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Bioconversion of Pear Pomace by Strains of *Pleurotus Ostreatus* During Mycelial Development

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Abstract

The cultivation of edible fungi is an ecological bioconversion system since it allows us to take advantage of lignocellulosic waste. In this way, the pear processing industries generate waste that is called pomace. Nevertheless, these kinds of residues could be considered the basement of substrate for these mushroom growths. Hence, the focus of this study was to analyse the modify of the content of different chemical components in pear pomace while the mycelial growth of Pl-P and Pl-J take place. These are two kinds of strains of *P. ostreatus*. Thus, the inoculated substrate was incubated in Petri dishes at 28°C in the dark for 8 weeks. A decrease in dry matter was observed, obtaining a 30% average bioconversion for the analysed strains. The chemical characterisation of the biodegraded pomace showed a significant decrease in all tested variables. The net content of non-structural carbohydrates had the greatest decrease (approximately 60%) in the analysed period. The content of dry matter, organic matter, crude protein, hemicellulose, and lignin was reduced to a lesser extent. The development of emerging end-of-pipe technologies and applications aimed at reducing the negative impact on the environment due to the accumulation of waste generated by industrial activities should be considered.

Keywords: biodegradation, agro-industrial by-products, lignocellulosic waste, ligninolytic microorganisms, *Pleurotus ostreatus*.

1. Introduction

The cultivation of edible mushrooms can be considered an ecological bioconversion system since it allows the production of food, enzymes, and physiologically active metabolites and provides technological tools to recycle agricultural and agro-industrial waste. Likewise, the biodegraded substrate can be used for animal feed and as organic fertiliser, thus reducing environmental pollution (Koutrotsios *et al*., 2014; Barshteyn & Krupodorova, 2016; Bellettini *et al*., 2019; Sadik *et al*., 2021).

The capacity of *Pleurotus* for generating extracellular enzymes makes that they can be cultivated on a vast diversity of lignocellulosic by-products, and they classified into the group of white-rot fungi (WRF). In this way, WRF have unique capabilities to depolymerise, cleave carbon–carbon linkages, and mineralise lignin to CO_2 and H_2O. Due to this capacity, WRF play a key role in carbon recycling in terrestrial ecosystems (Isroi *et al*., 2011; Anike *et al*., 2016; Yang *et al*., 2020; Del Cerro *et al*., 2021).

The raw material used as a substrate for the cultivation and production of these edible mushrooms includes cereal straw, leaves, plant crop residues destined for industrial use, and products result of agro-industries activities, such as oilseeds, distillation of different resources, sugar cane process, and factory of wood cut (Koutrotsios *et al*., 2014; Martínez *et al*., 2015; Barshteyn & Krupodorova, 2016; Rodríguez *et al*., 2018).

As well the mushrooms employ the substrates as nutrients sources for this reason the degrades materials are called "spent" mushroom substrate (SMS). Meanwhile, these elements are exhausted, the fungi grow. SMS is a lignocellulosic biomass that contains fungal mycelium and the biodegraded substrate. Currently, it is considered a valuable waste, as a source of raw material for new processes, but it is still named SMS (Ribas *et al*., 2009; Sánchez, 2010; Foluke *et al*., 2014; Koutrotsios *et al*., 2014; Hanafi *et al*., 2018).

SMS has been successfully reused as a component of substrate mixtures in the production of horticultural seedlings, according to Lopes *et al*. (2015); Moraes *et al*. (2020) in tomato (*Solanum lycopersicum*) and Liu *et al*. (2018) in lettuce (*Lactuca sativa* L.). Other authors have investigated the use of SMS as a diet feed supplement for broilers (Foluke *et al*., 2014, Cayolo *et al*., 2019, Bandara *et al*., 2021).

Previously, the authors of this work evaluated the mycelial growth of three strains of *Pleurotus ostreatus* and one strain of *Agrocybe aegerita* for their ability to colonize pear pomace generated by pear juice production plants from Río Negro (Argentina). In this study, the strains of *A.*

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aegerita analyzed showed a lower rate of colonisation than *P. ostreatus* (Martínez *et al*., 2015).

