Saudi Journal of Biological Sciences (2016) **23**, 621–627

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Nutritional value and proteases of *Lentinus citrinus* produced by solid state fermentation of lignocellulosic waste from tropical region

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Received 13 March 2015; revised 1 July 2015; accepted 22 July 2015
Available online 30 July 2015

KEYWORDS

Edible mushroom;
Basidiomata;
Nutritional value;
Proteases;
Solid state fermentation

Abstract This paper examined the growth and yield performance of *Lentinus citrinus* on cupuaçu exocarp (*Theobroma grandiflorum*) mixed with litter (CE + LI) or rice bran (*Oryza sativa*) (CE + RB) in the ratio of 2:1 (800 g:200 g) to investigate the nutritional composition and proteolytic potential of the fruiting body produced. Significance values of yield were determined on substrate combinations. In CE + LI the biological efficiency of the mushrooms was 93.5% and the content of fat (4.5%), fiber (11.0%), protein (27.0%) and amino acids were higher when compared with CE + RB. Among the amino acids, the amount of glutamic acid, aspartic acid, alanine, arginine and leucine was high. The biological efficiency on CE + RB reduced to 84.2% and based on the nutritional value, carbohydrates (53.59%), energy (324.33 kcal) and minerals such as zinc, iron, copper, potassium and phosphorus were higher in this substrate combination. Protease activity from fruiting body was significant in CE + LI (463.55 U/mL). This protease showed an optimal activity at 50 °C in neutral and alkaline pH with maximum stability at 30 °C at alkaline pH. This is the first report of *L. citrinus* fruiting body nutritional composition with potential for human food and application in industrial processes.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

Edible mushrooms are considered healthy food rich in protein, dietary fiber, reduced fat content and a broad spectrum of bioactivity, particulars that enable the inclusion of these fungi in human diet (Bukhari et al., 2014 and Cheung, 2013).

http://dx.doi.org/10.1016/j.sjbs.2015.07.002
1319-562X

Among the mushrooms, the genus *Lentinus* Fr., represented by around 63 species, is known as saprobe cosmopolitan basidiomycete of occurrence in tropical and temperate areas. Many of these macrofungi have distinct flavor and are foods with high amount of protein, fibers, minerals, vitamins and reduced lipid content (Karunarathna et al., 2011).

Lentinus edodes occupies a prominent place in world consumption of mushrooms when compared to other commercialized species (Balbi et al., 2013). Besides this species, *Lentinus cladopus* Lév., *Lentinus crinitus*, *Lentinus glabratus* Mont., (L.), *Lentinus subnudus* Fr., *Lentinus velutinus* Fr., *Lentinus cubensis* Berk and *Lentinus strigosus* (Schwein) Fr. are other examples recorded as edible mushrooms (Karunarathna et al., 2011 and Zent et al., 2004).

Other representatives of *Lentinus* genus as *L. crinitus* and *L. citrinus* produce proteases, *Lentinus tigrinus* produces esterases, *L. edodes* produces enzymes that are used for Leishmaniasis treatment and *L. strigosus* synthesizes compounds with immunomodulatory activity and properties to chemotherapy and Chagas disease (Kirsch et al., 2011; Sabotič et al., 2007 Souza-Fagundes et al., 2010 and Tahir et al., 2012).

In many ecosystems, mushrooms play an important role in the decomposition of organic matter. The white rot fungi cleavage the cellulose, hemicellulose and lignin from wood while the brown rot fungi only cleavage cellulose and hemicellulose (Fonseca et al., 2014).

Literature data show that between the bioactive compounds produced by *Lentinus*, proteases have an important regulatory role in nature with emphasis on morphogenesis, physiology and metabolism of mushrooms. They are a complex group of enzymes with a different substrate specificity, active site, catalytic mechanism, optimal pH and temperature and stability profile. Besides, proteolytic enzymes have applications in food processing, bakery, juices and cheese production. These properties contributed to the promotion of the predominance of these biocatalysts in the enzymes world market estimated in billion of dollars (Fonseca et al., 2014; Singhal et al., 2012 and Sumantha et al., 2006).

