



Research article

Evaluation of nutritional values of wild mushrooms and spent substrate of *Lentinus crinitus* (L.) FrLina R. Dávila G^{a,*}, Walter Murillo A^a, Cristian J. Zambrano F^a, Héctor Suárez M^b,
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ABSTRACT

In Colombia, despite the great diversity of mushrooms, most are yet unknown from the taxonomic point of view, and even less known from their nutritional composition or their possible application to obtain value-added products from agro-waste. The mycelial growth of *Lentinus crinitus* (L.) Fr strain was investigated on agro-waste in culture media agar and correlation analyses were performed. The proximate and mineral element composition was determinate in wild mushrooms and spent substrate of *L. crinitus*, obtained in the solid-state fermentation. The evaluation of the mycelial growth of the *L. crinitus* strain confirmed that it can grow on agro-waste. The treatment T6 (Orange peel and brand) was determined to be the best for the mycelial growth of *L. crinitus* (0.0790 cm/h), T7 (Bran, Orange peel and rice husk) and T5 (Rice hush and orange peel) followed, with mycelial growth rates of 0.0753 cm/h and .0720 cm/h, respectively. The growth rate was positively correlated with C/N ratios but negatively correlated with Zn, N and protein. The combination of the agro-waste (T6, T7 and T5) were used to obtain the spent substrate and assess its nutritional potential. The results showed that wild mushrooms of *L. crinitus* had protein contents of 14.42%, and fiber of 57.18%. The spent substrate of *L. crinitus* increased their protein content (10.5–11.22%), fiber (44.1–56%) and nitrogen (1.64–1.28%). These advances are promising for the use of *L. crinitus* as degrader of agro-waste to obtain different products of food and agro-industrial interest.

1. Introduction

Edible mushrooms are postulated as biological and genetic resources with nutritional value and biotechnological potential. They have high content in carbohydrates, phenolic compounds, vitamins, minerals and protein, that could be an alternative to the deficient intake of proteins of animal origin by many rural communities (Omarini et al., 2010; Ramos et al., 2019). They also provide polyunsaturated fatty acids known for their protective properties of the cardiovascular system (Sinanoglou et al., 2015). In addition, the mushrooms contain a very good proportion of dietary fiber, these, unlike cereals and given their chemical structure, exhibit immunostimulatory and anticancer activity (Cheung, 2013; Deng, 2013; Lemieszek and Rzeski, 2012). Other biological properties of the mushrooms are anti-tumor, anti-diabetic and anti-oxidant (Chaiyasut and Sivamaruthi, 2017; Meng et al., 2016; Nowacka-Jechalke et al., 2018).

Edible mushrooms are cultured in media that usually consist of lignocellulosic agricultural wastes such as crop straw (Huang et al.,

2019). The organic solid waste remaining after cultivation of edible mushrooms is the spent mushrooms substrate (SMS) (Gao et al., 2015). SMS can be used as compost, as animal feed, to promote health of animals, and to produce packaging and construction materials, biofuels, and enzymes (Grimm and Wösten, 2018). Hence, the mushroom cultivation is a value added process to convert these materials and represents one of the most efficient biological ways by which these residues can be recycled (Elenwo and Okere, 2007). This process can be used in regions with agricultural vocations. For example, Tolima-Colombia, is one of the largest generators of lignocellulose waste, being the rice crop one of the largest contributor with approximately 135,695 tons per year (Escalante et al., 2011).

In the present study, the mycelial growth of the *L. crinitus* strain was investigated under agro-waste and the correlation analyses were performed. The proximate and mineral element composition was determinate in wild mushrooms and spent substrate of *L. crinitus* obtained in the solid-state fermentation.

