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# Cultivation and nutritional studies of an edible mushroom from North Brazil

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The vertical mycelial growth to investigate the feasibility of *Pleurotus ostreatoroseus* DPUA 1720 production in lignocellulosic Amazonic residues was evaluated. Mycelial development was carried out in cupuaçu exocarp (*Theobroma grandiflorum* Willd Former Spreng Schum), açai seed (*Euterpe oleracea*) and sawdust as substrates. Each residue was supplemented with rice bran, crown and pineapple peel. The average speed of mycelial growth was determined using three replicates for 15 days at 25°C in the absence and presence of light and mycelial vigor and density were evaluated. Five replicates of the selected substrate were used in mushroom production. Vigorous mycelium and strongly dense growth were observed in cupuaçu exocarp treatment supplemented with rice bran. The biological efficiency, production rate and productivity were 22.90, 54.33 and 3.55%, respectively in this substrate. The basidiomata showed low levels of minerals and fat and can be considered as a source of protein (23.53%) and fiber (12.79%).

Key words: basidiomata, edible mushroom, agro-industrial wastes.

# INTRODUCTION

The importance of edible mushrooms has increased in recent years because of their gastronomic value, nutritional potential, medicinal properties and ability to degrade and recycle agro-industrial residues (Bonatti et al., 2004; Cheung and Cheung, 2005; Furlani and Godoy, 2005, Pedra et al., 2009).

*Pleurotus* is an important mushroom genus known for its high content of proteins, carbohydrates, minerals (calcium, phosphorus and iron) and vitamins (thiamine, riboflavin and niacin), as well as low fat content. These mushrooms have the ability to colonize and degrade a wide variety of lignocellulosic wastes with relatively short cycle (Justo et al., 1998; Manzi et al., 1999; Eira, 2004; Bonatti et al., 2004; Shashirekha et al., 2005; Pedra and Marino, 2006; Toro et al., 2006; Pedra et al., 2009; Menolli Junior et al., 2010; Omarini et al., 2010).

Among the edible *Pleurotus* genus, *Pleurotus ostreatoroseus* produces bioactive compounds with reducing action of triglycerides in the human body besides the meaty pink color basidiomata have good taste (Nascimento et al., 2008). This species has a worldwide distribution and can be found in tropical or

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Experiment	(A) Basal substrate	(%)	(B) Supplementation substrate	(%)
1	AS	80	PP	20
2	AS	80	PC	20
3	SAW	80	PP	20
4	SAW	80	PC	20
5	CE	80	RB	20

**Table 1.** Substrates used to evaluate the mycelial growth: (A) Basal substrate-açai seed (AS); sawdust (SAW); cupuaçu exocarp (CE) and (B) supplementation substrate- pineapple peel (PP); pineapple crown (PC); rice bran (RB).

subtropical forests (Guerrero and Homrich, 1999; Putzke, 2002; Rosado et al., 2002).

This mushroom has been produced *in vitro* in several agro-industrial substrates as castor plant peel, rice straw, sugarcane bagasse, cotton waste, soybean straw, corn cob, elephant grass, oat, rye grass, sunflower seed, eucalyptus sawdust, stillage and privet (Rosado et al., 2002; Donini et al., 2005; Bernardi et al., 2007; Minotto et al., 2008; Nascimento et al., 2008; Aguiar et al., 2010; Reis et al., 2010).

The cultivation of edible mushrooms in agro-industrial residues has been shown as an alternative to better utilization of the organic matter. Usually at the end of cycle the biomass obtained can be used as food due to its high nutritional value. These residues associated with mycelium also have a great potential for use as fodder animal and as fertilizer in agriculture (Bonatti et al., 2003; Shibata and Demiate, 2003).

The Amazon rainforest has an incalculable wealth diversity of micro-organisms, water, ores, plants and animals species. It is also a source of a large organic residues volume that can be used as substrate for the growth of fungi by fermentation processes to produce biomass with biological activity. Based on the availability of various agro-industrial residues from local logging and amazonic fruit production, the aim of this study was to evaluate the use of agro-industrial wastes in vertical mycelial growth of the edible mushroom Ρ. ostreatoroseus DPUA 1720, select a mixture of substrates for the mushroom production and verify their nutritional value and microbiological quality.

