Bioconversion of agroindustrial residues by *Pleurotus ostreatus* cultivation

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ORIGINAL

Bioconversión de residuos agroindustriales a través del cultivo de *Pleurotus ostreatus*

Resumen. Bioconversion of rice straw (*Oriza sativa*), coffee pulp (*Coffea arabica*) and banana leaves (*Musa sapiensis*) after *Pleurotus ostreatus* cultivation was evaluated. Nutrient availability and digestibility assays were performed both in fresh substrates and residues. Changes within substrates were analyzed through t (student) paired tests. Coffee pulp showed a decrease in lignin, cellulose, organic cell wall and low digestibility fractions ($P<0.05$); an increase in the C/L ratio, organic cell content and IVDMD but no change on the available protein, hemicellulose and high digestibility fraction. For banana leaves, available protein, lignin, cellulose hemicellulose, organic cell wall contents and the low digestibility fraction decreased ($P<0.05$); organic cell content and the high digestibility fraction showed a significant increase; C/L ratio and IVDMD showed no change. A decrease in the available protein, organic cell wall and low digestibility fraction was observed for rice straw ($P<0.05$). Organic cell content and the high digestibility fraction exhibited a significant increase.The rest of the response variables had no change on this substrate. Lignin reduction indicated a positive bioconversion effect over coffee pulp and banana leaves. Changes in organic cell contents, organic cell wall and low digestibility fraction indicated a positive bioconversion effect for all substrates.

Key words: *Pleurotus ostreatus*, fresh substrates, residues, nutrient availability, digestibility.

Abstract. Se evaluó la bioconversión de la paja de arroz (*Oriza sativa*), pulpa de café (*Coffea arabica*) y hojas de banano (*Musa sapiensis*) por efecto del cultivo de *Pleurotus ostreatus*. Los cambios en la disponibilidad de nutrientes y la digestibilidad dentro de cada sustrato fresco y residuos se analizaron con pruebas de t pareadas. Los contenidos de lignina, celulosa, pared celular orgánica y fracción de baja digestibilidad disminuyeron en la pulpa de café ($P<0.05$); la razón C/L, el contenido celular orgánico y DIVMS aumentaron ($P<0.05$). No hubo cambios en las demás variables. En la hoja de banano, la proteína disponible, lignina, celulosa, hemicelulosa, pared celular orgánica y fracción de baja digestibilidad disminuyeron ($P<0.05$). El contenido celular orgánico y la fracción de alta digestibilidad aumentaron ($P<0.05$). No hubo cambios en la razón C/L y DIVMS. En la paja de arroz, la proteína disponible, pared celular orgánica y fracción de baja digestibilidad disminuyeron ($P<0.05$); el contenido celular orgánico y la fracción de alta digestibilidad aumentaron ($P<0.05$). Las demás variables no mostraron cambios. Los cambios en el contenido celular orgánico, pared celular orgánica y fracción de baja digestibilidad en los tres sustratos y la reducción de la lignina en la pulpa de café y hoja de banano indican efectos de bioconversión positivos.

Palabras clave: *Pleurotus ostreatus*, sustratos frescos, residuos, disponibilidad de nutrientes, digestibilidad.

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Introduction

Edible mushrooms are able to bioconvert a wide variety of lignocellulosic materials due to the secretion of extracellular enzymes [3, 11]. Literature reports that the bioconverted materials show an increase in their protein contents and a decrease in their fiber content[10,15] being possibly used as ruminant feed supplements [4, 5].

Traditionally, the evaluation of the quality of the converted materials is under the same criteria as those applied to the evaluation of conventional forages [14]. Nutrient availability is evaluated by chemical analyses such as dry matter, ash, protein, lignin, cellulose and hemicellulose determinations. Digestibility is evaluated through *in vitro* and *in vivo* assays and palatability suppose feed selection trials with whole animals [1, 8].

There is an increasing tendency to avoid animal experimentation. This implies the usage of even more indirect assays for the estimation of digestibility. For instance, Japan Livestock Technology Association recommends an enzymatic fractionation of dry matter into cell contents and cell wall. In turn, these are also fractionated in organic cell contents and organic cell wall. Cell wall is further divided into high digestibility and low digestibility fractions. Organic cell contents and the high digestibility fraction are assigned with a 100% and 95-100% digestibility respectively. Depending on the assayed material, the low digestibility fraction may have from 40% to 50% digestibility [9].

With respect to the protein content it should be quoted that most of the work in biodegradation of lignocellulosic wastes discusses the changes on the total protein content as obtained by Kjeldahl analysis. Nevertheless the protein fraction potentially available to cattle is that fraction neither covalently associated with lignin nor compromised in Maillard polymers [14]. Therefore our research group considered important to judge the quality improvement of a biodegraded substrate in terms of the available protein contents.