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2. Material and methods

2.1. Reagents, standards and culture media

Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany), agar (Merck, Darmstadt, Germany), element standard solutions supplied by Sigma-Aldrich (C, P, Ca, Mg, K, Mn, Fe, Zn, Cu), hydrochloric acid (HCL, Sigma-Aldrich), petroleum ether (Sigma-Aldrich, Heidelberg, Germany), lanthanum (III) oxide (La₂O₃, Sigma-Aldrich, Heidelberg, Germany). For the proximate composition, sodium hydroxide 35%, boric acid 4%, sulfuric acid 95–98% and kjeldahl catalyst (K₂SO₄, CuSO₄ * 5H₂O) were used as reagents (Sigma-Aldrich, Heidelberg, Germany).

2.2. Fungal material

Wild mushrooms of *Lentinus crinitus* (L.) Fr were collected in Ibagué, Tolima. Mushrooms were cleaned, dried in an oven for 8 h at 30 °C and transferred in paper bags for description of microscopic and macroscopic features (Gómez-Montoya et al., 2017; Largent, 1986; Mata and Ryvar-den, 2010; Ryvar-den, 1991).

2.3. Mycelium isolation

Fresh mushrooms were cut with sterilized blades, then a small piece of the mushroom tissue was removed and placed on PDA medium and incubated at 27 °C for 7 days; several sub-cultures were made until pure cultures were obtained (Tudses, 2017). The strain obtained was deposited in the Collection of Microorganism of Corporation for Biological Researches (MicroCIB).

2.4. Agro-waste

Three types of waste generated from the agroindustry of Tolima were used: Bran (B), Orange peel (OP) and Rice husk (RH). The wastes were dried at 48 h, 40 °C (Memmert, Schwabach, Germany). Wastes were crushed until obtaining a particle size of 0.1 mm in diameter, then stored for later use in the preparation of agar media.

2.5. Mycelial growth evaluation on different agro-waste in culture media

The mycelia growth of *L. crinitus* was evaluated in different agro-waste in culture media. Seven culture media agars (bran, orange peel, rice husk, rice husk and brand, rice husk and orange peel, orange peel and brand and brand, orange peel and brand) were use. The media were prepared using 200g of the substrate and 15 g of agar in one liter of deionized water with adjustment to pH 6. The culture media agar with agro-waste were sterilized at 15 psi, 121 °C for 20 min and dispensed into sterile Petri plates. After sterilization, plate per medium were aseptically inoculated with a 5 mm mycelial disc from a seven-day old pure culture of the *L. crinitus* and inoculated centrally onto each plated medium. The inoculated plated were sealed and incubated at 28 °C under dark conditions. The test was carried out in triplicate, and diameter of mycelial growth was measured. Each set-up was replicated three times (48–72 and 120 h). The diameter was measured at different angles of 0°, 45°, 90° and 135°, taking the center of the inoculum as an intersection (Martínez et al., 2015).

2.6. Solid-state fermentation (SSF): spent substrate

2.6.1. Spawn preparation

Solid spawn was prepared in 500 ml flask with 200 g of wheat seeds. The water content was adjusted to approximately 65% and then sterilized at 15 psi, 121 °C for 20 min. After cooling to room temperature, the solid spawn was inoculated with 3 mycelial agar discs of *L. crinitus* (5 mm in diameter). The solid spawn was incubated at 28 °C until the wheat seeds was fully colonized.

2.6.2. Substrate preparation and inoculation

Based on the results of mycelial growth in culture media agar, three best combination agro-waste were used. A total of 300 g of each of the agro-waste was used to fill a polypropylene bag, with a collar at the bag mouth. The filled bags were autoclaved at 120 °C and 1 atm for 120 min. After cooling, each bag was then inoculated with 5% of solid spawn of *L. crinitus*. After inoculation, all bags were incubated in the dark at 25–26 °C. Once the mycelium invaded the entire substrate (Spent substrate) the bags were stored at 4 °C for further used.

2.7. Proximate analysis and mineral element composition

Wild mushrooms of *L. crinitus*, spent substrate and agro-waste were used for determination of proximate analysis and mineral element composition. Protein, ash and crude fiber were determined according to AOAC, 1995. Protein was calculated from total nitrogen using the conversion factor 6.25 and the Kjeldahl method involving three steps (i.e digestion, distillation and titration). Ash content was evaluated by carbonization of the dried sample followed by incineration in a muffle furnace at 550 °C. The proximate composition was expressed in grams per 100 g of dry basis.