Mushroom production as a sustainable alternative can contribute to reducing of organic waste and minimize the content of environmental pollutants. They also can aggregate value to low-cost products as agrowastes (Ahmed et al., 2013 and Dahmardeh, 2013).

In Amazon, agrowastes as cupuaçu exocarp, litter and rice bran exist in abundance, they are little explored but have important nutritional characteristics for usage in edible mushroom cultivation (Castillo, 2006). The aim of this study was to analyze the yield performance, nutritional quality and proteolytic potential of *L. citrinus* fruiting bodies produced in lignocellulosic substrates.

2. Material and methods

2.1. Mushroom production

L. citrinus DPUA 1535 obtained from DPUA Culture Collection, Federal University of Amazonas–UFAM, Brazil, was maintained on potato dextrose agar (PDA) supplemented with 0.5% (w/v) yeast extract, at 25 °C for 8 days in the absence of light.

The spawn was produced in wheat grain pre-cooked for 15 min and then added to calcium carbonate (3.5 g/kg grain, dry basis). In flasks of glass, with screw cap containing a central hole capped with a cotton plug, 300 g of these grains were stored and sterilized at 121 °C for 15 min. After cooling, each flask was inoculated with 12 mycelial agar disks. The cultures were maintained at 25 °C in the absence of light until the completion of growth of mycelium on substrate (Castillo, 2006).

The production of *L. citrinus* was performed in substrates in the ratio of 2:1 (800 g:200 g): CE + RB (cupuaçu exocarp + rice bran) and CE + LI (cupuaçu exocarp + litter), 60% moisture. Samples of 3 kg of each substrate mixture were filled in uncolored polyethene bags measuring 28 cm × 45 cm. The substrates were sterilized at 121 °C during 60 min for two consecutive days. After cooling, the bags were inoculated with the spawn (100 g/kg), and incubated at 25 °C in the absence of light, 80% moisture. When the mycelium had completely covered the substrates, the induction of primordia was made at 12 °C, 90% moisture, with alternating light every 12 h and fruiting body production at 25 °C, 80% humidity, with alternating light every 12 h. For each substrate mixture, fifteen repetitions were performed. The mushrooms were collected for determination of biological efficiency (Eq. (1)), productivity (Eq. (2)) and production rate (Eq. (3)) as described by Ahmed et al. (2013).

2.2. Proximate composition

The fruiting bodies of *L. citrinus* were analyzed for moisture content, crude fat, crude protein, ash and carbohydrates. The moisture was determined by drying, in a forced air circulation oven at 40 °C, until stable weight (AOAC, 2005); crude protein fractions by micro Kjeldahl method (AOAC, 2005), using 4.38 as conversion factor. Quantification of crude fat was determined by *Bligh* and *Dyer* method; ash by incineration of the material, in a muffle furnace at 550–660 °C, until constant weight (AOAC, 2005). Crude fibers were determined by acid-base digestion according to Weende methodology (AOAC, 2005); total carbohydrates were estimated by difference (100 g −- total grams of moisture, protein, fat and ash); and total energy was calculated using the conversion factor Atwater, both recommended by NEPA, 2006.

The determination of minerals content was performed according to Embrapa methods (Embrapa, 2009). The samples were dried in a forced air circulation oven at 40 °C and then submitted to acid digestion in HNO_3 + $HClO_4$ (3:1 ratio). Phosphorus content was determined by Ultraviolet–visible spectroscopy. Calcium, magnesium, potassium, copper, iron, manganese and zinc contents were determined by atomic absorption spectrophotometry (AAS). All analyzes were performed in triplicate. The amounts of macronutrients (Ca, P, Mg and K) were calculated in g/kg^{-1} and micronutrients (Fe, Cu, Mn, and Zn) in mg/kg^{-1}.

The amounts of amino acids were performed by high performance liquid chromatography (HPLC). The samples were submitted to hydrolysis with 6 N hydrochloric acid (HCl) followed by derivatization of amino acids with phenylisothiocyanate (PITC) and separation of the phenylthiocarbamyl derivative amino acids in reversed phase column with UV (Ultraviolet) detection at 254 nm. The quantification was performed by multilevel internal calibration with α-aminobutyric

acid (AABA) as internal standard for total amino acids. The determination of tryptophan was performed after hydrolysis with pronase enzyme and color reaction with *p*-dimethylamino benzaldehyde (DAB) according to Spies, 1967.