Large quantities of rice straw, coffee pulp and banana leaves are produced as agricultural wastes in Panama. These wastes are not well managed. Even though rice straw is known to be used in soil preparations, this application is not documented [6].

The cultivation of edible mushrooms on rice straw, coffee pulp and banana leaves may generate an additional value to fundamental produces. If the converted substrate proves to have the appropriate characteristics so as to be used in cattle feeding, then local agrobusiness would grow into a sustainable activity.

The objective of our research group was to perform a first approach on the bioconversion of the above mentioned agricultural by-products from the Republic of Panama after cultivation of a *Pleurotus ostreatus* strain.

Materials and methods

Pleurotus ostreatus cultivation

P. ostreatus (RN 8) was obtained from the culture collection of the Natural Resources Laboratory, Province of Chiriquí, Republic of Panama. The fungus was maintained at 23 ± 1 °C in potato dextrose agar media (PDA) with periodic transfers.

Substrates used in this experiment were rice straw, coffee pulp and banana leaves, obtained from different cultivars at the Province of Chiriqui, Republic of Panama. Spawn and substrate preparation, seeding, incubation and cropping stages were done accordingly to Guzmán *et al*. [7]. For pasteurization three baskets of 20 kg (wet substrate) were used. Each basket was divided into 10 kg sub-samples (see below for substrate sampling procedure). A 5% inoculation level was used on a total of 30 bags of 2 kg each (wet substrate) for each substrate.

Substrate sampling and chemical analyses

Substrate sampling was done in two different substrate stages: fresh substrate and residue. This residue corresponds to the spent substrate collected after two flushes within 15 days. The same hierarchy of baskets and sub-samples was applied to the fresh substrate. A quartering method was applied in order to assure proper sampling on both stages. The sub-samples were analysed as follows: lignin, acid detergent nitrogen, cellulose and hemicellulose were assayed according to the Japan Livestock Technology Association 2000 [9]. Available protein was calculated as the difference between the total protein obtained by Kjeldahl analysis [2] and the acid detergent nitrogen using a 6.25 factor. The cellulose to lignin ratio was also obtained.

Digestibility

In vitro dry matter digestibility (IVDMD) was done according to Tilley and Terry (1963) [13]. Dry matter fractionation was done by means of an enzymatic analysis. Samples were treated with á amylase and actynase and fractionated into cellular contents (CC) and cell wall (CW) that render upon incineration organic cell contents (OCC), organic cell wall (OCW). Cell wall was further fractionated into a high digestibility fraction (Oa) and a low digestibility fraction (Ob) by cellulase treatment [9].

Experimental design and statistical analyses

Data was analysed by means of a hierarchic model and random sampling techniques. Lignin, cellulose, hemicellulose and available protein contents, cellulose to lignin ratio (C/L ratio), organic cell wall (OCW), organic cell contents (OCC), high digestibility fraction (OA), low digestibility fraction (OB) and in vitro dry matter digestibility (IVDMD), were considered as response variables. Each variable was analysed with the proposed model both in the fresh substrates and the residues. The difference between means within each substrate was evaluated with t (student) paired tests [12]. Observations were generated for lignin and cellulose and then used to calculate a C/L ratio. The C/L ratios were analysed according to the statistical model; adjusted means and their standard error were calculated.

Table 1. Response variables means and standard error within substrates.

Variable	Rice straw		Coffee pulp		Banana leaves	
	Fresh	Residue	Fresh	Residue	Fresh	Residue
Lignin	5.74±0.63[a]	9.73±1.04[a]	20.97±0.73[a]	13.84±0.70[b]	11.75±0.63[a]	9.07±0.70[b]
Cellulose	33.91±1.24[a]	15.44±4.97[a]	36.41±1.43[a]	29.11±3.85[b]	23.15±1.24[a]	18.86±3.85[b]
Hemicellulose	26.80±2.94[a]	24.14±6.35[a]	5.05±3.39[a]	2.60±4.92[a]	19.10±2.94[a]	11.96±4.92[b]
C/L ratio	6.21±0.47[a]	2.51±0.23[a]	1.75±0.55[a]	2.11±0.15[b]	1.98±0.47[a]	2.24±0.15[a]
Available protein	8.96±0.66[a]	6.90±0.38[b]	10.17±0.65[a]	11.67±0.38[a]	10.68±0.65[a]	7.98±0.38[b]

Mean values in fresh and residue stages for each substrate with no common superscript differ (P<0.05).
All percentage values (dry weight basis).

Results and discussion

Table 1 shows means comparison within substrates through t (student) paired test for the chemical parameters or response variables.

After 60 days of cultivation period, there was not a significant change in the available protein and hemicellulose contents for coffee pulp (P>0.05), whereas the C/L ratio showed a significant increase (P<0.05). In turn, lignin and cellulose exhibited a significant decrease (P<0.05).

For banana leaves, the available protein content showed a significant decrease, as well as the lignin, cellulose and hemicellulose contents (P<0.05). The C/L ratio did not show significant changes on this substrate (P>0.05).

