.03

Results expressed as mean±standard deviation. N.D. = not detected.

42.65%, 29.51% and 25.77%, respectively (Figure 1). This result is consistent with the characteristics of the *L. edodes* mushroom, since this lignocellulolitic fungus has the ability of producing enzymes that degrade lignin, cellulose and hemicellulose, converting them into sugars that are used for its growth (Gomes-da-Costa et al., 2008). Thus, the organic material was consumed by the mycelia of *L. edodes* and transformed into its fruiting body.

Levels of moisture and dry organic matter levels of the wood decreased, both QA bark and in AS, after two consecutive production cycles of *L. edodes*, as show in Table 1. It was determined by mass balance. The moisture in the wood, bark and AS was reduced by 48.28%, 12.83% and 29.05%, respectively. The dry matter reduction was equivalent to 42.46%, 29.04% and 20.02% in wood, bark and AS, respectively (Table 1). Both the moisture and dry matter reduction in *L. edodes* culture media indicated that the mushrooms used liquid and solid compounds for their development.

The ash content of the wood and bark at the start and end times were not significantly different (Table 1), indicating that the proportionality of minerals, organic matter and water along the *L. edodes* cultivation were maintained. Conversely, the ash content for the AS was different at the initial and end times. *L. edodes* cultivation using this method caused a concentration of dry matter and minerals due to their lower level of consumption when compared to QA wood and bark.

Wood and QA bark extractives (1.04 to 2.63%) presented were similar to those obtained by Silvério et al. (2006) whose research studied eucalyptus woods. The extractives

refer to substances of low or medium molecular weight, such as fatty acids and esters, long chain alcohols, steroids, phenolics and glycosides that can be solubilized in organic solvents (Silvério et al., 2006). Extractives levels were higher in AS (5.23 and 3.98%) because AS is composed of eucalyptus wood, also containing wheat bran and corn germ, which increase lipophilic compound level that can be extracted with the solvents used (toluene:ethanol) in this research.

Total lignin decreased by 47.36%, 48.03% and 26.73% for wood, bark and AS, respectively (Table 2). Following the same pattern, holocelulose (cellulose + hemicellulose) also decreased after the second mushroom production cycle by 10.84% 21.58% and 13.90%. These polymers were hydrolyzed by enzymes produced by *L. edodes* as laccases, manganese peroxidase and lignin peroxidase (Regina et al., 2008), and converted into nutrients used for mushroom growth (Gomes-da-Costa et al., 2008).

The elemental composition (CHNSO) of the QA studied in this paper (Table 1) was similar to the composition of the same wood species studied by Lee et al. (2008). Table 2 shows that all the elementary constituents (CHNSO) exhibited weight reduction in both wood as AS, after the second production of *L. edodes*. This result confirms what the other analyses showed: reduction in macromolecule mass, such as holocelulose, lignin and extractives.

The nitrogen content initially present in AS was about 68.42% higher than the one found in the wood and 36.84% QA bark (Table 1). When calculating the C:N ratio of the initial culture media, we found that this ratio is 3 times higher for wood and 2.3 times higher for the QA bark than for AS. The high C:N correlation in QA is normal according to specifications of the United States Department of Agriculture (USDA, 2017) for the QA species. Andrade et al. (2011) point out that this ratio is also high for eucalyptus, about 200:1. Possibly the greatest bioavailability of N in AS renders this compound easier to be incorporated by *L. edodes*, resulting in an increased protein amount in the mushroom cultivated in AS, when compared to the same species cultivated in QA (Table 3). Özçelik and Peksen (2007) showed that the growth rate and biological efficiency of *L. edodes* is related to nitrogen bioavailability. They also mention that nitrogen has been recognized as a limiting factor to mushroom growth.

The fresh *L. edodes*, produced in *Quercus acutíssima* (QA) and axenic substrate (AS) showed significant differences ($p \leq 0.05$) in its moisture average percentage, quantified at 87.57% and 90.29%, respectively. Possibly the greatest water content in AS (Table 1) provided more moisture in mushrooms produced according to this method.