The relationship between the physical and chemical factors has variable effects in the colonization and fructification phases due to the influence of some conditions such as pH, temperature, time of cultivation, substrate composition, kind of inoculated strain and others. For this reason, it is important to determine these technical aspects to achieve adequate substrate management and an efficient production system (Sánchez, 2009, 2010; Pineda-Insuasti *et al*. 2015; Heredia-Solís *et al*., 2016; Bellettini *et al*., 2019).

In the Patagonia of Argentina, just on the Valle Medio y Alto Valle of Río Negro province, there are food agro-industries systems destined to produce concentrated juice, cider, dehydrated products preserves and liqueurs. This industry generates a large amount of a by-product known as bagasse of apple and pear, which is the product obtained (25–30% of the total weight of the fresh fruit) mainly from the pulp / peel (95%), seeds (2–4%), and peduncle (1%), as described by Bhushan *et al*. (2008). The pomace or bagasse presents a high content of lignocellulosic carbon sources, in addition to soluble simple sugars.

The objective of this study was to evaluate the changes in the chemical composition of the pear pomace during mycelial growth by Pl-P and Pl-J which are two different strains of *P. ostreatus*.

2. Materials and Methods

2.1. Fungal strains

Two strains of *Pleurotus ostreatus* (Jacq.: Fr.) Kummer were used. The strains were obtained from Cepario FACA-UNCo, Laboratorio de Hongos Comestibles y Medicinales:

Pl-J: Misiones, Argentina. Collector: Rodríguez G. FACA Pl-J 07.

Pl-P: Paraje Abra Ancha, Neuquén, Argentina. Collector: Rodríguez G. FACA Pl-P 12.

The strains have been maintained in malt extract agar (10% w/v) at 4°C in the dark. Meanwhile, the multiplication to obtain active mycelium discs has carried on potato dextrose agar (PDA) which has been formulated by an infusion of potato, agar-agar and D (+) glucose. (Merck, 2005).

2.2. Substrate

The characterization of the biodegradation process by *P. ostreatus* took place over pear (*Pyrus communis* L.) v. 'Williams Bon Chretien' pulp as a substrate. The wet bagasse of pears produced by company Jugos S.A. was first sundried on a rectangular mesh. Afterwards, dried substrate was crushed in a commercial grain grinder until a particle size between 0.1 to 0.5 mm in diameter was obtained. This company is in the Alto Valle of Río Negro Province in the Patagonian region of Argentina.

2.3. Mycelial growth

The dried and ground pomace was hydrated at 70% (w/w) with distilled water and placed in Petri dishes (approximately 30 g/dish). The Petri dishes (90 mm diameter) were sterilised in an autoclave for 30 minutes at 1 atm of pressure and kept sealed in a refrigerator at 4°C until inoculated. Subsequently, active mycelium discs with a diameter of 10 mm have inoculated into plates containing hydrated and sterile pomace. The inoculation process has carried on under a laminar flow cabinet, ensuring the environment and materials have been germ-free. Later, the Petri dishes with inoculated substrate have incubated in a culture stove at 28°C and environmental darkness for eight weeks. Thus, after this time, the Petri dishes have been stored at -20°C until their chemical characterization.

2.4. Physicochemical analysis of pomace

The chemical composition of the pear pomace was analysed before inoculation with the Pl-P and Pl-J strains (initial) and after eight weeks of incubation (final). At the end of the incubation period, the substrate contained mainly degraded lignocellulosic materials impregnated with fungal mycelium.

The substrates (initial and final) were dried to a constant weight at 65°C to obtain the dry matter content. The final product has been ground to pass through an l-mm screen in a Wiley grinder (Thomas Scientific, Swedesboro, NJ, USA), and the samples of each substrate were analyzed.

The total ash content (Ash) was determined by incineration in a muffle at 550°C; non-structural carbohydrates (NSCH) were determined by the Antrona method (Koehler, 1952). The crude protein (CP) content was determined by the Kjeldahl method (CP = N x 6.25) as well as acid and neutral detergent fibres (NDF and ADF) and acid detergent lignin (ADL = LIG) were determined by the sequential method of Van Soest *et al*. (Van Soest *et al*., 1991). The bioconversion efficiency was estimated as the decrease in the initial dry matter, expressed as a percentage. The organic matter content (OM) was obtained by the difference between dry matter (DM) and ash (OM = DM – Ash). Organic carbon (OC) was calculated as OM / 1.724 (AOAC, 2000). The net cellulose (CEL) and hemicellulose (HCEL) contents were calculated as CEL = ADF – ADL and HCEL = NDF – ADF, respectively (Van Soest *et al*., 1991).

