Nutritional composition of bioproducts generated from semi-solid fermentation of pineapple peel by edible mushrooms

Raiane Áila Teixeira Souza¹*, Tamiris Rio Branco da Fonseca¹, Larissa de Souza Kirsch², Larissa Svetlana Cavalcante Silva¹, Mircella Marialva Alecrim³, Raimundo Felipe da Cruz Filho¹ and Maria Francisca Simas Teixeira¹

¹Departamento de Parasitologia, Universidade Federal do Amazonas, Instituto de Ciências Biológicas, Coleção de Culturas DPUA –UFAM. Av. General Rodrigo Octávio, 6200, Coroado I, Cep: 69077-000, Manaus, Amazonas, Brazil.
²Uninorte Laureate Internacional Universities. Avenida Joaquim Nabuco, 1232, Centro, Manaus, Amazonas, Brazil.
³Departamento de Engenharia Agrícola e de Solos –UFAM. Av. Faculdade de Ciências Agrárias, Universidade Federal do Amazonas, Av. General Rodrigo Octávio, 6200, Coroado I, Cep: 69077-000, Manaus, Amazonas, Brazil.

Received 2 September, 2015; Accepted 30 October, 2015

Mushroom production is a very efficient alternative to agro-industrial residues recycling. The proximal composition, content of macro and micro minerals, amino acid and proteolytic activity were assessed in the pineapple peel and in the bioproducts generated from pineapple peel and mycelium of Pleurotus albidos, Lentinus citrinus and Pleurotus florida. The bioproducts were obtained by semi-solid fermentation and drying process. Nutritional analysis was performed following standard methodologies. The data showed that the percentage of protein and minerals of the bioproducts increased and the carbohydrate content reduced after the myceliation by the mushrooms. All essential amino acids were found in the bioproducts and no contamination was observed. There was significant difference between proteolytic activities of bioproducts samples, presenting L. citrinus’ bioproduct a higher value. These data show that the bioproducts produced have great nutritional value and can be used as an alternative food.

Key words: By-production, fermentation, mushroom, pineapple, protease.

INTRODUCTION

Edible mushrooms are macrofungi widely used as food and in folk medicine around the world since antiquity (Patel et al., 2012). These basidiomycetes are much appreciated for their culinary characteristics as they have high content of protein and fiber and are low in lipids, and still produce various metabolites such as antimicrobials, antioxidants and immunostimulants (Finimundy et al., 2013). Among the edible mushrooms, Pleurotus species

*Corresponding author. E-mail: aila_raiane@gmail.com. Tel/Fax: 0055(92)3305-4284.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
are becoming more popular throughout the world because of their ability to colonize various substrates and being primary decomposers. *Pleurotus* are cosmopolitan, found naturally in tropical and subtropical rainforests and commonly known as oyster mushrooms. The versatility of cultivation of this species has led to a great demand and oyster mushrooms are now the third largest produced mushrooms in the world (Adebayo and Martínez-Carrera, 2015).

Just like *Pleurotus*, the basidiomycetes of the genus *Lentinus*, can be found mainly in tropical and subtropical forests and their consumption has been increasing worldwide due to their nutritional and therapeutic potential (Manjunathan and Kaviyarasan, 2010). Only a few species of *Lentinus* are cultivated, *Lentinus edodes* being the main one (Bisen et al., 2010). However, recent study shows that *Lentinus citrinus* cultivated in substrates containing vegetable wastes has nutritional value and can be included in the human diet as an innovative product source of protein, essential amino acids and fiber (Machado et al., 2015).

Besides their nutritional value, mushrooms are also becoming an attractive source of bioactive compounds. In recent years, many researches had shown that edible mushrooms are sources of many enzymes, including proteases (Mokochinsk et al., 2015). The proteases participate in various physiological functions and they are very important technological tools in several areas such as food, detergent and pharmaceutical industries (Nakamura et al., 2011).

