



RESEARCH ARTICLE

NUTRITIONAL AND ENZYMATIC POTENTIAL OF THE *LENTINULA EDODES* STRAINS

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ABSTRACT

The species *Lentinula edodes*, also known as shiitake, presents marked utilization of cuisine and is one of the mushrooms most consumed in the world, due to the several functional properties such as anti-tumoral and hypocholesterolemic as well as its anti-microbial and antioxidant action. Nevertheless, its nutritional value is variable according to the strain, processing after harvest, developmental stage of the basidiome and of the growing substrate. Thus, the present work intended to evaluate the centesimal composition, antioxidant activity and lectin content of six strains of *Lentinula edodes* grown on substrate containing meals and sawdust and also to determine the enzymes present in the fruiting bodies (or fruit bodies) after harvest which can influence the browning and senescence of the mushroom. In the experiment, six strains of *Lentinula edodes* (LE1, LE2, LE3, LE4, LE5 e LE6) were utilized and the centesimal composition, antioxidant activity (DPPH), tyrosinase activity and laccase activity of the samples were evaluated. Strain LE6 stood out nutritionally, nevertheless, as that strain presented greater laccase activity, it may be subjected to a faster browning of mushrooms. The antioxidant activity of strain LE4 was greater, so, those two lines can be selected for future genetic breeding program.

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INTRODUCTION

Mushrooms are much appreciated foods since the Ancient Ages, for they believed in their high nutritive value and in its medicinal potential, in addition to being classified as a noble spice in cuisine dishes (Furlany and Godoy, 2007). It is noticed that, in Brazil, there has been an increase in the consumption of mushrooms and, consequently, in the production and commercialization of such a product. That fact occurs mainly for the greatest dissemination of its nutritive and medicinal value. In the latest years, the interest in human health has risen and the nutrition and prevention of diseases has increased consumers' demand for functional foods. Under this context, mushrooms have stood out due to their low content of fat, energy and high dietary fiber content and functional compounds (Hung et al., 2012). Numerous mushroom-synthesized molecules are known for being bioactive, including polysaccharides, glycoproteins, terpenoids and

lectins (Santos-Neves et al., 2008). In addition to the nutritional properties, it was pointed out that mushrooms possess medicinal properties such as anti-inflammatory, anti-tumor, antibacterial and antioxidant (García-Lafuente et al., 2010; Patel and Goyal, 2012; Valverde et al., 2015). Besides, they show antiallergic, blood glucose-reducing and hematological properties and are involved in immunomodulatory therapy (Guillamón et al., 2010). The species under study *Lentinula edodes* (Berk.) Pegler, also known as shiitake, is one of the mushrooms most consumed in the world, owing to its several functional properties such as anti-tumoral and hypocholesterolemic as well as antimicrobial and antioxidant action (Kitzberger et al., 2007). The shiitake mushroom is highly nutritive, with low calories and high amounts of vitamins, proteins and minerals and contain some elements essential to human nutrition such as calcium, copper, phosphorus, manganese, magnesium and zinc (Emery, 2005; Kalač, 2013). But very little is known about the quality of the edible mushrooms grown in Brazil, especially concerning their nutritional value. Even in the international literature, the data found are scarce and are concerned with mushrooms cultivated

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under conditions different from those found in Brazil (Furlany and Godoy, 2007). Information about the composition of foods has become more and more important to evaluate their quality. Before that picture, the need for a survey which demonstrates the real nutritional value of edible mushrooms appears. Then, the present work aimed to evaluate the centesimal composition, antioxidant activity and lectin content of six strains of *Lentinula edodes* cultivated on substrate containing meals and sawdust and also to determine the enzymes present in the fruiting bodies after harvest which can influence the browning and senescence of the mushroom.

MATERIALS AND METHODS

Microorganisms

In the experiment, six strains of *Lentinula edodes* (LE1, LE2, LE3, LE4, LE5 and LE6) belonging to the collection of the Edible Mushroom Laboratory at the Federal University of Lavras (UFLA) were utilized. The stock cultures were kept in a Agar Malt medium (15.0 g L⁻¹ of glucose; 15.0 g L⁻¹ of malt extract; 15.0 g L⁻¹ of agar) and incubated at 25°C.