2.5. Experimental design and statistical analyses

An experimental design with two factors and two levels was considered to characterise the biodegradation process during the mycelial development of two strains of *P. ostreatus*. For this, the following were considered:

- Strains: Pl-P and Pl-J
- Moments: Initial (not biodegraded) - Final (biodegraded after eight weeks)

The tests were carried out with 5 replications of each sample.

The analysed variables were N (g); C (g), C/N; OM (g), CP (g), NSCH (g), HCEL (g), CEL (g), and LIG (g).

The means of the treatments or their linear combinations were evaluated by analysis of variance (ANOVA) and subsequently contrasted using the multiple comparisons method proposed by Fisher (LSD Fisher) with a significance level of $\alpha = 0.01$. The normal distribution of the data was previously verified by applying the Shapiro-Wilks test (Di Rienzo *et al*., 2012).

3. Results

3.1. ANOVA model

A 2x2 full factorial experiment was considered in the treatment design; that is, two factors (Strain and Moment) with two levels each. In the first instance, the level of significance of each source of variation and the possible interaction (Strain * Moment) in the model for each of the response variables was evaluated using the value of *p*.

For each observation (Y), the model that was subjected to statistical analysis was:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_{ij} + \varepsilon_{ijk}$

where $i = 1,2$; $j = 1,2$; $k = 1,..,5$

Y_{ijk} represents the response of the k-th repetition at the i-th level of factor A (Strain) and the j-th level of factor B (Moment), μ represents a general mean, α_i is the effect produced by the i-th level of factor A, β_j corresponds to the effect of the j-th level of factor B, δ_{ij} is the additional effect (interaction) for the combination of levels i of factor A and j of factor B and ε_{ijk} is the associated random error to the ijk-th observation (Di Rienzo *et al*., 2012).

Table 1 shows the results obtained from the statistical analysis by means of an ANOVA for one of the dependent variables, DM, using models with and without interaction. Similar results were observed for the rest of the variables (data not shown).

Table 1. Analysis of the variance (type III SoS) for the variable dry matter expressed in g (DM, g), considering the Moment of sampling as factors and Strains as classification variables, with the interaction Strain * Moment and without strain (additive bifactorial model). N = 20, $R^2 = 0.72$.

Variation source	Interaction	*p* value
Model	Yes	0.0001
	No	< 0.0001
Factor A: Strain	Yes	0.6018
	No	0.5912
Factor B: Moment	Yes	< 0.0001
	No	< 0.0001
Strain * Moment	Yes	0.7882

The *p* value <0.0001, less than the nominal significance level of the test ($\alpha = 0.01$), for the effect of the Moment factor implies that it had a statistically different effect from zero on the average chemical composition of the substrate. The results obtained also suggest that there were no effects associated with the interaction of the Strain * Moment factors ($p = 0.7882$) or the Strain factor ($p = 0.60187$), since the value of *p* in both cases was greater than the chosen significance level.

In other words, the strain factor did not interact with the sampling moments, so a new ANOVA was carried out by applying an additive bifactorial model without the interaction term δ_{ij}. Table 1 shows the results obtained from the statistical analysis with this model for one of the dependent variables, DM. Similar results were observed for the rest of the variables (data not shown).

The biodegradation process (Moment factor) induced highly significant variation in the chemical composition of the substrate ($p < 0.0001$), and this variation did not depend on the genetic characteristics of the analysed strains ($p = 0.5912$).

3.2. Biodegradation of pear pomace during mycelial growth

Table 2 shows the results of the post-ANOVA analysis, using the LSD Fisher mean comparison test, for the net DM content, using the factors Strain (Pl-P and Pl-J) and Moment (initial and final) as classification variables. Similar effects were observed for the rest of the response variables (data not shown). Post-ANOVA analysis confirmed that the variation in each of the components examined in the substrate was exclusively due to the biodegradation process and not to the strain type.

Table 2. Post-ANOVA analysis for the variable dry matter (g) for the comparison of means by using the Fisher test.