## MATERIAL AND METHODS

#### Organism and culturing conditions

*P. ostreatoroseus* DPUA 1720 was obtained from Culture Collection DPUA/Federal University of Amazonas (UFAM). The culture was maintained in Potato Dextrose Agar+0.5% Yeast Extract (w/v) (PDA+YE) under refrigeration (4°C). The matrix culture was prepared in the same medium at 25°C for 8 days in absence of light.

#### Measurements of vertical mycelial growth

P. ostreatoroseus was cultivated in the following substrates:

cupuaçu exocarp [*Theobroma glandiflorum* Willd Ex-Spreng Schum (CE)], açai seed [*Euterpe oleracea* (AS)] and sawdust (SAW) [80% (w/v)] supplemented with rice bran [*Oryza sativa* (RB)], pineapple crown [*Ananas* sp. (PC)] and pineapple peel [*Ananas* sp. (PP)] [20% (w/v)] on dry basis, respectively (Table 1) to determinate the vertical micelial growth. The substrates were treated with Pury Vitta® (0.96% p/p active chlorine) according to the manufacturer's recommendations. Excess water was drained and the medium adjusted to 60% moisture and pH 6.5.

The formulations made with the wastes (Table 1) were placed in test tubes (200 x 25 mm) and were sterilized at 121°C for 60 min in three consecutive days. After cooling, three discs (8 mm of diameter) from the matrix culture were inoculated on the substrates surface. Millimeter tapes of 150 mm were placed in three equal distant points of the test tube to follow the growth of mycelium. The cultures were maintained at 25°C, 60% humidity, in absence (experiment 1) and presence of light (experiment 2). The vertical mycelial growth was measured (mm) every 24 h, for 15 days. All the experiments were performed with three replicates.

The mycelial vigor was assessed according to the following classification: weakly dense, moderately dense or strongly dense (Marino et al., 2008). Daily mycelial growth average speed (VMC) was calculated in cm/day according to Equation 1 (Israel, 2005; Palheta et al., 2011).

$$VMC = \frac{Vf - Vi}{Tf}$$
(1)

Onde: VMC = Daily mycelial growth average speed; Vf = Final measure of micelial growth (cm); Vi = Initial measure of micelia growth (cm); Tf = Final time (days).

## Production of P. ostreatoroseus in agro-residues

#### Spawn production

The spawn was prepared according to Rollan (2003) using wheat grains. The grains were washed, pre-cooked for 15 min and treated with 0.3% calcium carbonate (w/v, dry basis). In flasks of glass (1000 mL), with screw cap containing a central hole capped with a cotton plug, the grains were included and sterilized at 121°C for 60 min. After cooling, 12 discs (8 cm of diameter) from the matrix culture were inoculated into the surface of the substrate. Inoculated flasks were maintained at 25°C in absence of light until the completion of growth of mycelium on substrate.

#### P. ostreatoroseus solid fermentation

The production of *P. ostreatoroseus* was made in cupuaçu exocarp supplemented with rice bran (CE+RB). The residues on dry weight basis were mixed in ratio 4:1 (800:200, w/w) and treated with 0.3%

calcium carbonate (w/v), pH 6.5. The substrate was distributed in polypropylene bags and sterilized at  $121^{\circ}$ C for 60 min during three consecutive days. After cooling, the spawn were inoculated in the substrate and a total of five replicates were made.

The incubation was carried out in two cycles. The first one at  $25^{\circ}$ C, 60% moisture and absence of light until full mycelial colonization of the substrate and the second at  $15^{\circ}$ C, for 24 h to induce primordia and at  $25^{\circ}$ C, 90% moisture to basidiomata formation.

During the growing cycle, the cultures were submitted to automatic control of temperature, lightening (12 h a day), moisture and air exchange. In this process the formation and development of the mushrooms and total time of cultivation were evaluated. The basidiomata were collected, weighed and dehydrated at 40°C in a forced air circulation oven. Four parameters of production performance were analyzed: biological efficiency (EB) (Equation 2), productivity (P) (Equation III), production rate (TP) (Equation IV) and loss of organic matter determination (PMO) (Equation V) (Dias et al., 2003;Oliveira et al., 2007; Holtz et al., 2009):

Biological efficiency (EB) = 
$$\frac{\text{mushroom mass (wet basis)}}{\text{substrate mass (dry basis)}} \times 100$$
 (2)