Spawn Preparation

The spawn was prepared by utilizing sawdust (80%), wheat meal (20%), limestone (2%) and gypsum (2%). The moisture was adjusted to 60% and 170g of that substrate were distributed in glass flasks. The flasks were autoclaved three times for 2 hours with a 24-hour interval. After inoculation, the flasks were incubated at room temperature (25 ± 2 °C) till the substrate was completely colonized by the fungus.

Axenic cultivation of the strains

The substrate for axenic cultivation of the strains of *Lentinula edodes* was prepared by utilizing sawdust (25 Kg); rice bran (2 Kg); wheat meal (0.3 Kg); cottonseed meal (0.3 Kg); coarse cornmeal (0.3 Kg) and calcium carbonate (0.06 Kg). The moisture was adjusted to 60% and 1 Kg of substrate was distributed in high density polyethylene bags. Two autoclaving processes of 2 hours with a 24-hour were carried out. The inoculation of the strains was done by utilizing 3% of spawn and the inoculated substrates were incubated at room temperature (25 ± 2 °C) for 90 days.

Induction of fruiting and harvest of *Lentinula edodes*

At 90 days of growth, the induction of fruiting was done. The blocks were removed from the bags and placed into the growing room at 18 ± 2 °C and humidity of 89 ± 5%. The harvest was conducted for 99 days, considering all production cycles. The second production cycle was started after 36 days, when the blocks were soaked in water at the temperature of 14 ± 2 °C for 12 hours, in order to replace water and to induce fruiting. After that, the blocks were put back into the mushroom growing room and a new harvest period of 33 days was carried out. After that period, a third production cycle was started, following the same procedure as described before, with 30 days' duration.

Analysis of the centesimal composition

For the determination of ether extract, the continuous extraction method was utilized by using ethylic ether as a solvent. The fixed mineral residue (ashes) was determined by ashing the sample in a muffle oven at 550 °C till the obtaining of light-colored ashes. The value of crude protein was obtained by the micro-Kjeldahl method through nitrogen determination. The fiber fraction was determined according to the gravimetric method after acidic medium digestion. The glucidic fraction was obtained by difference of 100% of the sum of the other components (AOAC, 2005).

Determination of the antioxidant activity by the DPPH method

DPPHs are widely utilized as substrates for the evaluation of the capacity of eliminating free radicals. The method is based upon the reduction of the methanolic solution of DPPH• in the non-radical form DPPH-H in the presence of antioxidant hydrogen (Li *et al.*, 2007). The quantitative evaluation of the antioxidant activity was done by monitoring the consumption of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) by the samples through the measure of the decrease of the absorbance of solutions of different concentrations. The absorbance measures were done in a spectrophotometer at 515 nm, having as positive controls 3,5-Di-tert-butyl-4-hidroxytoluene and L-ascorbic acid (Brand-Williams *et al.*, 1995).

Preparation of crude protein extract

The crude extract for lectin analyses was prepared according to Vetchinkina *et al.* (2008a), with modifications. The mushrooms were sliced, macerated and extracted for 2h with 20 mL of Tris-HCl 20 mmol L⁻¹ buffer (pH 8.0) for each 200 mg of mushroom, centrifuged and filtered. The obtained solution was utilized in hemagglutination assays. For analyses of tyrosinase and ligninases, the crude extract was prepared according to Luz *et al.* (2012). The mushrooms were sliced and 20 g were placed in an Erlenmeyer (250 mL) with com 100 mL of sodium citrate buffer (50 mmol L⁻¹, pH 4.8) and shaken at 150 rpm for 2 h. The crude enzymatic extract was obtained by filtering after separation of the residues by utilizing filter paper (Whatman n. 1).