In particular, proteases play an essential role in the food industry, acting as agents for modifying the functional properties of proteins, in the processing of cheese, in obtaining protein hydrolyzates, improving the flavor of some foods and also in baking (Inacio et al., 2015). These enzymes participate in some physiological processes such as sporulation, conidial discharge, germination, regulation of gene expression and protein turnover (Khaund and Joshi, 2014).

In many ecosystems, mushrooms play an important role in the decomposition of organic matter, cleaving the cellulose, hemicellulose and lignin from wood (Fonseca et al., 2014). Therefore, mushroom production is an extremely efficient alternative to recycling, reducing the environmental impacts arising from the disposal of organic wastes. The production of these macro fungi using agro wastes as substrates adds value to these low-cost products and allows the production of biomass with biological activity that can be use as food due to its high nutritional value. These residues also have a great potential for use as animal food and as fertilizer in agriculture (Ahmed et al., 2013; Fonseca et al., 2015; Sales-Campos and Andrade, 2011).

The pineapple is considered the third most important fruit in the world and besides the fruit consumption in natura, the pineapple is processed in order to obtain canned slices, crush and juice, which generates a great quantity of wastes (Bresolin et al., 2013). In the processing industries of pineapple, only a small percentage of the inflorescence is used, since the edible part represents about 22.5% of its volume (Silva and Zambiasi, 2008). The waste from the pineapple processing has nutritional constituents suitable for use in human food and is an example of debris that can be used for the cultivation of edible mushrooms (Martin et al., 2012).

The pineapple peel has superior nutritional content than the edible parts, with a high content of dietary fibers and even proteins. However, the pineapple peel has not received enough attention and nowadays it is used mainly in animal feed or as soil amendment (Fortkamp and Knob, 2014).

The objective of this study was to develop three different bioproducts formulated with pineapple peel and the mycelium of the edible mushrooms *Pleurotus albidus*, *Pleurotus florida* and *Lentinus citrinus* to be used in the food industry as an ingredient in the preparation of new products.

**MATERIALS AND METHODS**

**Mushrooms**

*P. albidus* DPUA 1692, *P. florida* DPUA 1534 and *L. citrinus* DPUA 1535 from DPUA Collection of Federal University of Amazonas-UFAM were cultured in Sabouraud agar supplemented with 0.5% yeast extract [w/v (SAB + YE)] for five days at 25°C in the absence of light, to obtain the matrice cultures.

**Inoculum preparation**

From the matrices of each fungus, 20 mycelial discs, measuring 10 mm, were taken and inoculated into 50 mL of BC-liquid medium (9.8% banana and 0.2% cupuaçu extract). The fermentation was conducted at 25°C, 150 rpm for five days. At the end of the fermentation, the biomass was separated from the supernatant by filtration on an aluminum tea sieve (diameter = 75 mm) for subsequent substrate inoculation.

**Semi-solid fermentation**

The fresh pineapple peels were washed in running water and immersed in sodium hypochlorite solution 2.5% (v/v) for 15 min. The peel was ground in a METIVISA® food processor and dried at 60°C in forced air oven for 12 h. The fermentation was carried out in 1000 mL glass flasks containing 100 g of dried pineapple peel, previously sterilized at 121°C, 60 min for two consecutive days. In each flask, all the mycelial mass of *P. albidus*, *P. florida* and *L. citrinus*, recovered from the liquid medium, were inoculated superficially in the substrate. The fermentation was conducted at 25°C until complete vertical myceliation of substrate. For each mushroom, three repetitions were performed.