Productivity (P) = 
$$\frac{\text{mushroom mass (dry basis)}}{\text{substrate mass (dry basis)}} \times 100$$
 (3)

Production rate (TP) = 
$$\frac{\text{biological efficiency}}{\text{total days of cultivatio n}} x100$$
 (4)

Loss of organic matter (PMO) = <u>residual substrate mass (dry basis)</u> x100 (5) initial substrate mass (dry basis)

#### Proximal composition of substrates and P. ostreatoroseus

The dehydrated residues and basidiomata were analyzed for moisture level, protein, ash, fat, carbohydrates, fiber and calories. The protein content was calculated using a correction factor of 4.38 to basidiomata and 6.25 to substrates (AOAC, 1997; Furlani and Godoy, 2005; Silva et al., 2007; Pauli, 2010).

#### Minerals analysis of P. ostreatoroseus

Minerals analysis was performed according to Embrapa methods. The samples were dried in a forced air circulation oven at 40 °C and then submitted to acid digestion in  $HNO_3 + HCI O_4$  (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 macrominerals (Ca, P, Mg and K) were calculated in g.kg<sup>-1</sup> and trace elements (Fe, Cu, Mn, and Zn) in mg.kg<sup>-1</sup>.

#### Amino acid analysis of P. ostreatoroseus biomass

The amounts of amino acids analysis were performed by high performance liquid chromatography (HPLC). The samples were submitted to hydrolysis in 6N hydrochloric acid (HCI) 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  $\alpha$ -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-dimethylaminobenzaldehyde (DAB) according to Spies (1967).

# Microbiological analysis of *P. ostreatoroseus* dehydrated basidiomata

The health and hygiene conditions of *P. ostreatoroseus* biomass were made according to Brazilian legislation (Brasil, 2001). Analysis of moulds and yeasts were also made although it is not required by the same legislation (WHO, 1998).

In microbiological analyses, 25 g of the dehydrated basidiomata were mixed with 225 mL of peptone water. From this solution, successive dilutions were prepared in tube tests containing 9 mL of 0,1 % peptone water (w/v) until  $10^{-3}$  dilution. Volumes of 100 and 200 µL were removed from  $10^{-1}$  to  $10^{-3}$  dilutions to determinate moulds and yeasts, total and thermotorelant coliforms, *Salmonella* sp. and *Staphylococcus aureus*.

#### Moulds and yeasts analysis

From each dilution made, a volume of 200  $\mu$ L was spread in the surface of Rose Bengal agar with 0.001 % chloramphenicol (w/v). The Petri dishes were incubated at 25°C for seven days. All the experiments were made in triplicates and the results were expressed as colony forming units per gram product (CFU/g) (Silva et al., 2007).

# Total and thermotorelant coliforms, *Salmonella* sp. and *Staphylococcus aureus* determination

Most Probable Number test (MPN.g<sup>-1</sup>) of total and thermotorelant coliforms and *Salmonella* sp. were made removing a volume of 1000  $\mu$ L to 3 test tubes containing 9 mL of Brila broth (Himedia®, Mumbai-India) with reversed Duhrantubes. The tubes were incubated at 37°C for 24-48 h. From the positive results (gas formation), confirmation tests were made. The confirmation for total coliforms was made in Brila broth (Himedia®, Mumbai-India) at 35°C for 24-48 h. Thermotolerant coliforms confirmation was made in *Escherichia coli* broth (EC) (Himedia®, Mumbai-India) at 45°C for 24 h. The values of NMP.g<sup>-1</sup> were calculated according the methodology of Silva et al. (2007).

Salmonella sp. test were made from the test tubes with gas formation and maintained at 35 °C. An aliquot was removed and inoculated in Bright Green agar (BG) (Himedia®, Mumbai-India) at 35°C for 24 h. The suspect colonies were tested by biochemical identification of *Salmonella* (Silva et al., 2007).

The quantification of coagulase positive Staphylococci were made from dilutions  $10^{-1}$  to  $10^{-3}$ . A volume of  $100 \ \mu$ L were removed and inoculated in 15 ml of Mannitol agar melted and then cooled until 45°C. After mixture and solidification of the medium, the dishes were incubated at 37°C. The measure was made after 24 to 48 h. The results were considered positive by the color change varying from red to yellow and to confirm coagulase positive, three colonies that promoted the color change, were selected with other three colonies atypical to the same test. These colonies were transferred, separately, to 2mL of Brain Heart Infusion broth (BHI) and maintained at 37°C. After 24 h, 300  $\mu$ L of fermented BHI were transferred to rabbit plasma and incubated at 37°C for 6 h. The positive result was determined by the presence of clot (Reis, 2010).

