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

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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 albidus*, *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

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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 Kaviyaran, 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 used 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 bioproduct 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 4.38 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 $\text{HNO}_3 + \text{HCl O}_4$ (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 (PITC) 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 α -aminobutyric acid (AAAB) as internal standard for total amino acids (White et al., 1986). The determination of tryptophan 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 ($\alpha \leq 0.05$), 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*

Table 1. Mean of chemical composition of the pineapple peel before myceliation and the bioproducts made with pineapple peel and edible mushrooms (*P. albidus*, *P. florida* and *L. citrinus*).

Parameters	Pineapple peel	Bioproduct made with <i>P. albidus</i> and pineapple peel	Bioproduct made with <i>P. florida</i> and pineapple peel	Bioproduct made with <i>L. citrinus</i> and pineapple peel
Moisture (%)	9.93±0.10a	5.42±0.10b	4.09±0.11c	4.21±0.06c
Ash (g.kg ⁻¹)	34.08±0.09b	52.60±0.99a	57.70±0.04 ^a	60.10±0.02a
Lipids(g.kg ⁻¹)	14.70±0.12 ^a	13.80±0.27 ^a	13.20±0.15 ^a	18.60±0.31 ^a
Protein (g.kg ⁻¹)	79.80±0.49 ^b	86.10±0.52 ^b	102.70±0.45 ^a	81.90±0.01 ^b
Total Fiber (g.kg ⁻¹)	92.30±0.35 ^b	142.30±0.20 ^a	143.60±0.46 ^a	139.60±0.60 ^a
Carbohydrates (g.kg ⁻¹)	678.90±0.15 ^a	650.80±0.86 ^b	641.70±0.96 ^b	657.50±0.93 ^b
Calories (Kcal)	316.78±1.51 ^a	307.25±2.70 ^c	309.65±1.28 ^{bc}	312.51±1.31 ^c

*In each row, different letters means significant differences between the samples.

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

Table 2. Mineral concentration in pineapple peel before myceliation and in the bioproducts with mycelium.

Substrates	Macronutrients (g.kg ⁻¹)				Micronutrients (mg.kg ⁻¹)			
	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Pineapple peel	1.21±0.01 ^l	15.13±0.03 ^d	2.80±0.01 ^h	0.56±0.01 ^o	1.46±0.01 ^o	28.40±0.01 ^g	24.56±0.02 ^h	8.97±0.07 ⁿ
Bioproduct made with <i>P. albidus</i> and pineapple peel	1.50±0.01 ^j	16.76±0.01 ^b	3.94±0.01 ^f	0.82±0.01 ⁿ	10.10±0.10 ^l	27.93±0.01 ^d	59.47±0.02 ^b	22.10±0.02 ⁱ
Bioproduct made with <i>P. florida</i> and pineapple peel	1.72±0.02 ⁱ	16.90±0.01 ^a	4.38±0.01 ^e	0.90±0.01 ^m	10.03±0.01 ^l	25.57±0.01 ^e	63.92±0.02 ^a	21.07±0.01 ^j
Bioproduct made with <i>L. citrinus</i> and pineapple peel	1.40±0.02 ^k	15.99±0.01 ^c	3.75±0.01 ^g	0.81±0.01 ⁿ	9.13±0.010 ^m	24.98±0.01 ^f	57.69±0.01 ^c	20.85±0.01 ^k

*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*).

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

* 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

Table 4. Proteolytic activity of the three types of flours formulated with pineapple peel and edible mushrooms.

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

*Means not sharing a letter are significantly different.

essential amino acids in a protein is a major determinant of quality of the protein (Afiuka et al., 2015).

The bio-product of pineapple peel and *P. albidus* mycelium showed the highest tryptophan content (1.10 g.kg⁻¹) and the ones with *P. florida* and *L. citrinus* biomass in its composition had the highest proline content (1.40 and 1.20 g.kg⁻¹). This study also revealed glutamic acid as the most abundant amino acid in all samples, whereas the least occurring amino acid was cysteine. Similar result was found by Afiukwa et al. (2015) that observed the predominance of glutamic acid and the least abundance of cysteine in four edible mushroom species from Nigeria. Jaworska and Bernas (2011) also detected glutamic acid as the most abundant amino acid in mushrooms. Glutamic acid has an important role as brain stimulatory neurotransmitters and enhancing food flavor (Fonseca et al., 2015). Machado et al. (2015) observed amino acids values close to those presented in this study.

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.

Determination of proteolytic activity

The results showed the protease activity in all bioproducts samples (Table 4). Despite the fact that all the extracts have presented very significant enzymatic activity, the higher proteolytic activity was determined in aqueous extract obtained from the bioproduct with *L. citrinus* mycelium and pineapple peel (296.55 U/mL). The proteolytic activities in the bioproducts with *P. albidus* and *P. florida* biomass was 270.44 and 280.55 U/mL, respectively, with significant difference between all the three values of enzymatic activities found.

The results obtained for the genus *Pleurotus* were higher than those found by Fonseca et al. (2014), who evaluated various substrates with *P. ostreatoroseus*. The

values obtained for the edible mushroom *L. citrinus* was also superior to that obtained by Kirsch et al. (2011) who studied the mushroom of the same species. And still, values higher than that of protease activity (50.03 U/mL) was obtained by Khaund and Joshi (2014) that investigated the enzymatic profiling of wild edible mushrooms consumed by the ethnic tribes of India. Detailed studies on the pineapple peel proteolytic activity are currently under way.

Many enzymes produced by mushrooms are used in food processing for improvement of the quality and nutrition of food products. Ahmed et al. (2015) studied the influence of four different types of commercial enzymes on dough rheology and end quality of cookie. These authors found that proteases affect the degree of softening substantially, breaking the gluten network that was responsible for water retention, migration and evaporation during baking and protein denaturation.

From the results, it was concluded that the biotransformation of pineapple peel and the enrichment with biomass of *P. albidus*, *P. florida* and *L. citrinus* provides a bioproduct that can be used to increase nutritional and biological value of products as well as to create new features of the product and raise its quality, especially in bakery industry.

Conflict of Interests

The authors have not declared any conflict of interests.

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