Test: LSD Fisher $\alpha = 0.01$ LSD = 2.21816 error: 2.9288 df: 17

Strain	Mean	n	E.E.	Moment	Mean	n	E.E.
Pl-J	13.29[a]	10	0.54	Initial	10.96[a]	10	0.54
Pl-P	13.71[a]	10	0.54	Final	16.04[b]	10	0.54

Means with the same letter are not significantly different ($p > 0.01$).

Consequently, the results presented below (Table 3) correspond to the analysis of the variation in the chemical composition of the pear pomace associated only with the biodegradation process by *P. ostreatus*.

As can be seen in Table 3, the mycelial development of *P. ostreatus* on the substrate caused a significant decrease in all considered variables. To analyse the results in greater detail, the percentage of loss for each variable was also calculated.

Table 3. Chemical composition of pear pomace prior to inoculation (initial) with *Pleurotus ostreatus* strains (Pl-J and Pl-P) and after eight weeks of incubation (final). Results are expressed as net content per Petri dish in grams, and the percentage of loss was calculated.

	Initial	Final	Loss, %
Dry Matter, g	16.04[a] ± 1.91	10.96[b] ± 1.41	31.66 ± 3.70
Nitrogen, g	0.11[a] ± 0.01	0.08[b] ± 0.01	25.41 ± 4.51
Carbon, g	9.10[a] ± 1.08	6.18[b] ± 0.79	32.07 ± 3.64
C/N	80.59[a] ± 0.01	73.62[b] ± 5.71	11.37 ± 4.66
Organic Matter, g	15.69[a] ± 1.86	10.66[b] ± 1.36	32.07 ± 3.64
Crude Protein, g	0.71[a] ± 0.08	0.52[b] ± 0.06	25.41 ± 4.51
Non-structural carbohydrates, g	0.70[a] ± 0.08	0.30[b] ± 0.05	57.03 ± 4.75
Cellulose, g	4.93[a] ± 0.59	4.05[b] ± 0.49	17.85 ± 2.82
Hemicellulose, g	3.05[a] ± 0.36	2.14[b] ± 0.24	29.82 ± 2.95
Lignin, g	3.54[a] ± 0.42	2.56[b] ± 0.42	27.80 ± 7.90

The values correspond to the mean of 10 replications per treatment ± SD. Means with the same letter for the same row are not significantly different ($p > 0.01$).

After allowing mycelial development on pear pomace for 8 weeks at 28°C in Petri dishes, a decrease in the dry matter (16.04 ± 1.91 g to 10.96 ± 1.41 g) was observed, indicating a 30% average bioconversion efficiency by *P. ostreatus*.

The net content of NSCH (0.70 g ± 0.08 vs. 0.30 g ± 0.05) was the one that presented the greatest decrease in the analysed period, approximately 60% on average for both strains. The biodegraded substrate impregnated with fungal mycelium showed a lower reduction in OM, CP,

HCEL, and LIG by almost 30%. The least fluctuation was observed in the fraction corresponding to CEL, which only decreased by about 20%.

4. Discussion

Due to the environmental problems related to the accumulation of solid waste with a high organic content, such as the generates in the food industries related to extraction of pear juice the authors of this work investigated alternatives to reuse these wastes and involve the agricultural and environmental sectors (Martínez *et al*., 2015; Maldonado *et al*., 2018; Rodríguez *et al*., 2018; Maldonado *et al*., 2021).

In this work, the biodegradation process of pear pomace during the stage of colonisation of the substrate by the Pl-P and Pl-J strains of *P. ostreatus* were chemically characterised, as these strains presented the best performance when analysing the mycelial growth curves in previous studies (Martínez *et al*., 2015).

The variation in the chemical composition of the substrate was analyzed using an additive factorial model. Therefore, the terms in the model showed that the interaction between the classification variables, Moment and Strain, were absent (Table 1). This model was used to study the main effects associated with the biodegradation process, since ANOVA indicated that the Strain and Moment factors did not interact with each other (Di Rienzo *et al*., 2012).

The obtained results indicated that the variation in the chemical composition of the substrate would be directly associated with the biodegradation process (Moment factor), regardless of the Strain type ($p < 0.01$).