#### Statistical analysis

All experiments were submitted to descriptive analysis (tables,

**Table 2.** Mycelial growth average speed (average) and mycelial vigor of *P. ostreatoroseus* DPUA 1720 cultivated on agrowastes in the presence and absence of light.

Tractment	Presence of light		Absence of light	
Treatment	VMC (cm/day)	Vigor	VMC (cm/day)	Vigor
AS + PP	$0.27 \pm 0.06^{ef}$	2	$0.41 \pm 0.04^{bcd}$	2
AS + PC	$0.52 \pm 0.05^{b}$	2	$0.75 \pm 0.02^{a}$	2
SAW+ PP	$0.25 \pm 0.02^{f}$	1	0.37 ± 0.001 <sup>de</sup>	1
SAW + PC	$0.33 \pm 0.03^{def}$	1	$0.50 \pm 0.06^{bcd}$	1
CE + RB	$0.38 \pm 0.02^{cde}$	3	$0.41 \pm 0.07^{bcd}$	3

AS+PP= açai seed+pineapple peel; AS+PC= açai seed+pineapple crown; SAW+PP= sawdust+pineapple peel; SAW+PC= sawdust+pineapple crown; CE+RB= cupuaçu exocarp+rice bran. Means followed by same letters do not differ between them by Tukey test (p $\leq$  5%). 1, weakly dense; 2, moderately dense and; 3, strongly dense.

graphics and frequency distribution), variance analysis and the means were compared by Tukey test (5% of significance) using Minitab software, version 16.0.

# **RESULTS AND DISCUSSION**

Agro-industrial wastes are raw materials useful to biotransformation by micro-organisms to develop products or compounds with various optional uses or applicability in different sectors of the economy (Ezejiofor et al., 2012). Table 2 presents the results of vertical mycelial growth average speed (VMC) and mycelial vigor of *P. ostreatoroseus* DPUA 1720 in AS+PP, AS+PC, SAW+PP, SAW+PC, CE+RB. The data demonstrated the influence of light for mycelial growth. In açai seed with pineapple crown (AS+PC) mycelial developed was significant. According to the following classification: weakly dense, moderately dense, and strongly dense, the mycelial vigor was moderately dense in presence and absence of light.

In cupuaçu exocarp with rice bran (CE+RB) the mycelial vigor was strongly dense in both cultivation conditions. Similar data were obtained by Palheta et al. (2011) with *P. florida* and *P. ostreatus* also cultivated in CE+RB (20% w/w). The fruit exocarps or peels used as substrate or supplement were more efficient to *P. ostreatoroseus* growing because they proportioned satisfactory mycelial vigor. The growing ability of a fungus species, its reproduction and basidiomata development in lignocellulosic substrates is associated to mycelial vigor and the capacity of activating physiological mechanisms during the developing cycle (Albuquerque et al., 2012).

The mycelial development resulting of the experiments probably is not only associated with mushroom cultivation conditions but also to other interfering factors as high concentrations of  $CO_2$  that compromise the enzyme activity. The substrate used as supplement can alter the medium composition. The size of the particles also can

be difficult, the gas changes and retard the growing of the apical hyphae modifying the mycelial speed formation in the bottom of substrate (Yang et al., 2015).

Some factors affect mycelial growth in mushroom cultivation as the culture media, temperature, carbon and nitrogen sources, availability of nutrients and genetic potential (Hoa and Wang, 2015). In this study, *P. ostreatoroseus* expressed distinct values of mycelial vigor in the presence and absence of light. The higher-level mycelial biomass was in the cultivations prepared with barks and seeds from Amazon fruit. Similar results were presented by Rivas et al. (2010) evaluating parameters that could confirm the viability of pectinolytic (banana peel and skin of passion fruit) and lignocellulosic (sawdust) substrates in *Pleurotus* spp. cultivation. The authors only confirmed the viability of pectinolytic substrates to mushroom cultivation.