The bioprodoot generated from semi-solid fermentation was crumbled and dried at 40°C in a forced air oven, followed by milling in a METIVISA® food processor and after that were sifted through an 18 mesh sieve and stored at room temperature in glass flasks (Bou Rached et al., 2006).
Determination of chemical composition

The pineapple peel and the bioproducts were subjected to analyses of moisture, lipid, protein, ash, carbohydrate and total energy. All tests were performed according to the procedures described by Ajayi et al. (2015). The moisture was determined by drying in a forced air oven at 105°C (pineapple peel) and at 40°C (bioproducts) (gravimetric method) until constant weight. The determination of the protein fraction was performed according to the micro Kjeldahl method, applying the conversion factor of 6.25 for the substrate and 6.82 for the fungi (Silva et al., 2002). Quantification of lipids was determined by Bligh and Dyer method and the ash was determined by the material incineration in furnace at 550 - 660°C until constant weight (AOAC, 2000). The crude fiber was determined by acid-basic digestion according to Weende methodology (AOAC, 2000). The total carbohydrates were estimated by difference and the total metabolizable energy was calculated by the conversion factor of Atwater, both recommended by Latinfoods (2011).

Determination of macro and micro minerals

The determination of minerals was performed according to the methods proposed by AOAC (2000). The samples were dried in a forced air oven at 40°C, then dehydrated and subjected to wet digestion HNO₃ + HCl O₃ (3:1). The phosphorus content was determined by spectrophotometry with molybdenum blue and calcium, magnesium, potassium, sodium, copper, iron, manganese and zinc 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 the values of micronutrients (Na, Fe, Cu, Mn, and Zn) in mg kg⁻¹.

Determination of amino acids

The determination of amino acid contents was performed by high performance liquid chromatography (HPLC). The samples underwent prior hydrolyzing with 6 N hydrochloric acid (HCl), followed by derivation of amino acids with phenylisothiocyanate (PTIC) and the separation of phenylthio-carbamyl amino acid derivatives in reverse phase column with UV detection at 254 nm. Quantification was performed by multilevel internal calibration, using γ-aminobutyric acid (AAAB) as internal standard for total amino acids (White et al., 1986). The determination of trypophan was performed after enzymatic hydrolysis with pronase and color reaction with p-dimethylamino benzaldehyde (DAB) according to Spies (1967).

Determination of microbiological quality

Microbiological analysis was performed based on determining the presence of yeast and mold and the most probable number of positive coagulase staphylococci and Bacillus cereus (MPN/g) (Beuchat and Cousin, 2001), total coliforms, fecal coliforms or Escherichia coli (Kornacki and Johnson, 2001) and Salmonella (ISO 6579:2002, 2002).

Protease extraction process

For protease extraction, 2 g of the crumbled bioproducts were added to 20 mL of sterile distilled water in 125 mL Erlenmeyer flasks and kept in a shaker at 30°C, 180 rpm. After 30 min, the crude extracts were recovered by filtration on cotton cloth, passed through a 0.22 µm membrane to remove the cells, and used as crude protease solution (Fonseca, 2014).

Determination of proteolytic activity of the flours

Proteolytic activity was determined using 150 µL of crude extract added to 250 µL of 1% azocasein (w/v), prepared in 0.1 M Tris-HCl buffer, pH 7.2. Samples and blanks were incubated at 25°C for 1 h in a dark chamber. The reaction was stopped with 1.2 ml of trichloroacetic acid (TCA) [10% (w/v)] and centrifuged for 10 min at 4°C. From the supernatant, 800 µL was removed and 1.4 mL of 1 M NaOH was added to it. The samples were prepared in triplicate and measured in a spectrophotometer at 440 nm. One proteolytic unit was defined as the amount of enzyme capable of producing an increase in absorbance at 440 nm of 0.1 in 1 h.

Statistical analysis

The results were submitted to descriptive statistics (mean and standard deviation) and also variance analysis (ANOVA) and the means were compared by Tukey test (p≤0.05), using Minitab 16.0 Software.