The DM loss of the pear pomace substrate was 31.66%. This is a substantial mass reduction from the standpoint of lignocellulosic residue removal. Similar values were obtained by Zhang *et al*. (2002) in the cultivation of *Pleurotus sajor-caju* on rice and wheat straw. Sánchez *et al*. (2002) found that the bioconversion of vineyard pruning and grape pomace by *Pleurotus* spp. was from 16.7 to 38.8%, respectively. As demonstrated by Anike *et al*. (2016), the use of *Pleurotus ostreatus* for the bio convert of substrates as peanut shells and cornstalks during the stage of mycelial development in an array of proportions assayed is an encouraging and healthy environment way to the bioconversion of different substrates.

As shown in Table 3, the OM and C content of pear pomace used as a substrate was reduced by 32%. Several authors have reported that the loss of carbon from the resulting biomass (fungus and substrate) can be explained in part by its assimilation into the mycelium of the fungus and in part by its loss to the atmosphere as carbon dioxide due to the respiration of the fungus. The N loss might be due to volatilisation during the N mineralisation process (Zhang *et al*., 2002; Isroi *et al*., 2011; Anike *et al*., 2016; Del Cerro *et al*., 2021).

Principally, the loss of C and OM could be due to intracellular catabolism of central carbon metabolism (Del Cerro *et al*., 2021) and not only to lignin degradation.

In this study, a statistically significant decrease in the C/N ratio from 80.6 to 73.6 (11% loss) was found in the mycelial development stage.

The analysis of this relationship was carried out due to the importance of the C/N ratio as one of the most important factors to balance biomass and biocomposite production. The excess or lack of nitrogen content in the substrate may be a limiting factor for fungal growth (D'Agostini *et al*., 2011). The decrease obtained during mycelial development is important to consider since according to Hoa *et al*. (2015), a higher C/N ratio favours mycelium growth and a lower C/N ratio favours fruiting body growth. Previously, in fruiting body production trials with the same substrate, the authors found that the Pl-P strain caused a significant decrease in the C/N ratio (26% loss), while Pl-J practically did not modify this variable (Rodríguez *et al*., 2018). This variation in the C/N ratio indicates that the substrate, even after biodegradation during the mycelial development of the analysed strains (SMS), could be used in productive cycles to obtain edible carpophores.

As can be seen in Table 3, NSCH were the carbohydrates that decreased the most (approximately 60%), while cellulose, hemicellulose, and lignin fibres had a decrease between 20 to 30%. This metabolic profile could be attributed to the fact that the expression of genes of enzymes related to the use of NSCH are constitutive, while the lignocellulolytic exoenzymes are inducible and would be expressed only when the levels of NSCH decrease. The presence of metabolised sugars could cause the analysed *P. ostreatus* strains to produce ligninolytic enzymes, thus obtaining the carbon source and inducers for enzymatic synthesis (Akpinar and Urek, 2012; Rouches *et al*., 2016; Ergun and Urek, 2017).

Thereby, variation minimal in the fibre fraction was detected in the cellular wall correspondent to CEL. The result was similar to the findings of Zhang *et al*. (2002), implying that the fungi utilised a greater percentage of hemicellulose than cellulose. The fungi also used some lignin.

In previous studies (Rodríguez *et al*., 2018), the authors evaluated the biological potential of different strains of *P. ostreatus* to develop on the same substrate, observing that pear pomace produced edible carpophores. They also evaluated the chemical composition during the substrate colonisation and fruiting phases. At the end of the productive period, similar results were obtained in terms of the variation of the chemical composition; the fibers (FDN, FDA, and LDA) decreased 10 to 20% while the crude protein content was not substantially modified.

At this time, the authors of this work are testing the use of agro-industrial wastes biodegraded by *P. ostreatus* as a feed supplement for broilers (Cayolo *et al*., 2019) and as a substitute for non-renewable resources, such as peat, for seedling production.

5. Conclusion

This work showed that *Pleurotus* mycelial development on pear pomace is possible. This, together with the fruiting results published by the authors previously, indicates that it is possible to consider the cultivation of edible mushrooms of the genus *Pleurotus* over this substrate taking advantage of an agro-industrial waste product.

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Conflict of interest

The authors declare that there is no conflict of interests.

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