Table 3: Chemical and mineral composition of *L. edodes* produced in *Quercus acutíssima* (QA) and axenic substrate (AS)

Analysis	*L. edodes* (QA)	*L. edodes* (AS)
Proteins (% dm)	14.45^{b}±0.14	18.00^{a}±0.58
Lipids (% dm)	2.08^{b}±0.16	2.82^{a}±0.29
Ashes (% dm)	7.64^{a}±0.04	7.38^{b}±0.08
Total carbohydrates (% dm)	75.83^{a}±2.66	71.80^{b}±5.40
Dietary fiber (% dm)		
Insoluble	40.97^{a}±1.85	32.49^{b}±3.61
Soluble	6.45^{a}±0.47	6.67^{a}±0.84
Total	47.42^{a}±2.32	39.16^{b}±4.45
Glucans (mg/g dm)		
α-glucan	4.45^{a}±0.09	1.14^{b}±0.02
β-glucan	21.82^{a}±0.21	16.64^{b}±0.36
Total glucan	26.28^{a}±0.28	17.78^{b}±0.36
Macrominerals (mg/100g dm)		
Phosphorus	330.00^{b}±2.83	774.00^{a}±19.80
Potassium	1950.00^{a}±98.99	2050.00^{a}±155.56
Calcium	12.50^{a}±0.19	16.30^{a}±1.77
Magnesium	108.80^{b}±1.77	155.00^{a}±3.54
Microminerals (mg/100g dm)		
Iron	4.20^{a}±0.03	3.40^{a}±0.28
Manganese	3.00^{a}±0.57	1.90^{a}±0.28
Copper	0.45^{a}±0.07	0.55^{a}±0.07
Zinc	3.65^{b}±0.07	8.00^{a}±0.04
Sodium	4.00^{b}±1.41	10.50^{a}±0.71

Results expressed as mean±standard deviation. Means followed by the same letter in the line are not differ significantly by Student's T-test ($p \leq 0.05$). dm = dry matter.

The chemical and mineral composition of the mushrooms is presented in Table 3. The main components of the mushroom fruit bodies are total carbohydrates, dietary fiber (soluble and insoluble) and proteins.

Total dietary fiber (insoluble and soluble) appeared high, both for mushrooms produced on QA (46.74%) and the one grown on AS (39.17%). Reguła and Siwulski (2007) analyzed *L. edodes* grown on AS and obtained similar results for total dietary fiber content. According to Vetter (2007), chitin is a polysaccharide, which presents the main fraction of insoluble dietary fiber, in addition to cellulose, some hemicelluloses and lignin. The soluble fiber fraction is composed of β-glucans, gums, proteins and some hemicellulose (Finimundy et al., 2014). It is worth mentioning that the consumption of foods with high fiber content presented benefits to the individual's health by reducing the incidence of diseases due to the increased volume of feces and reduced intestinal transit time. The consumption of foods with high fiber content stimulates intestinal flora development and reduces fat and sugar absorption, thus reducing the level of blood cholesterol and glucose levels (Dhingra et al., 2012).

According to Rop, Mlcek and Jurikova (2009), fiber content variation may be related mainly to genetic factors, which

determine the amount and type of saccharides present in fungal cell walls. However, as the analyzed mushrooms belong to the same species (*L. edodes*), it is believed that the higher insoluble fiber content of the *L. edodes* produced in QA is a result of less favorable conditions in obtaining nutrients. QA wood structure is more resistant than the compounds that form SA (sawdust and eucalyptus brush, wheat bran, corn germ and limestone). Thus, *L. edodes* produced on QA would have undergone more demands regarding its resistance, production and enzyme secretion (laccase, manganese peroxidase and lignin peroxidase) to obtain nutrients (Chen, 2005; Regina et al., 2009) when compared to *L. edodes* produced on AS, where nutrients were more readily available for absorption.

As observed in dietary fiber content, the glucan content was higher in the *L. edodes* mushroom produced in QA. β-glucan content ranged from 16.64 to 21.82 mg g^{-1}. The β-glucans make up about 50% of the cell wall of fungi and their concentration varies according to species, growth conditions and maturity of the mushroom fruit body. The genus *L. edodes* and *Pleurotus spp*. are the most important sources of β-glucans and Lentinan is the active substance in *L. edodes* (Bach et al., 2017). The active β-glucan compounds help in treating several chronic diseases, and it presents antioxidant, anti-inflammatory and hipocholesterolemic activities, radioprotective effects, antitumor potential, immunopotentiation, among others (Bach et al., 2017; Maity et al., 2014; Pillai; Devi, 2013; Ren et al., 2012; Silveira et al., 2014).