Available protein content showed a significant decrease on rice straw (P<0.05), while no significant changes were seen on the lignin, cellulose and hemicellulose contents, as well as on the C/L ratio.

In terms of the chosen chemical indicators or response variables the process had an evident bioconversion effect over coffee pulp, except for the available protein and hemicellulose contents. For banana leaves, these response variables also evidenced a bioconversion process except for the C/L ratio. Results for the fibrous components for rice straw did not support a bioconversion effect. The observed decrease on the available protein showed a negative bioconversion effect over rice straw and banana leaves, since an increase in the availabe protein content may be expected after mushroom growth. A more detailed study is needed.

Present results in coffee pulp and banana leaves with respect to the lignin content could be regarded as positive from the standpoint of feed quality. Ruminants are adapted to food sources which are fiber rich. Fiber quality is judged partly in terms of the degree of delignification since lignin limits the availability of cellulose, hemicellulose and nitrogen [4, 14]. In turn, cellulose to lignin ratios can be used as delignification indicators. Our results show a positive bioconversion effect over coffee pulp in terms of fiber quality and degree of delignification.

Table 2 shows means comparison within substrates through t (student) paired tests for the digestibility response variables.

After 60 days of mushroom cultivation coffee pulp showed significant changes for OCC, OCW and Ob (P<0.05). These changes are positive from the standpoint of digestibility improvement since it is expected to have an increase in the OCC and a decrease in the low digestibility fractions due to mushroom growth. Oa showed no significant change on this substrate (P>0.05). IVDMD in turn, showed a significant increase (P<0.05). Therefore for coffee pulp the results for the dry matter fractionation analysis agree with those for IVDMD.

Banana leaves exhibited a significant increase in OCC and Oa (P<0.05). OCW and Ob showed a significant decrease. These results indicate a positive bioconversion effect over this substrate. No change on the IVDMD was observed.

There was a significant increase (P<0.05) on OCC and Oa for rice straw. OCW contents and Ob exhibited significant decreases (P<0.05) on this substrate. These results are positive indicators of the bioconversion of rice straw and suggest an improvement tendency on digestibility. Nevertheless IVDMD had no change. Comparing results from Table 1 and Table 2 for rice straw, it can be said that dry matter enzymatic fractionation variables showed a bioconversion effect over rice straw.

There was a bioconversion effect due to mushroom growth for all substrates. Also there was a quality improvement in terms of the lignin content reduction for coffee pulp and banana leaves. In the case of coffee pulp the increase in the C/L ratio suggests a degree of delignification.

The cultivation process was also positive in terms of the increase in organic cell contents and the decrease in the organic cell wall and the low digestibility fraction. For rice straw and banana leaves results for Oa also reflect a positive effect. The process also improved the IVDMD for coffee pulp.

Finally, the bioconversion effect due to mushroom growth over rice straw was observed by means of the response variables obtained through the dry matter enzymatic fractionation.

It is advisable to develop experimental designs to correlate IVDMD assays with other assays such as the enzymatic fractionation of dry matter.

Other strains and substrates should be evaluated. It is also important to take into account other substrate related factors such as particle size, and the evaluation of different parts of the plant material. Age should be observed, specially for rice straw.

More research should be done with respect to the consideration of the available protein, as well as correlation studies between biological efficiency and the degree of bioconversion of the substrates.

For coffee pulp it is necessary to evaluate changes in the presence of anti-physiological substances such as tannins and caffeine.

Table 2. Digestibility response variables means and standard error within each substrate.

Variable	Rice straw		Coffee pulp		Banana leaves	
	Fresh	Residue	Fresh	Residue	Fresh	Residue
OCC	21.24±0.69[a]	35.32±1.92[b]	26.47±0.69[a]	45.88±1.92[b]	17.70±0.69[a]	51.66±1.92[b]
OCW	67.89±1.00[a]	51.34±1.55[b]	62.65±1.00[a]	52.26±1.55[b]	76.26±1.00[a]	56.80±1.55[b]
O_A	2.31±1.37[a]	11.51±0.83[b]	0.42±1.37[a]	1.17±0.83[a]	0.10±1.37[a]	7.78±0.83[b]
O_B	65.58±1.01[a]	39.82±1.03[b]	62.23±1.01[a]	51.09±1.03[b]	76.15±1.01[a]	49.02±1.03[b]
IVDMD	37.17±2.55[a]	32.25±1.51[a]	21.56±2.55[a]	37.88±1.51[b]	36.14±2.55[a]	36.96±1.51[a]

Mean values in fresh and residue stages for each substrate with no common superscript differ (P<0.05).
All percentage values (dry weight basis).
OCC=organic cell contents; OCW= organic cell wall; O_A=high digestibility fraction; O_B= low digestibility fraction; IVDMD= in vitro dry matter digestibility.

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