Hemagglutination assay

Hemagglutination was carried out to verify the lectin activity. The assay was done by a serial dilution of the sample using the TOXO-HAI Gold Analisa kit (BH-MG, Brazil) which contains the diluent and a suspension of 2% avian erythrocytes. The activity was expressed in terms of mass percentages (T), which represents the inverse of the greatest dilution of the sample which caused detectable hemagglutination after 2 hours at room temperature (Tsivileva *et al.*, 2001).

Enzymatic assays

Tyrosinase (E.C.1.14.18.1)

The tyrosinase activity was determined by the rate of oxidation of L-dihydroxyphenylalanine (L-DOPA) 2mmol L⁻¹ in Tris-HCl 50 mmol L⁻¹ buffer (pH 7.5). The oxidation of L-DOPA to

DOPA quinone was measured by the increase of absorbance at 475 nm (ϵ_{475} 3700 M⁻¹cm⁻¹) (Pomerantz and Murthy, 1974). A unit of enzyme activity was defined as the amount of enzyme capable of oxidizing 1 μ mol of L-dihydroxyphenylalanine (L-DOPA) per minute, per kg.

Laccase (EC 1.10.3.2)

Laccase activity was determined by indirect spectrophotometric method by utilizing 2,2-azino-bis ethylbenzothiazoline (ABTS) in reaction mixture of 1mL containing 0.3 mL of sodium acetate 0.1 mol L⁻¹ buffer (pH 5,0); 0.1 mL of ABTS 1 mmol L⁻¹ (in water) and 0.6 mL of the enzymatic source. The reaction mixture was incubated for 10 minutes at 37°C and the ABTS oxidation was measured by the increase of absorbance at 420 nm (Buswell *et al.*, 1995). A unit of enzymatic activity was defined as the amount of enzyme capable of oxidizing 1 μ mol of ABST per minute (ϵ_{420} = 3.6 x 10⁴ mol⁻¹ L cm⁻¹), por kg.

Statistics

In the experiment of mushroom growing, the completely randomized design (DIC) with factorial scheme of 10 x 6 (10 replications and 6 strains) was used. The chemical and enzymatic analyses were done in triplicate and the means of each variable analyzed were compared statistically by the Tukey test at 5% of probability.

RESULTS AND DISCUSSION

Centesimal Composition

The chemical composition ranged among the strains of *Lentinula edodes* (Table 1). The greatest contents of moisture were presented by strains LE3 and LE4, while the strain LE5 presented the lowest content. The short shelf-life of mushrooms is ascribed to the high water content they contain. The moisture content varies among the mushroom species; *Lentinula edodes* showed a smaller moisture content as compared with *Agaricus bisporus*, *Pleurotus ostreatus*, *Pleurotus eryngii* and *Flammulina velutipes*. Strain LE1 presented the greatest lipid content. Reis *et al.* (2012) determined the nutritional value of five mushroom species and among the evaluated species, *Lentinula edodes* presented the greatest lipid content, nevertheless, the lipid content described by those authors for this species was only compatible with the content determined for strain LE5. Andrade *et al.* (2008) reported lipid contents of strains of *Lentinula edodes* varying from 2 to 3%. Furlani e Godoy (2007) reported a lipid content for *Lentinula edodes* close to that of *Pleurotus ostreatus* and smaller than in *Agaricus bisporus*. In mushrooms, the fat content is very low as compared with both carbohydrates and proteins (Wani *et al.*, 2010).

As to the contents of proteins and ashes, strains LE2, LE4 and LE6 presented the highest contents. The ashes furnish an indication of the richness of the sample in mineral elements. The main constituents in the ashes is potassium and depending on the mushroom, phosphorus, magnesium, in addition to calcium, copper, iron and zinc (Guillamón *et al.*, 2010). According to Carneiro *et al.* (2013), the contents of lipids,