The declaration of the carbohydrate content of the same food may vary widely, because it can be expressed as the sum of its total carbohydrates (mono, di, oligo and polysaccharides), or simply the sum of its simplest compounds, when dietary or crude fiber analysis is performed separately. In our research, we presented the total carbohydrate content (including dietary fiber) presented by *L. edodes* mushrooms. The carbohydrate content was significantly ($p \leq 0.05$) different among the mushrooms analyzed. The *L. edodes* produced in QA had a higher content (75.83%) of total carbohydrates when compared to the mushroom produced in AS (71.80%). Ulziijargal and Mau (2011) reported total a carbohydrate content ranging from 47.61 to 91.77% in some mushrooms.

The protein content of edible mushrooms is expressive and important to the food's nutritional quality. The mushrooms studied here showed levels of 14.45% (dm) when grown on QA and 18.00% (dm) when grown in AS (Table 3). These levels differ statistically ($p \leq 0.05$). This difference in protein content may be related to the mushroom's stage of development or availability of nitrogen present in the culture medium (Cohen et al., 2014) considering that they belong to the same species (*L. edodes*) were harvested at the same time of year and are the products obtained from the second fruiting held in QA and the AS.

Both mushrooms contained low lipid levels: 2.08% and 2.82% (dm). These values are similar to those reported in the literature for this kind of mushroom (1.70% to 2.89%) (Reis et al., 2012; Wang et al., 2014). The low fat content of mushrooms is a relevant factor for the nutritional aspect of this food, given that the caloric value remains below many products with the same protein content.

The mineral content (ash) of the *L. edodes* produced in QA and AS was 7.64% and 7.38% (dm), respectively. Gaitán-Hernández and Mata (2004) quantified 7.49% ash in sample *L. edodes* grown in AS. The potassium content was high for both mushrooms. The *L. edodes* cultivated in QA showed a higher iron and manganese content, whereas *L. edodes* produced in AS had higher levels for phosphorus, potassium, calcium, magnesium, copper, zinc and sodium. The higher amount of minerals of mushrooms produced in the AS may be associated with the bioavailability of these components in the culture medium, considering that the AS besides being a woody material, is also composed of wheat bran and corn germ. Wheat bran and corn germ contain all minerals (El-Sharnouby et al., 2012) that are forming part of the composition of the studied fungi. Gaitán-Hernández and Mata (2004) quantified the minerals content of *L. edodes* produced in wheat straw and they found Mg, Ca, Na, Cu, Fe, Zn, slightly higher than those quantified in mushrooms studied in this research. On the other hand, Mallikarjuna et al. (2013) analyzed *L. edodes* cultivated in a sawdust substrate and found higher Na, Ca, Zn and Cu levels than the ones quantified in this study.

CONCLUSION

This study analyzed the chemical composition *L. edodes* fruiting body produced by two cultivation methods and showed that the physicochemical composition of the culture medium influences the mushroom's nutritional composition. The moisture, protein, lipids, minerals and insoluble dietary fiber, of *L. edodes* cultivated in *Quercus acutíssima* (QA) and axenic substrate (AS) were statistically different. The mushrooms grown in AS presented higher moisture than those grown on QA, and this feature was also observed in culture media, indicating a positive correlation between this variable and the production method. An inverse correlation was found between the C:N ratio of the selected cultivation method and the protein content of *L. edodes*. The density of QA and AS decreased due to the consumption of substrate constituents, such as lignin, holocelulose (cellulose + hemicellulose), extractives and

minerals. Organic matter consumption of the culture medium was confirmed by global and partial mass balance and elementary components analysis (CNHSO).

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Author's contributions

Bach, F. collected the test data, interpreted the results and drafted the manuscript. Lima, E. A. de. assisted in conducting the experiments and in the results discussion. Bellettini, M. B. analyzed the results and corrected the english text. Helm, C. V. and Haminiuk, C. W. I. guide the research, results analysis and the paper writing.

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