2.3. Extraction of mushroom

The enzymatic extract of *L. citrinus* (dehydrated) was obtained by water extraction (distilled water, 1:10 ratio) and buffer extraction (0.2 M Tris–HCl, pH 7.2, 1:10 ratio). The samples were submitted to constant agitation of 150 rpm on orbital shaker (Certomat MO) at 25 °C for 24 h followed by vacuum filtration on Whatman filter paper no. 1.

2.4. Protease assay

2.4.1. Proteolytic activity

Proteolytic activity was determined according to methodology described by Leighton et al., 1976. A mixture containing 0.15 mL of crude extract and 0.25 mL of substrate [1% (w/v) azocasein in 0.2 M Tris–HCl buffer, pH = 7.2] was incubated for 60 min. The reaction was interrupted by addition of 10% (w/v) trichloroacetic acid and centrifuged (8000 rpm) for 15 min at 4 °C. One unit of proteolytic activity was defined as the amount of enzyme that produces a 0.01 increase of absorbance in one hour at 440 nm.

2.4.2. Effect of pH and temperature on activity and stability

To assay optimum pH, proteolytic activity was determined at 25 °C with different pH ranges using the following 0.2 M buffer solutions: citrate–phosphate (5.0 and 6.0), Tris–HCl (7.0 and 8.0) and glycine-NaOH (9.0 and 10.0). Optimum temperature was determined by incubating the enzyme extract at different temperatures ranging from 25 °C to 70 °C and assaying the activity at the pH determined as optimum.

For the pH stability, the crude extract was dispersed (1:1) in the following 0.2 M buffer solutions: citrate–phosphate (5.0 and 6.0), Tris–HCl (7.0 and 8.0) and glycine–NaOH (9.0 and 10.0) and maintained at optimum temperature for 0, 30, 60, 90 and 120 min. In thermal stability, the extracts were incubated at different temperatures ranging from 25 °C to 70 °C for 0, 30, 60, 90 and 120 min. All samples were prepared in triplicate.

2.5. Statistical analysis

The results were statistically analyzed at a significance level of 95% by Tukey's test carried out with Minitab software, version 16.0 (Minitab, 2010).

3. Results and discussion

L. citrinus micelium colonized completely the substrates (CE + LI and CE + RB) and no significative difference in mushrooms number and in the weight of *in natura* biomass was observed (Table 1). These data are in agreement with those reported by Chang and Miles, 2004.

Lentinus and *Pleurotus* are fungi that grow on substrates with low content of nitrogen in the range from 0.03% to 1.0%. The agrowastes cupuaçu exocarp, rice bran and litter used for cultivation of *L. citrinus* in this study are substrates that contain 0.29%, 1.2% and 0.21% of nitrogen, respectively.

Table 1 Characteristics and total yield of *Lentinus citrinus*.

Variables	Substrates	
	CE + RB	CE + Li
N (mushrooms)	(95 ± 2.5)[a]	(120 ± 2.8)[a]
weight/g	(98 ± 38)[a]	(116 ± 54)[a]
Total yield		
Biological efficiency/%	(84.2 ± 2.2)[a]	(93.5 ± 3.1)[a]
Productivity/%	(20.52 ± 1.9)[a]	(24.17 ± 2.1)[a]
Production rate/%	(20.32 ± 8)[b]	(34.15 ± 14)[a]
Mushrooms dimensions		
Pileus thickness/cm	(3.7 ± 0.8)[a]	(2.4 ± 0.8)[b]
Pileus width/cm	(3.8 ± 0.4)[a]	(2.5 ± 0.6)[b]
Stipe length/cm	(3.50 ± 0.3)[a]	(2.8 ± 0.4)[b]

Means followed by the same letters did not differ from one another by the Tukey test ($p < 0.05$).

After inoculation, the development of primordia occurred in 15 and 23 days in CE + Li and CE + RB, respectively. The formation of *L. citrinus* fruiting bodies occurred in 19 days (CE + LI) and in 27 days (CE + RB). The first harvest of mushrooms in all substrates was taken around 4 days. In CE + LI, regardless of production flows, the amount of fresh weight was 145 g.