Mineral content was determined using the atomic absorption spectrophotometer, SHIMADZU AA-6300. Dry ashing of digestion procedure was applied (Tüzen, 2003). 1 g of dried sample was placed in a Furnace, 550 °C up to 2–4 h until ash was obtained. The residue was dissolved in 5 ml of HCL (10% v/v) and filtered into a 10 ml volumetric flask using Whatman filter paper (N° 42). This mixture was used for determine the following metals: Cu, Zn, Fe, Cd, Cr, Pb. 1 ml of the mixture was dissolved in 1 ml of La₂O₃ (10% v/v) and was transferred to 10-ml volumetric flask made up to volume. This mixture was used for the determined the following metals: Na, Ca, Mg y K. Certified standards of each metal were used to make the calibration curves. The spectrometer operating requirements were adapted according to the "Atomic absorption spectrophotometry cookbook" for each element.

2.8. Data analysis

Experimental results were given as the mean ± standard deviation (SD). All measures were performed at least three or two time and were subjected to two-way analysis of variance (ANOVA) followed by Fishers Least Significant Difference (LSD) test. Pearson correlation analysis was performed between the growth rate and the variables of the proximal and mineral composition of agro-waste These analyzes were performed using the R-studio program (<https://www.rstudio.com/>).

3. Results and discussion

3.1. Wild mushrooms of *L. crinitus*

The genus *Lentinus* is distributed worldwide, but species of the sub-genus *Lentinus*, specifically *L. crinitus*, are distributed throughout the Americas (Justo et al., 2015). For Colombia, *L. crinitus* has been reported in Amazonas, Antioquia, Caquetá, Cesar, Choco, Cundinamarca and Valle del Cauca, at an elevation of 50–2800 MASL (Vasco-Palacios and Franco-Molano, 2013). Ibague Tolima is given as a new locality record for *L. crinitus*.

Wild mushrooms of *L. crinitus* presented content of 14.42% protein and 57.18% fiber (Table 1), higher than the reported values for Rios Hurtado and Hicela Mosquera Mosquera (2015) and Silva-neto et al (2019). This protein content can be compared with products found in common grocery items such as beans, corn, rice and wheat, which contain protein values of 28%, 10.2%, 7.6% and 14.3%, respectively (Chapingo et al., 2006; Koziol, 1992) and other mushrooms as *Ganoderma lucidum* (8.59 %), *Lentinus brunneofloccosus* (8.13%) and *Pleurotus ostreatus* (9.56%) (Sharif et al., 2017; Zoho Bi et al., 2016).

Table 1. Proximate and mineral analysis of spent substrate (SMS) and wild mushroom of *L. crinitus* (dry basis %, mg/kg, w/w).

Variable	SMS-1	SMS-2	SMS-3	WM
% N	1.69	1.80	1.72	2.31
% Protein	10.54	11.28	10.77	14.42
% Fiber	44.11	56.33	56.17	57.18
% Ash	39.05	48.90	47.84	50.53
% C	35.35	29.64	30.25	28.69
% P	1.10	1.15	0.28	0.41
% Ca	0.014	0.007	0.005	0.25
% Mg	0.226	0.28	0.28	0.005
% K	0.701	8.75	4.28	4.04
Mn (mg/kg)	115.41	44.02	88.83	120.34
Fe (mg/kg)	71.03	78.76	99.00	129.26
Zn (mg/kg)	40.84	41.52	27.12	59.59
Cu (mg/kg)	93.25	10.99	7.21	11.23

Data are expressed as mean (n = 2). SMS: Spent mushroom substrate; -1 Rice husk and orange peel, -2 Orange peel and bran, -3 Rice husk, orange peel and bran. WM: Wild mushrooms of *L. crinitus*.

Table 2. Growth rate (GR) of *L. crinitus* in the treatments evaluated.