RESULTS AND DISCUSSION

Chemical composition

The results of the nutritional composition of pineapple peel and the three bioproducts enriched with mycelium biomass of P. albidus, P. florida and L. citrinus are shown in Table 1. Pineapple peel presented the highest moisture content (9.93%), while the bioproducts samples ranged from 4.0 to 5.0%. This significant moisture reduction favors nutrients concentration as well as decreases microorganism contamination. Similar conclusion was cited by Ackom and Tano-Debrah (2012) that studied the use of processed pineapple pulp as a dietary fiber supplement.

The ash content was significantly increased after substrate colonization by the mushrooms. The increased ash content was also verified by Okano et al. (2007) that noted an ash content increase from 38 to 74 g/kg after cultivating Pleurotus eryngii sugarcane bagasse.. Bento et al. (2015) observed a great ash content after cultivating Pleurotus ostreatus and Lentinus edodes in eucalyptus sawdust, eucalyptus bark, coffee bark, sugarcane bagasse, corncobs and coconut fiber.

The lipid content did not show variation, and the highest amount was determined in the bioproduct containing L. citrinus mycelium biomass. Close values were observed for Dundar et al. (2008) that studied nutritional composition of some mushrooms cultivated on wheat stalk; lipid content values obtained were 1.15, 2.60 and 7.50 g for Pleurotus sajor-caju, Pleurotus ostreatus and Pleurotus respectively.

The data in Table 1 also show a significant change in protein content in the bioproduct composed by Pleurotus Florida mycelium and pineapple peel. After the myceliation, the protein content was increased by 30%. The bioproducts made with P. albidus and L. citrinus
mycelium exhibited increase of 7.8 and 2.5%, respectively, and did not show significance in comparison with pineapple peel. Tuyen et al. (2013) also identified significant increase in protein content in corn straw, rice straw, oil palm leaf and sugarcane bagasse after 6 weeks of incubation with *P. eryngii*, *P. ostreatus* and *L. edodes*. Gonçalves et al. (2010) found 11-19% increase in the amount of proteins on different substrates colonized by *P. sajor-caju*, which is less than the values found in this study. Koutrotsios et al. (2014) observed the same behavior in the cultivation of *P. ostreatus* in grape marc plus cotton gin trash and olive mill by-products (leaves and two phase olive mill waste). The protein increase probably was due to the addition of fungal protein during the mycelial growth, indicating the mushrooms’ ability to contribute a deposit of proteins in the residue.

Regarding the fiber content, there was a significant increase after the mushroom myceliation in the three bioproducts analyzed. Gonçalves et al. (2010) found a similar behavior with increased fiber content in the colonized substrate. According to them these results can be explained due to the production of different enzymes during vegetative and reproductive stages of mushrooms, and the enzymes responsible for degradation of cellulose are secreted only in the reproductive phase. Thus, there was no significant reduction in fiber content in the bioproducts samples since fungi do not reach reproductive phase. Still, in the study of Gonçalves et al. (2010), the fiber contents decrease after production and harvesting of mushrooms. Being a potential source of dietary fibers, mushrooms raise the possibility of its inclusion in the highly competitive market of fiber-enriched food products, which seriously demands the exploration of alternative source and preparation methods of dietary fibers (Fernandes et al., 2015).

The carbohydrate content of the three bioproducts decreased significantly in comparison with the values found on the pineapple peel itself. This result can be associated with the heterotrophic habit of fungi that consume carbon sources to meet the nutritional requirements necessary for apical growth of the mycelium.

In this study, the differences between protein, fiber and carbohydrates contents of the bioproducts composed by *P. albidus*, *P. florida* and *L. citrinus* mycelium and pineapple peel might be due to growth conditions, genetic factors and also geographical variations (Saiqa et al., 2008).