The biological efficiency and productivity in CE + LI were 93.5% and 24.17%, respectively. These values did not differ significantly when compared to the same variables determined in cultivations using CE + RB. The exception observed was between the values of production rate that was significant in CE + LI (Table 1). Literature data show that the efficiency of mushroom production is directly related to the genetic characteristics of each species, as well as the structure, nutritional quality of the substrate and conditions of cultivation. Furthermore, the supplementation of the substrate with cereal brans or the use of new combinations may promote increased productivity and biological efficacy of the fungus (Donini et al., 2009 and Samuel and Eugene, 2012).

The following morphological characteristics of *L. citrinus* fruiting bodies after harvest were observed: the diameter was averaging 3.7 × 3.8 cm (CE + RB) and 2.4 × 2.5 cm (CE + LI) and the stipe measured 3.50 cm (CE + RB) and 2.8 cm (CE + LI) showing significant differences between them. The dimensions of mushrooms influence on their weight and yield and these parameters are also positively correlated with productivity, content of carbohydrates and proteins (Ahmed et al., 2013 and Samuel and Eugene, 2012).

Moisture content of the mushrooms grown in CE + LI and CE + RB was 10.94% and 11.88%, respectively. Protein content was 27% in CE + LI cultivation and 25.92% in CE + RB (Table 2). These results are close to the ones found in *Pleurotus ostreatus* that showed 28–31% of protein in dry basis (Ahmed et al., 2013).

Fat content in CE + RB was 3.33% and 4.50% in CE + LI (Table 2). This value is close to the result obtained by Alam et al. (2007) using *Pleurotus florida* (4.30–4.50%). Other similar results were reported for species of edible mushroom sources of low lipid content that are usually constituted of more unsaturated fatty acids than saturated ones.

Table 2 Centesimal composition of *L. citrinus* (g/100 g of substrate in dry basis).

Variables	Substrates	
	CE + RB	CE + Li
Humidity	(11.88 ± 0.4)[a]	(10.94 ± 0.1)[b]
Ash	(4.90 ± 0.7)[a]	(4.95 ± 0.4)[a]
Fat	(3.33 ± 0.3)[b]	(4.50 ± 0.1)[a]
Nitrogen	(4.67 ± 0.1)[b]	(6.30 ± 0.2)[a]
Protein/Nx 4,38	(20.00 ± 0.2)[b]	(27.00 ± 0.3)[a]
Total fiber	(6.30 ± 0.4)[b]	(11.20 ± 0.2)[a]
Total carbohydrates	(53.59 ± 0.1)[a]	(41.41 ± 0.1)[b]
E/kcal	(324.33 ± 0.1)[a]	(314.14 ± 0.1)[b]

Means followed by the same letters did not differ from one another by the Tukey test ($p < 0.05$).

Unsaturated fatty acids are beneficial because of their prophylactic action in cholesterol and triglyceride reduction (Chang and Miles, 2004 and Manjunathan and Kaviyarasan, 2011).

L. citrinus ash levels were 4.90% and 4.95% in CE + RB and CE + LI, respectively. In CE + RB, carbohydrate content was 53.59% and fiber 6.30%. In CE + LI, carbohydrate content was 41.41% and fiber 11.30% (Table 2). These fiber values are close to those described for *Pleurotus florida* (11.5%) and are higher than *Lentinus tuberregium* (3.6%) fiber content. Fibers are essential nutrients that comprise a healthy diet and have a preventive action in reducing cholesterol (Chang and Miles, 2004 and Manjunathan and Kaviyarasan, 2011). Energy content represented 324.33 kcal/100 g in CE + RB and 314.14 kcal/100 g in CE + LI, dry basis.

Singh and Singh, 2012 reported that species of mushrooms that have low calories and high protein content can be compared to food sources of essential nutrients to organism, as raw rice (7.2 g/100 g), wheat (9.8 g/100 g), egg (13.0 g/100 g), some vegetables and milk (25.0 g/100 g) (Taco, 2011).