Treatment	Agro-waste	GR (cm/h)
T1	Bran (B)	0.0419 ± 0.0025 e
T2	Orange peel (OP)	0.0710 ± 0.0014 b,c
T3	Rice husk (RH)	0.0673 ± 0.0049 c
T4	RH + B	0.0563 ± 0.0021 d
T5	RH + OP	0.0720 ± 0.0023 b
T6	OP + B	0.0790 ± 0.0018 a
T7	RH + OP + B	0.0753 ± 0.0014 a,b

Data are expressed as mean ± sd (n = 3). Means with the same letter are not significantly different at $P > 0.05$. (Fisher's LSD method). Bran (B), Orange peel (OP) and Rice husk (RH).

3.2. Mycelial growth on agro-waste in culture media agar

Based on the results obtained from mycelial growth in culture media agar, there were significant differences ($P < 0.05$) between the different agro-waste. The treatment T6 (Orange peel and bran) was determined to be the best for the mycelial growth of *L. crinitus* (0.0790 cm/h), T7 (Bran, Orange peel and rice husk) and T5 (Rice husk and orange peel) followed, with mycelial growth rates of 0.0753 cm/h and 0.0720 cm/h, respectively (Table 2). This information is complemented with the Figure 1, which shows a larger mycelium diameter of *L. crinitus* in these treatments at 72 h. The Pearson correlation coefficient test detected strong positive and negative correlation among growth rate and agro-waste composition variables (Table 3). Growth rate was negatively correlated with Zn, N and protein, but it was positively correlated with C/N ratios (Figure 2). This preliminary test can be used to select the most appropriate agro-waste and define the variables that can affect mycelial growth, and therefore the yield and productivity of mushrooms in solid cultivation (Torres López et al., 2011). This ratio of C/N is essential for the growth of the fungus, especially because for its anaerobic growth, it is necessary to have high contents of C and low contents of N, and its value will depend on the type of strain (Sánchez and Roysse, 2001).

In addition, other elements such as zinc, sulfur, phosphorus, potassium and magnesium are essential for the growth of fungi as they are part of the synthesis of amino acids, nucleic acids, and ATP, and because they participate as cofactors in various enzymatic reactions among many other functions (Gow and Gadd, 1995). In spite of the fact that the correlation of components such as calcium, magnesium and manganese was evaluated, these did not show a correlation with the growth rate and other variables that must be included, such as the content of vitamins and amino acids present in agro-waste, which can influence mycelial growth (Marino et al., 2008).

3.3. Solid-state fermentation (SSF): spent substrate

The combination of the agro-waste (T6, T7 and T5) were used to obtain the spent substrate and assess its nutritional potential. The spent substrate by *L. crinitus* obtained in the SSF, increased its content of protein, fiber, nitrogen, phosphorus, manganese, iron, zinc and copper, as well as decreased the content of carbon, calcium and magnesium (Table 1). When comparing the obtained values of protein,