proteins and ashes are greater in *Agaricus blazei* than in *Lentinula edodes*. The value of ashes reported by those authors for *Lentinula edodes* is close to that described in this work, but the protein content described in the present work is higher than that reported by those authors. The results of proteins and ashes reported in this work are close to the ones reported by Andrade *et al.* (2008). Proteins are important constituents of mushrooms which normally contain between 19 and 35% of protein as compared with 7.3% in rice, 12.7% in wheat, 38.1% in soybean and 9.4% in corn (Wani *et al.*, 2010). Strain LE5 presented the highest carbohydrate content. It is possible to observe that, in the composition of all the strains, carbohydrates are the main nutritional constituents. According to Wani *et al.* (2010), the carbohydrate content represents the majority of the fruiting body occurring between 50 and 65% on a dry matter basis. The carbohydrate content of *Lentinula edodes* according to Furlani and Godoy (2007) is greater than that of *Agaricus bisporus*. Carneiro *et al.* (2013) reported a carbohydrate content in *Lentinula edodes* higher than the maximum content described in that work. The fiber content was greater for strain LE6. Furlani and Godoy (2007) reported a fiber content for *Lentinula edodes* far higher than the contents reported in this work. Those authors showed that the fiber content of *Lentinula edodes* is about equal to that found in *Pleurotus ostreatus* and higher than that found in *Agaricus bisporus*. Fibers in mushrooms comprehend predominately water-insoluble fibers, chiefly chitin and β -glucans with low levels of water-soluble fibers (less than 10%) (Cheung, 2010).

Antioxidant activity

The antioxidant capacity, based upon the DPPH•free radical scavenging method was measured in the different strains of *Lentinula edodes*. The results showed that all the strains had capacity of eliminating the DPPH• radical, that is, presented antioxidant activity, but this antioxidant potential was different among the strains, as illustrates Table 2. Those results corroborate with those of other studies which stressed an antioxidant potential in *Lentinula edodes* mushrooms.

Lectin activity

The strains of *Lentinula edodes* presented different significant lectin contents as Figure 1 illustrates. The LE1 strain presented the lowest content while LE3 and LE6 strains showed the highest lectin content. Tsivileva *et al.* (2001) reported lectin activity in strains of *Lentinula edodes* varying from 4 to 128 UH 50 μ L⁻¹ and found as the fruiting body ripened the lectin activity decreased. In addition to the polysaccharide fraction which is extensively studied, bioactive proteins constitute the second most plentiful functional component in mushroom. Mushrooms produce a great number of proteins with biological activities including lectins (Xu *et al.*, 2011).

Lectins are proteins which can link to the cell surface carbohydrates with a capacity of producing cell agglutination and which show several antiproliferative and anti-tumoral activities against tumoral cells (Zhang *et al.*, 2009; Zhang *et al.*, 2010; 2010b). Lectins are found in several mushroom species, including *Agaricus bisporus*, *Pleurotus ostreatus*, *Tricholoma mongolicum*, *Agaricus subrufescens* and *Grifola frondosa* (Wang *et al.* 1998).

Enzyme activity

The laccase enzyme is one of the proteins produced by mushrooms which hold bioactive properties as immunomodulatory, antitumor and antimicrobial activity (Li *et al.*, 2010b; Zhang *et al.*, 2010b). The strains of *Lentinula edodes* produced that enzyme and the greatest activity was detected for strain LE6 (Table 3). Although, that enzyme possesses a great deal of beneficial qualities, after harvest of the mushroom, an increase in the laccase activity could cause its browning, resulting in a short shelf life, since the mushroom appearance becomes disagreeable (Ye *et al.*, 2012). Nagai *et al.* (2003) showed that the laccase activity increased proportionately to the mushroom browning of *Lentinula edodes*, showing the involvement of this enzyme in the post-harvest browning process. The increase of the tyrosinase activity is also responsible for the post-harvest browning process (Sato *et al.*, 2009). However, no tyrosinase activity was detected or any strain of *Lentinula edodes*, so this enzyme activity cannot be related to browning process in *Lentinula edodes*.

Conclusion

Strain LE6 stood out for the lowest moisture content, high content of ashes, proteins, fibers and lectin, while LE4 showed the highest antioxidant activity.

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