Mineral content in CE + RB had good values of potassium, phosphorus, iron, sodium, zinc and copper (Table 3). In CE + LI cultivation, calcium and sodium were predominant. The amount of sodium was 446 mg/kg[1] in dry basis or 4.46 mg/10 g in wet basis. These values are lower than the ones determined by Brazilian administrative rule No. 24/1998, which deals with the classification of foods in relation to sodium quantitative: "foods classified as no sodium content present levels equal to or less than 5 mg Na/l0 g of solids" (Anvisa, 1998). Data presented by Gucia et al., 2012 showed that in *Boletus edulis*, potassium content was on average 25–29 g/kg^{-1} in the pileus and 20–40% in the stipe when compared with the previous value.

Table 3 Biomass minerals of *L. citrinus* cultivated in different substrates.

Nutrients	Substrates	
	CE + RB	CE + Li
P (g kg^{-1})	(10.94 ± 0.01)[a]	(0.01 ± 0.01)[b]
K (g kg^{-1})	(22.25 ± 0.02)[a]	(0.96 ± 0.03)[b]
Ca (g kg^{-1})	(0.09 ± 0.01)[b]	(40.00 ± 0.02)[a]
Mg (g kg^{-1})	(1.26 ± 0.04)[a]	(0.58 ± 0.01)[b]
Fe (mg kg^{-1})	(53.34 ± 0.02)[a]	(0.57 ± 0.03)[b]
Na (mg kg^{-1})	(174.42 ± 0.02)[b]	(446.0 ± 0.52)[a]
Cu (mg kg^{-1})	(34.84 ± 0.01)[a]	(0.27 ± 0.02)[b]
Mn (mg kg^{-1})	(9.05 ± 0.02)[a]	ND
Zn (mg kg^{-1})	(66.21 ± 0.02)[a]	(0.10 ± 0.01)[b]

Means followed by the same letters did not differ from one another by the Tukey test ($p < 0.05$). ND: no determined.

Mushrooms can be considered sources of K, P, Cu and Zn, which are, among the mineral nutrients, essential to the human body acting as regulators and/or co-factors for many enzymes (Gucia et al., 2012 and Koyyalamudi et al., 2013). Singh and Singh, 2012 reported the existence of a large variation of mineral content in mushrooms of the same species due to genetic variation, substrates and cultivation technologies that affect its composition. In combination, the variation in the content of minerals probably reflects the mineral composition of the substrate used in different cultivations (Gucia et al., 2012).

Amino acid content of *L. citrinus* grown in CE + LI and CE + RB showed a total of 27.0 and 20.1 g/100 g, respectively (Table 4). The most abundant components between the essential and nonessential amino acids were leucine (2.07 mg/100 g) and glutamic acid (4.41 mg/100 g) in CE + LI. These data compared to CE + RB showed a difference of 55% and 9.2%, respectively. The values presented in this study are higher than those found by Lee et al., 2011 with *Agrocybe chaxingu*. Singh et al., 2008 reported that mushrooms with high protein content also show high levels of essential amino acids.

Tryptophan (0.37 mg/100 g) and serine (1.52 mg/100 g) concentrations were lower in CE + LI than CE + RB (1.83 and 0.41 mg/100 g, respectively). The total amount of essential amino acids was 36% higher in CE + LI. These results are considered significant when compared to those made by Lee et al.,

Table 4 Amino acids presented in the biomass of *Lentinus citrinus* cultivated in different substrates (mg/100/g protein).

Lentinus citrinus		
Amino acids	CE + RB	CE + Li
Aspartic acid	(2.49 ± 0.01)[b]	(2.57 ± 0.02)[a]
Glutamic acid	(4.00 ± 0.01)[b]	(4.41 ± 0.03)[a]
Serine	(1.83 ± 0.03)[a]	(1.52 ± 0.01)[b]
Glycine	(1.24 ± 0.01)[b]	(1.45 ± 0.02)[a]
Histidine	(0.49 ± 0.03)[b]	(0.70 ± 0.03)[a]
Arginine	(1.47 ± 0.02)[b]	(2.28 ± 0.02)[a]
Threonine	(0.96 ± 0.01)[b]	(1.30 ± 0.01)[a]
Alanine	(1.11 ± 0.02)[b]	(1.78 ± 0.01)[a]
Proline	(0.49 ± 0.01)[b]	(1.24 ± 0.03)[a]
Tyrosine	(0.46 ± 0.03)[b]	(1.10 ± 0.02)[a]
Valine	(1.25 ± 0.01)[b]	(1.55 ± 0.01)[a]
Methionine	(0.18 ± 0.03)[b]	(0.48 ± 0.03)[a]
Cysteine	(0.04 ± 0.02)[b]	(0.15 ± 0.01)[a]
Isoleucine	(0.65 ± 0.01)[b]	(1.27 ± 0.02)[a]
Leucine	(0.93 ± 0.01)[b]	(2.07 ± 0.02)[a]
Phenylalanine	(0.77 ± 0.01)[b]	(1.28 ± 0.01)[a]
Lysine	(1.33 ± 0.02)[b]	(1.85 ± 0.01)[a]
Tryptophan	(0.41 ± 0.03)[b]	(0.37 ± 0.01)[a]
Total	2010	2700