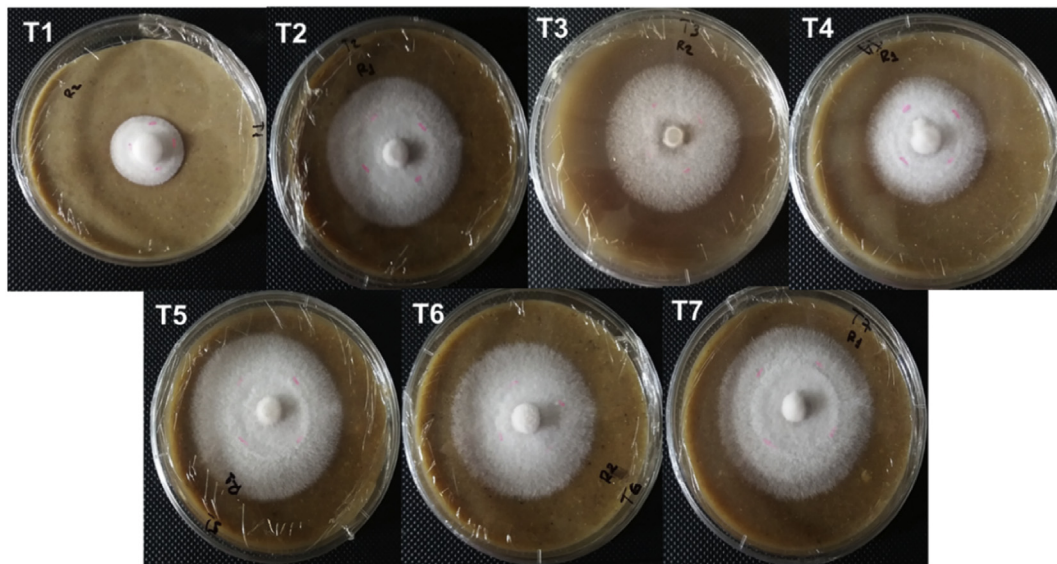


Figure 1. Mycelial growth of *L. crinitus* at 72 h in each of the treatments. T1 = Bran, T2 = Orange peel, T3 = Rice husk, T4 = Rice husk and bran, T5 = Rice husk and orange peel, T6 = Orange peel and bran, T7 = Rice husk, orange peel and bran.

Table 3. Proximate composition of agro-waste (dry basis %, mg/kg, w/w).

Variable	T1	T2	T3	T4	T5	T6	T7
% N	1.94	0.95	0.79	1.35	1.40	0.93	0.86
% Protein	8.4	8.6	3.1	6.2	8.75	5.81	5.36
% Fiber	15.2	10.3	38.1	28	22.333	38.626	42.061
% Ash	32.25	28.33	34.09	33.22	46.66	47.51	57.51
% C	26.22	29.11	25.32	23.52	55.220	32.520	33.353
% P	2.84	0.224	0.32	1.58	0.96	0.254	0.73
% Ca	0.55	1.37	0.58	0.59	0.095	0.254	0.246
% Mg	0.11	0.21	0.12	0.14	0.237	0.216	0.398
% K	0.31	0.27	0.07	0.19	9.556	0.507	1.443
Mn (mg/kg)	47.8	29	24	35.9	29.963	25.107	89.330
Fe (mg/kg)	28.4	22.4	32.3	30.35	61.365	111.000	44.577
Zn (mg/kg)	63.5	15.5	29.6	46.55	38.711	9.892	33.925
Cu (mg/kg)	0.55	1.56	1.13	0.84	1.793	6.474	11.330
C/N	13.51	30.64	32.05	17.42	39.443	35.003	38.881

Data are expressed as mean (n = 2). T1 = Bran, T2 = Orange peel, T3 = Rice husk, T4 = Rice husk and bran, T5 = Rice husk and orange peel, T6 = Orange peel and bran, T7 = Rice husk, orange peel and bran.

fiber and nitrogen, these increased by 10–20% in comparison with the negative controls (Agro-waste without inoculum mycelium). In each of the treatments, the values were similar and can be compared with other spent mushroom substrates of *Pleurotus* species, where they presented protein values of 15.7–29% (Pathmashini et al., 2008) or 26.9–59.4% (Koutrotsios et al., 2014), similar to those obtained in this investigation.

These increases in the nutritional composition of the substrates suggests an alternative biotransformation of agro-waste for the

generation of aggregate products as a source of animal protein; especially because the use of agro-waste for animal feed is limited by low digestibility and availability of nutrients (Sánchez and Royse, 2001). The treatments to reduce the lignin, and to increase the digestibility of the cellulose remaining in said residues, are highly expensive (Pandey and Soccol, 2000). This biotransformation of agro-waste by mushrooms is considered the only technology that allows human and animal food to be obtained (Bermúdez Savón et al., 2014).