Marino et al. (2008) confirmed that coconut bark sawdust supplemented with wheat bran and rice promoted growing and mycelial vigor of three *Pleurotus ostreatus* strains. Bernardi et al. (2007) used black oat supplemented with 20% wheat bran and observed an expressive colonization of the substrate by *Pleurotus ostreatoroseus*. It probably happened due the relation between carbon and nitrogen sources.

Rice bran was the substrate that presented the highest contents of protein and fat (17.37 and 19.41%, respectively). Pineapple crown, cupuaçu exocarp, pineapple peel, açai seed and sawdust presented protein contents of 16.14, 12.42, 7.99, 7.85, 5.44%, respectively (Table 3).

Fiber content was higher in sawdust (63.53%) and pineapple crown (28.36%). The others substrates presented contents between 0.37 to 9.2%. Ash content was 9.14, 4.53, 3.96 and 3.45% in rice bran, cupuaçu exocarp, pineapple crown and pineapple peel, respectively. The higher values of carbohydrates were determined in açai seed (85.69%), cupuaçu exocarp (71.09%) and pineapple peel (67.96%). Total energy was 415.25 kcal in rice bran and 391.18 kcal in açai seed. In the other substrates, these values were between 134.03 and 351.85 kcal. Different values of centesimal composition were presented in other studies with the same substrates (Costa et al., 2007; Sales-Campos et al., 2010; Jafarpour et al., 2010; Sousa et al., 2011).

The production of *P. ostreatoroseus* in CE+RB 20% (w/w) presented an average of total myceliation, primordia formation and total cultivation in 15.2; 4.2 and 42.2 days, respectively (Table 4). Vega et al. (2006) cited that the total myceliation and primordia formation of *P. djamor* occurred in 13 to 20 days and 42 to 51 days.

*P. florida* cultivated in different agrowastes presented total substrate myceliation in 21 days, primordial formation in 4 days and total cultivation time in 30 days. In other study, the cultivation of *P. florida* in cotton residue supplemented of 5% (w/v) rice bran presented

Parameter	Sawdust	Açai seed	Cupuaçu exocarp	Pineapple crown	Pineapple peel	Rice bran
Humidity*	3.42	0.79	9.61	1.56	9.93	9.24
Ash*	1.33	1.68	4.53	3.96	3.45	9.14
Nitrogen*	0.87	1.26	1.99	2.58	1.28	2.78
Protein* (Nx6.25)	5.44	7.85	1.42	16.14	7.99	17.37
Fat*	1.43	1.89	1.98	2.28	1.47	19.41
Fiber*	63.53	2.1	0.37	28.36	9.2	2.07
Carbohydrate*	24.85	85.69	71.09	47.7	67.96	42.76
Energy (kcal)	134.03	391.18	351.85	275.88	317.01	415.25

Table 3. Average proximal composition of the agro-industrial wastes used in the solid state fermentation.

\*Percent (%).

**Table 4.** Analyzed parameters during *P.*ostreatoroseusproduced in cupuaçuexocapsupplemented with rice bran.

Parameter	Average
Myceliation (days)	15.2 ± 1.3
Primordia formation (days)	$4.2 \pm 0.84$
Total time of cultivation (days)	42.2 ± 2.77
Biological efficiency (%)	22.90 ± 2.27
Production rate (%)	54.33 ± 4.95
Productivity (%)	3.55 ± 0.61
Organic matter loss (%)	37.68 ± 1.39

mycelial growth in 20 days, total cultivation in 43.4 days. *P. ostreatoroseus* also cultivated in the same residue presented mycelial growth in 20 days, body fruiting bodies formation in 11 days and total cultivation time in 35.6 to 36.8 days (Reis et al., 2010; Figueiró and Graciolli, 2011).

The biological efficiency (EB), production rate (TP) and productivity (P) of *P. ostreatoroseus* DPUA 1720 were 22.90, 54.33 and 3.55% in CE+RB (800 g: 200 g), 60% humidity in dry basis, respectively. Close values were obtained by Oliveira et al. (2007) using peanut bark to produce *P. pulmonarius*. The biological efficiency was around 23% and productivity was 4.58%.

*P. ostreatoroseus* produced in cotton residue with or without supplementation of 5 % rice bran showed biological efficiency of 104% and 92.5 %, respectively (Reis et al., 2010). Sales-Campos et al. (2010) presented high values of biological efficiency to *P. ostreatus* (64.6% and 125.6%) using pejibaye trunk and balsa wood sawdust as substrates. Low values of biological efficiency can be explained by the organism genetics, culture conditions, substrates composition and its proportion used in the process. Besides, the biological efficiency can interfere in environmental factors as temperature, humidity, luminosity and pH (Oliveira et al., 2007).

The degraded organic material percentage in CE+RB was 37.68% in cultures of *P. ostreatoroseus* DPUA 1720

Table 5. Proxin	mal analysis of P.			
ostreatoroseus basidiomata				
produced in o	cupuaçu exocarp			
supplemented with rice bran.				

Parameters	%	
Humidity	7.15 ± 0.01	
Protein	23.53 ± 0.13	
Fat	$3.08 \pm 0.35$	
Ash	$6.49 \pm 0.01$	
Fiber	12.79 ± 0.09	
Carbohydrates	46.98 ± 0.57	

(Table 4). In Holtz et al. (2009) only 24.10% of the organic matter in cotton spinning residue was degraded after *P. ostreatus* cultivation, however, PMO was 59.91 to 71.83% and 53.58 to 58.75% when the cultivation was made in balsa wood sawdust and pejibaye trunk. *Pleurotus* spp. degradation of passion fruit peel, banana peel and sawdust were 16.63%, 18.59% and 39.79%, respectively (Rivas et al., 2010; Sales-Campos et al., 2010).

Table 5 shows proximate analysis of *P. ostreatoroseus* DPUA 1720 basidiomata. Protein content was 23.53%. This values is according to others recorded to *Pleurotus* species (10.5 to 30.4%) (Furlani and Godoy, 2005). Shimeji mushroom (*P. ostreatus*) commercialized in the city of Campinas presented protein content of 22.22%. *P. ostreatus* and two strains of *P. sajor-caju* cultivated in elephant grass showed protein content of 22.59, 29.24 and 25.51%, respectively. These values are close to the ones obtained by *P. ostreatoroseus* DPUA 1720 (Bernardi et al., 2009; Furlani and Godoy, 2007).

Young and mature mushrooms basidiomata from south of Nigeria presented protein content between 5.1 and 34.1%. Young *P. florida* and *Psathyerella atroumbonata* protein content (15.3 and 18.5%, respectively) were higher than the mature ones (Gbolagade et al., 2006). In Rampinelli et al. (2010) study were determined the proximate composition of two fluxos of *P. djamor*. The

Macromineral	(g kg⁻¹)	Trace element	(mg.Kg⁻¹)
Р	10.39±0.005 <sup>b</sup>	Na	30.85±0.01 <sup>°</sup>
К	24.19±0.006 <sup>ª</sup>	Cu	12.47±0.01 <sup>e</sup>
Ca	0.21±0.005 <sup>d</sup>	Fe	72.34±0.01 <sup>b</sup>
Mg	1.46±0.005 <sup>°</sup>	Mn	13.17±0.01 <sup>d</sup>
		Zn	78.06±0.01 <sup>a</sup>

**Table 6.** Macrominerals and trace elements of *P. ostratoroseus* produced in cupuaçu exocarp supplemented with rice bran.

cultivation in banana tree straw presented protein content of 20.5 and 19.8% and fat of 1.12 and 1.09%. Other species like *P. ostreatus* (0.88%), *P. sajor-caju* (0.30 and 0.54%) and *P. florida* (0.9 and 1.2%) also showed low content of fat.

P. ostreatus isolated from Amazon presented 3.08% of fat. This value is considered according to some dry basis mushrooms biomass (1.1 and 8.3%) and close to Shimeji mushrooms (P. ostreatus) commercialized in São Paulo (Gbolagade et al., 2006; Furlani and Godoy, 2007; Bernardi et al., 2009). Ash and crude fiber of P. ostreatoroseus DPUA 1720 was 6.49 and 12.79%, respectively (Table 5). Mshandete and Cuff (2007) obtained 6.1% of ash and 11% of fiber in P. flabellatus basidiomata cultivated on sisal waste. In the same study, P. djamor cultivated in banana tree straw presented 6.34% of ash and 12.69% of crude fiber. P. ostreatus BF24 presented 18.25% of crude fiber and P. sajor-caju PSC96/03 and P. sajor-caju PSC01/06 presented 10.32 and 15.34% of crude fiber, respectively (Bernardi et al., 2009; Rampinelli et al., 2010).

Carbohydrates (46.98%) were the main component in *P. ostreatoroseus* DPUA 1720 basidiomata. Other mushrooms like *P. ostreatus*, *P. flabellatus*, *P. sajor-caju* and *P. djamor* presented an average of 25.69 to 60% of carbohydrates. These differences are according to species and cultivation substrate. Total energy of *P. ostreatoroseus* DPUA 1720 presented 309.7 kcal while in *P. flabellatus* and *Volvariella volvaceae* were 302 and 305 kcal, respectively (Mshandete and Cuff, 2007; Bernardi et al., 2009; Rampinelli et al., 2010).

The nutritional characteristics of mushroom species related in different publications can be associated to some conditions as climate, growth condition, regional characteristics and type of management. In *P. ostreatoroseus* mushrooms the macrominerals K and P were significant while Mg and Ca were determined in minor amounts. Among the trace elements, Zn and Fe had the highest concentrations and Na, Mn and Cu were present in small quantities (Table 6). The mineral elements are essential for many metabolic processes and play an important biological role on the function and cellular structure (Masamba and Kazombo-Mwale, 2010; Soetan et al., 2010; Osredkar and Sustar, 2011; Mallikarjuna et al., 2013). *P. flabellatus* presented 16.2

**Table 7.** Amino acids concentration in *P.*ostreatoroseus DPUA 1720 basidiomata.

Amino acids (g/100 g of basidiomata)	Values
Lisine (Lys)	1.298
Metionine (Met)	0.298
Valine (Val)	1.134
Triptofano (Trp)	0.330
Treonine (Thr)	0.937
Isoleucine (IIe)	0.751
Leucine (Leu)	1.304
Fenilalanina (Fen)	0.805
Histidine (Hys)*	0.379
Arginine (Arg)*	1.891
Tirosine (Tyr)	0.743
Aspartato (Asp)	2.061
Serine (Ser)	1.466
Glicine (Gly)	1.037
Proline (Pro)	0.772
Cisteíne (Cys)	0.040
Glutamato (Glu)	3.592
Alanine (Ala)	1.432
Total	20.27 g

\*Conditionally essential amino acids.

and 15.37 g.kg<sup>-1</sup> of phosphorus and potassium, respectively. *P. ostreatoroseus* from São Paulo presented 91.0, 25591, 51.5 and 93.4 mg.kg<sup>-1</sup> of iron, potassium, cupper and manganese, respectively, while *P. eryngii* presented 16.7 and 20.3 mg.kg<sup>-1</sup> of cupper and manganese (Mshandete and Cuff, 2007; Moura, 2008; Gençcelep et al., 2009).

Bender (2004) reports that *P. ostreatoroseus* contains eight essential amino acids. In *P. ostreatoroseus* DPUA 1720 the most abundant were valine, lisine and leucine ranging from 1.134 to 1.304 g/100 g (Table 7). The content of glutamate and aspartate (nonessential aminoacids) were 3.592 and 2.061 g/100 g. Both have important roles as brain stimulatory neurotransmitters and enhancing foodflavor (Rodrigues et al., 2004).

Histidine and alanine values (Table 7) were close to the ones obtained in *Agrocybe chaxingu* (0.30 and 1.03

g/100 g). However, amino acids concentration in *Flammulina velutipes* and *P. ostreatus* cultivated in wheat, cotton and soy were lower (Dundar et al., 2009; Lee et al., 2011).

The microbiological assessment of *P. ostreatoroseus* mycelial biomass showed absence of molds, yeasts, *Salmonella* sp., total and thermotolerant coliforms or *E. coli*, coagulase positive *Staphylococcus*, mesophilic bacteria and *Bacillus cereus*. Therefore, microbiological analysis revealed that the mushroom was within microbial safety standard specifications and can be considered as safe food.

## Conclusion

*P. ostreatoroseus* grew in all substrates, however the higher level mycelial biomass was in cupuaçu exocarp with rice bran (80:20%) in the presence of light. These Amazon residues properties show that they can be used as substrates in edible mushrooms production, which promotes the reduction of environmental contamination and enable the developing of new protein-rich food. *P. ostreaturoseus* has good appearance, texture and flavor with great contents of crude fiber and proteins, low content of fat and the presence of amino acids and minerals.

## **Conflict of interests**

The authors did not declare any conflict of interest.

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