Means followed by the same letters did not differ from one another by Tukey's test ($p < 0.05$).

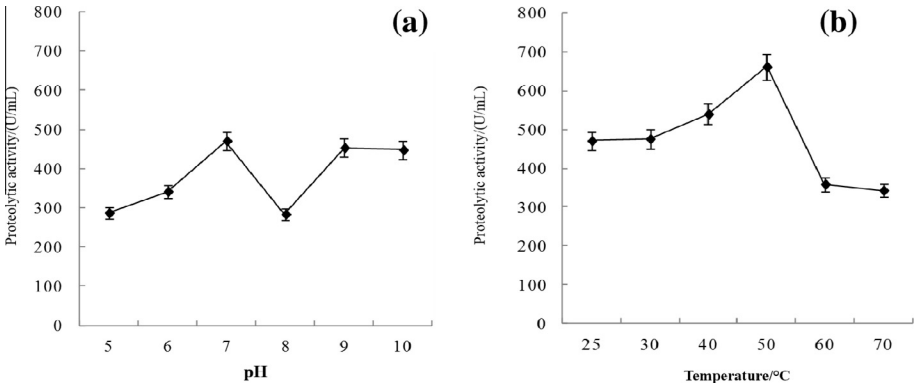

Figure 1 Effect of pH (A) and temperature (B) on proteolytic activity of *Lentinus citrinus* proteases.

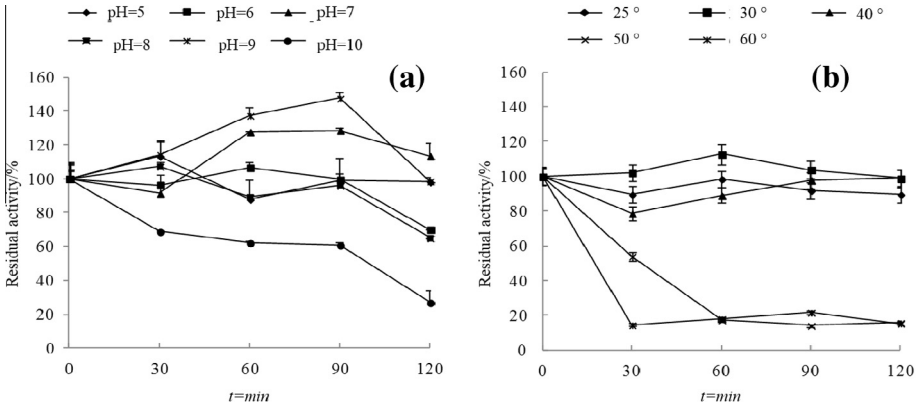

Figure 2 Effect of pH (A) and temperature (B) on stability of *Lentinus citrinus* proteases.

2011 with *Agrocybe chaxingu* (2.70 g/100 g), *Pleurotus ostreatus* (2.38 g/100 g) and *Flammulina velutipes* (1.66 g/100 g).

Singh and Singh (2012) and Singh et al. (2008) reported that mushrooms produce extracellular enzymes that affect the increase of its nutritional value. In the present work, the mushrooms cultivated in CE + LI had high qualitative protein content due to the presence of essential amino acids in its composition (leucine, isoleucine, valine, threonine, methionine, tryptophan and phenylalanine) and by its protein content.