Other studies show that spent substrate by mycelium of *Pleurotus ostreatus* and *Pleurotus eryngii*, have high percentages of primary nutrients, useful to fertilize or being used as soil conditioners (Alberto et al., 2013). This alternative has a great advantage of containing the necessary elements to guarantee the balance of the microbiota and the induction of resistance for the plants. For example, spent substrates of *Pleurotus ostreatus* and *Agaricus bisporus* have been evaluated as soil biofertilizers for the growth of pepper plants (*Capsicum annum* L), demonstrating that these substrates have a positive effect on plant growth, as well as the mobilization of soil phosphate, an increase in chlorophyll content, and protein content in leaves and fruits (Roy et al., 2015).

The results obtained from spent substrate with *L. crinitus*, suggests an alternative for the transformation of agroindustrial waste from the department of Tolima, in order to generate value added products.

4. Conclusion

Mushrooms of *L. crinitus* are reported for the first time in Tolima, with a great nutritional potential, especially because these can be used as a food source, given their protein, fiber and mineral contents. In addition, given the capacity of the *L. crinitus* mycelium to transform agro-waste with high protein, fiber and nitrogen values, these can have a potential to be used as a source of animal feed or soil conditioners.

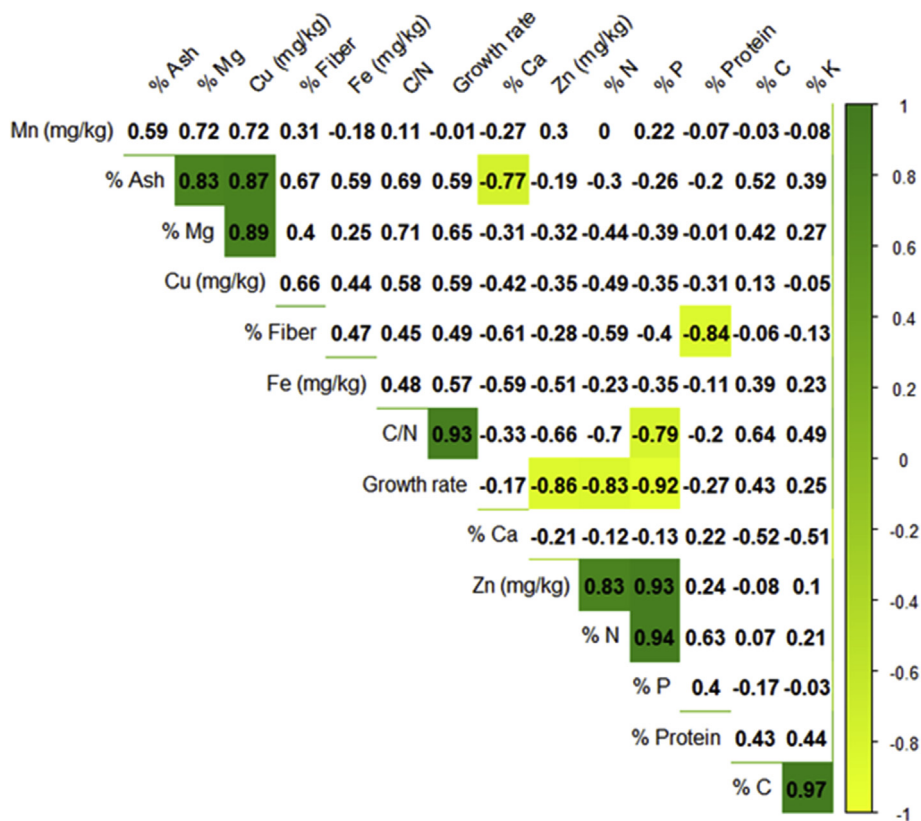


Figure 2. Correlation matrix of Pearson between growth rate (GR) and agro-waste composition variables. Light green color indicates negative relation of the variables, dark green-inverse correlation. Only significant correlations are filled with color (confidence = 0.95).

Declarations

Author contribution statement

Lina R. Dávila G: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Walter Murillo A: Conceived and designed the experiments; Wrote the paper.

Cristian J. Zambrano F: Conceived and designed the experiments.

Héctor Suárez M: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Jonh J. Méndez A: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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