**Mineral content**

The results of mineral content are shown in Table 2. Among the macronutrients, potassium showed the highest values in all samples evaluated, ranging from 15 to 17 g, followed by calcium, phosphorus and magnesium. Fonseca et al. (2015) also found a significant value for potassium (24 g.kg⁻¹) in *P. ostreatus*. Among the micronutrients, manganese content was higher, followed by iron, zinc and copper. Machado et al. (2015) had good values of potassium, phosphorus, iron, zinc and copper as in this study. Lee et al. (2009) and Medina et al. (2009) also observed increase in the concentration of minerals in the substrates after cultivation. According to Lee et al. (2009), the mineral increasing is probably due to the supply of mineral elements through moisture during the cultivation. Lee et al. (2009) found potassium and zinc values close to those found in this work (10.44 g/kg and 29 mg/kg, respectively). The amounts of phosphorus, potassium, copper and manganese in this study were higher than those found by Medina et al. (2009). Minerals are indispensable in human metabolism, part of important reactions, transmission of nerve impulses, bone development and regulation of salt and water balance (Okoro and Achuba, 2012).

**Amino acid content**

Table 3 shows the levels of essential and non-essential amino acids found in the bioproducts. The results showed that the amino acids threonine, valine, methionine and isoleucine did not differ significantly across the pineapple peel.
Table 2. Mineral concentration in pineapple peel before myceliation and in the bioproducts with mycelium.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Macronutrients (g.kg(^{-1}))</th>
<th>Micronutrients (mg.kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>Pineapple peel</td>
<td>1.21±0.01(^{a})</td>
<td>15.13±0.03(^{d})</td>
</tr>
<tr>
<td>Bioproduct made with <em>P. albidus</em> and pineapple peel</td>
<td>1.50±0.01(^{l})</td>
<td>16.76±0.01(^{h})</td>
</tr>
<tr>
<td>Bioproduct made with <em>P. florida</em> and pineapple peel</td>
<td>1.72±0.02(^{b})</td>
<td>16.90±0.01(^{h})</td>
</tr>
<tr>
<td>Bioproduct made with <em>L. citrinus</em> and pineapple peel</td>
<td>1.40±0.02(^{k})</td>
<td>15.99±0.01(^{c})</td>
</tr>
</tbody>
</table>

*Means not sharing a letter are significantly different.

Table 3. Amino acidic profile (in g.kg\(^{-1}\)) of the pineapple peel before myceliation and of the bioproducts made with peel and edible mushrooms (*P. albidus, P. florida* and *L. citrinus*).