Protease activity was determined using the mushroom crude extracts obtained from extraction in sterile distilled water and in buffer 0.2 M Tris–HCl, pH = 7.2. Among these extracts, the enzyme activity was significantly higher in the aqueous extract of the mushroom produced in CE + LI (463.55 U/mL). On the same substrate, when buffer was used in the extraction, the proteolytic activity was 6.04% lower when compared to sterile distilled water.

The extracts from the mushrooms produced in CE + RB showed proteolytic activities of 246.88 U/mL (distilled water) and 365.55 U/mL (buffer). Comparing the substrates used for growing of *L. citrinus*, cupuaçu exocarp mixed with litter (CE + LI) was the substrate which promoted the development of mushrooms with a greater quantity of proteases. There are several parameters that affect the production of enzymes; however, a major factor is the source of nitrogen (Singh et al., 2008). In this study, the type of substrate promoted an influence in protease quantity. It probably happened because of the relation with the protein content of the substrates used in the bioprocess, since the mean value of the proteolytic activity was determined in the substrate composed by cupuaçu exocarp mixed with litter (CE + LI).

The crude extract selected for partial characterization of proteases was that one obtained from *L. citrinus* cultivated in CE + LI (Figs. 1 and 2). The optimal pH (Fig. 1A) was determined in pH = 7.0 and 9.0 (470.3 U/mL and 453.0 U/mL, respectively). In the study by Cui et al., 2007, *in natura* mushroom proteases of *Pleurotus citrinopileatus* showed an optimal pH of activity at pH = 10.0. Proteases with optimal activity at neutral and alkaline conditions have potential use as detergent additives in leather processing, silver recovery, medical purposes, food processing, animal feed production and in chemical industries as well as for the treatment of agro-industrial wastes (Haddar et al., 2009 and Ravikumar et al., 2012).

The optimal temperature (Fig. 1B) for protease activity was determined at 50 °C (660.0 U/mL). Similar data have been shown in extracts from *in natura* mushrooms of *P. citrinopileatus* (50 °C), *Hypsizygus marmoreus* (50 °C) and *Termitomyces albuminosus* (60 °C) (Cui et al., 2007 and Zhang et al., 2010 and Zheng et al., 2011).

L. citrinus proteases showed better stability at pH = 7.0 and 9.0 retaining more than 100% of its initial activity for 90 min (Fig. 2A). At pH = 10.0, however, the relative activity decreased to almost 70% in 30 min and decreased after 90 min. Nishiwaki et al., 2009 obtained a stability of 70% in *Grifola*

frondosa proteases at pH range from 4.5 to 8.5 after incubation at 30 °C for 30 min.

Thermal stability (Fig. 2B) showed that the enzymes were active at all temperatures with higher activity at 30 °C during 60 min (112.7% of residual activity). At 25 °C and 40 °C the activity was maintained in 98.5% and 97.8% for 60 min, respectively. At 50 °C and 60 °C 54.1% and 14.5% of enzyme activity were observed, respectively. Different results of protease characterization were obtained by Kirsch et al., 2013 using the crude extract of *L. citrinus* produced in liquid medium. In the same culture conditions these proteases expressed optimal activity at pH = 7.0 and 40 °C. These enzymes were stable at pH = 5.0, 6.0 and 7.0 with retention of 85% of activity after 90 min. Thermal stability was observed at 25 °C and 40 °C with retention of activity higher than 85%.

4. Conclusion

The results of this research indicate that the combination of cupuaçu exocarp with litter or cupuaçu exocarp with rice bran can be used as alternative substrates for *L. citrinus* cultivation and production of proteases. *Lentinus citrinus* cultivated in substrates containing vegetable wastes have nutritional property for inclusion in the human diet as an innovative product source of protein, essential amino acids and fiber. They can be consumed *in natura*, dried or as supplement of food products. The proteases extracted from these mushrooms expressed optimal activity in neutral and alkaline pH and at 50 °C showing maximum stability in alkaline pH and at 30 °C. Therefore, this study also reveals potential use of *L. citrinus* proteases for industrial application.

Acknowledgments

The authors wish special thanks to the financial support from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Appendix A.

g of fresh mushroom/dry substrate weight × 100 (1)

g of fresh mushroom/fresh substrate weight × 100 (2)

biological efficiency/days crop (3)

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