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Pineapple peel</th>
<th>Bioproduct made with <em>P. albidus</em> and pineapple peel</th>
<th>Bioproduct made with <em>P. florida</em> and pineapple peel</th>
<th>Bioproduct made with <em>L. citrinus</em> and pineapple peel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine*</td>
<td>1.50±0.01(^{a})</td>
<td>1.70±0.01(^{a})</td>
<td>1.60±0.01(^{a})</td>
<td>1.60±0.01(^{a})</td>
</tr>
<tr>
<td>Valine*</td>
<td>2.00±0.01(^{a})</td>
<td>2.20±0.01(^{a})</td>
<td>1.90±0.01(^{b})</td>
<td>1.90±0.01(^{b})</td>
</tr>
<tr>
<td>Methionine*</td>
<td>0.40±0.01(^{a})</td>
<td>0.40±0.01(^{a})</td>
<td>0.40±0.01(^{a})</td>
<td>0.40±0.01(^{a})</td>
</tr>
<tr>
<td>Isoleucine*</td>
<td>1.00±0.01(^{a})</td>
<td>1.20±0.01(^{a})</td>
<td>1.10±0.01(^{a})</td>
<td>1.10±0.01(^{a})</td>
</tr>
<tr>
<td>Leucine*</td>
<td>1.20±0.01(^{b})</td>
<td>1.70±0.01(^{a})</td>
<td>1.70±0.01(^{a})</td>
<td>1.50±0.01(^{a})</td>
</tr>
<tr>
<td>Phenylalanine*</td>
<td>0.80±0.01(^{c})</td>
<td>2.80±0.01(^{a})</td>
<td>1.90±0.01(^{b})</td>
<td>2.10±0.01(^{b})</td>
</tr>
<tr>
<td>Lysine*</td>
<td>2.10±0.01(^{a})</td>
<td>0.60±0.01(^{b})</td>
<td>0.60±0.01(^{b})</td>
<td>0.50±0.01(^{b})</td>
</tr>
<tr>
<td>Tryptophan*</td>
<td>0.90±0.01(^{ab})</td>
<td>1.10±0.01(^{a})</td>
<td>0.80±0.01(^{b})</td>
<td>1.00±0.01(^{ab})</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>11.30±0.01(^{a})</td>
<td>1.10±0.01(^{b})</td>
<td>3.60±0.01(^{c})</td>
<td>3.70±0.01(^{c})</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>6.30±0.01(^{a})</td>
<td>4.70±0.01(^{b})</td>
<td>4.00±0.01(^{c})</td>
<td>4.10±0.01(^{c})</td>
</tr>
<tr>
<td>Serine</td>
<td>3.20±0.01(^{a})</td>
<td>2.90±0.01(^{b})</td>
<td>2.40±0.01(^{c})</td>
<td>2.40±0.01(^{c})</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.50±0.01(^{a})</td>
<td>2.30±0.01(^{ab})</td>
<td>2.10±0.01(^{b})</td>
<td>2.20±0.01(^{b})</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.60±0.01(^{a})</td>
<td>0.30±0.01(^{b})</td>
<td>0.30±0.01(^{b})</td>
<td>0.30±0.01(^{b})</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.70±0.01(^{a})</td>
<td>0.90±0.01(^{c})</td>
<td>1.20±0.01(^{b})</td>
<td>1.00±0.01(^{bc})</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.10±0.01(^{b})</td>
<td>2.90±0.01(^{a})</td>
<td>2.80±0.01(^{a})</td>
<td>2.80±0.01(^{a})</td>
</tr>
<tr>
<td>Proline</td>
<td>0.70±0.01(^{b})</td>
<td>0.90±0.01(^{b})</td>
<td>1.40±0.01(^{a})</td>
<td>1.20±0.01(^{a})</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.80±0.01(^{a})</td>
<td>0.90±0.01(^{a})</td>
<td>0.90±0.01(^{a})</td>
<td>1.00±0.01(^{a})</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.10±0.01(^{b})</td>
<td>0.10±0.01(^{a})</td>
<td>0.10±0.01(^{a})</td>
<td>0.10±0.01(^{a})</td>
</tr>
</tbody>
</table>

* Essential amino acids; **Means not sharing a letter are significantly different.

peel and bioproducts studied. All the essential amino acids are presented in the samples analyzed in this study. The most abundant essential amino in the pineapple peel was aspartic acid, whereas in the bioproduct, it was glutamic acid. The presence and relative abundance of
Determination of proteolytic activity

The results showed that all samples were negative for molds, yeasts, Salmonella sp., total and fecal coliforms, E. coli, coagulase positive Staphylococcus, mesophilic bacteria and Bacillus cereus. These results revealed that the samples were within the standards specifications and can be consumed as a safe food.

<table>
<thead>
<tr>
<th>Bioproducts</th>
<th>Proteolytic activity (u/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pineapple peel and Lentinus citrinus</td>
<td>296.55 ± 2.41^a</td>
</tr>
<tr>
<td>Pineapple peel and Pleurotus florida</td>
<td>280.55 ± 3.87^b</td>
</tr>
<tr>
<td>Pineapple peel and Pleurotus albidus</td>
<td>270.44 ± 1.39^c</td>
</tr>
</tbody>
</table>

*Means not sharing a letter are significantly different.

Microbiological analysis

The results showed that all samples were negative for molds, yeasts, Salmonella sp., total and fecal coliforms, E. coli, coagulase positive Staphylococcus, mesophilic bacteria and Bacillus cereus. These results revealed that the samples were within the standards specifications and can be consumed as a safe food.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES


