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Exploring local lignocellulosic substrates for the production of edible mushrooms in Northwestern Argentina

Explorando sustratos lignocelulósicos locales para la producción de hongos comestibles en el Noroeste de Argentina

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ABSTRACT

La Rioja province annually produces approximately 75,000 tons of agricultural residues and derived materials from agro-industrial activities, which could potentially be incorporated into oyster mushroom cultivation. This study aimed to evaluate the viability of this lignocellulosic biomass as a substrate for the cultivation of edible mushrooms belonging to the genus *Pleurotus*. Initially, the mycelial growth of two species (*P. ostreatus* and *P. djamor*) was assessed by formulating combinations of local substrates. Experimental crops were grown employing the most promising substrates, which were subsequently selected for chemical characterization. It was found that both strains exhibited maximum mycelial growth in the substrate formulated

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with jojoba leaf litter. A comparison of the two strains revealed no direct correlation between mycelium growth and productive performance. The highest biological efficiency (BE) values were obtained when *P. ostreatus* was cultivated in treatments combining jojoba leaf litter and grape pomace with olive pomace. Furthermore, these treatments showed suitable chemical properties and were formulated from problematic waste generated in large quantities in the region without proper processing and disposal methods. In this context, there is potential to ensure a continuous supply of this lignocellulosic biomass for cultivating these mushroom species over an extended period of time, thus providing a sustainable alternative for these regional by-products.

Keywords: *Pleurotus* species; olive and grape pomace; jojoba litter; La Rioja province.

RESUMEN

La provincia de La Rioja produce anualmente aproximadamente 75.000 toneladas de residuos agrícolas y subproductos agroindustriales, los cuales potencialmente podrían incorporarse al cultivo de hongos ostra. El objetivo de este estudio fue evaluar la factibilidad de esta biomasa lignocelulósica como sustrato para el cultivo de hongos comestibles del género *Pleurotus*. Inicialmente, se determinó el crecimiento micelial de dos especies (*P. ostreatus* y *P. djamor*) utilizando formulaciones de sustratos locales. Luego se realizaron cultivos experimentales utilizando los tratamientos más óptimos, y su posterior caracterización química. Ambas cepas exhibieron un crecimiento micelial máximo en el sustrato formulado con hojarasca de jojoba. Al comparar las dos cepas no se detectó una correlación directa entre el crecimiento micelial y el comportamiento productivo. Los valores más altos de eficiencia biológica (EB) se obtuvieron cuando se cultivó *P. ostreatus* en tratamientos que combinaban hojarasca de jojoba y orujo de vid con orujo de olivo. Además, estos tratamientos presentaron propiedades químicas adecuadas, y fueron formulados a partir de residuos problemáticos, que se generan en grandes cantidades en la región, y que carecen de métodos adecuados de procesamiento y eliminación. En este contexto, existe la posibilidad de garantizar un suministro continuo de esta biomasa lignocelulósica para el cultivo de estas especies de hongos durante un período largo de tiempo, generando así una alternativa sustentable para estos subproductos regionales.

Palabras clave: Especies de *Pleurotus*; orujo de olivo y vid; hojarasca de jojoba; provincia de La Rioja.

INTRODUCTION

In the last twenty years, *Pleurotus* species (commonly known as oyster mushrooms) has been intensely studied and cultivated in many regions worldwide owing to its great gastronomic and medicinal value (Sardar *et al*., 2017; Chanakya *et al*., 2015). *Pleurotus* species are recognized as functional food sources due to their high protein, fiber, carbohydrate, vitamin, and mineral content (Patel *et al*., 2012; Feeney *et al*., 2014; Sadh *et al*., 2018). Additionally, their basidiomes are characterized by a low proportion in fat and cholesterol and a high polyunsaturated fatty acids content, which contributes to their excellent aroma and taste (Wani *et al*., 2010; Heleno *et al*., 2010). Numerous studies have reported the health-promoting effects of these mushrooms, including their antitumor, antibiotic, antiviral, hypocholesterolic, hypoglycaemic and immunomodulary activities, among others (Wasser, 2002; Correa *et al*., 2016; Jayachandran *et al*., 2017).

Over the last decade, the popularity of *Pleurotus* species has been on the rise because of several advantages associated with their cultivation (Ritota and Manzi, 2019; Jegadeesh *et al*., 2021). These isolates are easy to propagate, have shorter growth and fruiting times compared to other edible mushrooms, and require minimal environmental controls during cultivation (Yingyue *et al*., 2014; Khan *et al*., 2013). A wide range of enzymes capable of breaking down complex biomass rich in lignin and cellulose are secreted by these species (Lallawmsanga *et al*., 2019; Knop *et al*., 2015). Cultivation substrates do not require composting or complete sterilization, even *P. ostreatus* has evolved protective mechanisms against their major pest, the mold mite (*Tyrophagus putrescentiae*) (Wendiro *et al*., 2019; Jang *et al*., 2016; Huiping *et al*., 2022). Family-scale production methods generally involve simple and cost-effective technology, without requiring large land areas or significant capital and infrastructure investments, although these conditions change at production scale (Jegadeesh *et al*., 2018; Chang, 2014). This makes it a valuable alternative for developing regions where employment opportunities and access to nutritious food are limited (Kimenju *et al*., 2009**;** Ishara *et al*., 2018; Tesfaw *et al*., 2015). Consequently, rural communities could be encouraged to edible mushrooms production on local wastes to generate additional income (Bandara *et al*., 2021; Rizki & Tamai, 2011).

Commercially, *Pleurotus* spp. production it is one of the most important after *Lentinula edodes* (shiitake) and *Auricularia* spp., accounting for 19% of total world production (Sun *et al*., 2020; Correa *et al*., 2016; Sanchez and Royse, 2017). Diverse agricultural residues and by-products have been explored as substrates for cultivating these oyster mushrooms, depending on their availability in different regions (Koutrotsios *et al*., 2014; Sardar *et al*., 2016). In Argentina, these species have traditionally been cultivated using different cereal straws such as wheat, corn, and sunflower, mainly sourced from extensive annual crops in the Pampas region (Jaramillo & Albertó,

2013; Lechner & Albertó, 2011). In the Patagonia region these species are cultivated on poplar logs, on debris generated from of the forest industry, or on residues from beer brewing and winery by-products (Albertó *et al*., 2010; Rugolo *et al.*, 2020; Luque *et al*., 2021). While in the NEA region, Pleurotus species are evaluated on wastes of citrus agroindustry for the enzymatic production (Fonseca *et al*., 2020). However, the rising costs of some substrates and the large amount of lignocellulosic biomass generated annually in the Northwestern region of the country need to develop new production strategies. Furthermore, in alignment with a transition towards a circular economy, it is crucial to incorporate this lignocellulosic biomass into sustainable and environmentally friendly solutions.

A prior investigation estimated that La Rioja province generates around 75,000 tons of agricultural residues and by-products from agro-industrial activities annually (Fracchia *et al*., 2022). Moreover, this region is characterized by limited availability of high-quality foods and escalating environmental issues. Therefore, the bioconversion of these lignocellulosic wastes into edible mushrooms could provide a promising and economically feasible solution. Several studies have demonstrated the potential of agro-industrial by-products generated in wineries and oil-olive factories for *Pleurotus* spp. mushroom cultivation (Petre *et al*., 2016; Melanouri *et al*., 2022). However, to our knowledge, there is little information in the literature about mushroom cultivation on substrates such as jojoba litter (*Simmondsia chinensis)* pure or combined with other previously characterized substrates. Hence, this study aimed to valorize these problematic lignocellulosic residues as substrates for cultivating *Pleurotus* spp. through 1) assessing the initial mycelial growth of two species *P. ostreatus* and *P. djamor* in various substrate formulations; 2) conducting experimental cultures of these species using the most promising substrates identified in the previous step; and 3) selecting the most suitable substrates or combinations based on productivity and fungal strain performance for their further chemical characterization.

MATERIALS AND METHODS

Pleurotus strains and culture conditions

The strains used in this study were *P. ostreatus* BAFC1723 and *P. djamor* BAFC1765, which are stored in the Fungal Collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires BAFC. The strains were maintained on malt extract agar (MEA) in Petri dishes at 4°C for *P. ostreatus* and 25°C for *P. djamor*.

Substrates preparation

4 different agro-industrial wastes were used. The jojoba litter (J) was obtained from AGRINSA SA. and Fuerte del Bañado SA., two agricultural companies located in Aimogasta. Red and white grape pomaces (RP and WP) were sourced from the San Huberto SA winery in Anillaco. Olive pomace (OP) was provided by HILAL factory, also located in Aimogasta. All substrates were sun-dried for approximately one week to remove moisture and prevent microbial contamination. They were then stored in polyethylene bags until further use. 10 treatments were formulated: the 4 pure substrates and their combinations in the ratio of 1:1 (w:w).

Treatments:

Jojoba litter (J) 100%
White grape pomace (WP) 100%
Red grape pomace (RP) 100%
Olive pomace (OP) 100%
RP-WP 50/50%
RP-J 50/50%
RP-OP 50/50%
WP-OP 50/50%
WP-J 50/50%
J-OP 50/50%

The treatments were then soaked in tap water to achieve a moisture content of around 65-70%. The percentage of dry matter was measured after drying the substrates in a forced hot air oven at 70 °C for 48 h, and the pH values were determined by pre-hydrating the substrates at 70% (w/w).

Mycelial growth measurements of *Pleurotus* species

Mycelial growth was assessed in Petri dishes of 10 mm diam. for the 10 treatments. Five replicates for each treatment and fungal strain were performed. In each Petri dish, 25 gr of lignocellulosic substrate were placed and then sterilized in an autoclave at 121 ºC for 45 min. After cooling, petri dishes were centrally inoculated with a 0.5cm diam mycelium plug of each fungal strain. Inoculums were taken from the edge of the active colony grown in an MEA plate (Sastre-Ahuatzi, 2007). The fungal cultures were incubated at 26-28 ºC under dark conditions (Hoa and Wang, 2015). The mycelial growth was recorded daily using the Image J program until the colony diameters covered more than 90% of the plate (Barrales and Mata, 2016; Abràmoff *et al.*, 2004). Growth rates were determined as cm^2/d by averaging the growth of replicates at 7 days. In addition, the mycelial density was qualitatively determined in 3 categories: compact (+++), semi-compact growth of mycelium (++) and poor mycelial density (+) (Villegas,

2007). The number of days from inoculation to complete colonization of the plate was recorded (Obodai *et al*., 2003).

Basidiome production assay

To experimental cultures were statistically selected 3 treatments into account two parameters (mycelial growth and fungal density) for each *Pleurotus* strain. The basidiome production assay was carried out in 5-liter plastic containers (5 replicates for each treatment). Substrates were hydrated overnight in an appropriate container, washed with tap water, and drained to remove excess water. Approximately 2 kg of the substrates were weighed and mixed in equal proportions (1:1 v/v), and 2 % of $CaCO_3$ was added to adjust pH level. The plastic containers containing the different substrate treatments were sterilized twice for 15 minutes at maximum power in a microwave oven and then cooled to room temperature. Each plastic container was inoculated with colonized oat seeds at 5% W/W within a laminar flow chamber. The cultures were sealed with the caps, but not hermetically, were incubated at 26±3°C in darkness. After 10 days were checked to evaluate the progress of colonization and possible contaminations. Once the substrate was fully colonized, the colonization time in days was recorded, and the treatments were transferred to a chamber for subsequent basidiome production. For this step, the conditions were temperatures of 15±3ºC, a light intensity of 1000 lux m^2, natural photoperiod (14h light, 10h dark), and relative humidity of 80±5%. Adequate ventilation was provided through an air extractor. Basidiome production continued until the third harvest.

The point of harvest was determined visually when the basidiomes attained their full size, but before the edge of pileus rolls up above, following the method described by Gaitán-Hernández *et al.* (2006). Basidiomes were harvested and weighed immediately to prevent any loss of moisture. The biological efficiency (BE) and production rate were calculated with the data from the first three harvests. The BE was calculated as the fresh weight of basidiomes x 100/ the dry weight of substrate, while the production rate (PR) was calculated using the formula: BE%/production days (Banik and Nandi, 2004; Dundar *et al*., 2009). The diameter of basidiomes in each treatment was measured and categorized into three size groups: G1: <5cm, G2: between 5 and 9cm, and G3: >10cm according to Merlo and Mata (2005).

Chemical characterization of the substrates

The chemical composition of the selected substrates for stage of production was evaluated. Previously, the substrates were dried in an oven at 70 °C to constant weight, ground, and refrigerated at 4 °C. The following analyses were performed: moisture, pH, lipids, carbohydrates, sugars and total proteins. The analyses for moisture, total lipid and total carbohydrates were

carried out through AOAC user and total sugars were obtained following the protocol described by Dubois et al., (1956). The total protein was determined from the total nitrogen content, using the correction factor 4.38 (Breene, 1990). The total nitrogen content was determined by the Kjeldahl method. The determinations were performed in duplicate.

Data analysis

All data were statistically analyzed by one-way ANOVAs using INFOSTAT software (Di Rienzo *et al*., 2002). When significant differences were detected by ANOVA ($P < 0.05$), *Tukey's* test for means comparison was performed, to assess differences between means of the individual parameters. Prior to analyses, the assumptions of normality and homogeneity of variance were verified with Levene and Shapiro–Wilkes tests, respectively.

RESULTS

Mycelial growth of *Pleurotus* species

Regarding mycelial growth, oyster mushrooms *P. ostreatus* and *P. djamor* were able to colonize successfully all evaluated substrates, although evidencing variable behavior between them (Fig. 1).

The *P. ostreatus* strain exhibited the highest mycelial growth on jojoba litter and the combination of jojoba litter and olive pomace (84.23 ± 1.54 and 67.96 ± 3.41, respectively, mean ± SD, in cm^2, Fig. 1). Conversely, the lowest growth rate was observed in treatments involving red grape pomace and the mix of red and white grape pomace (9.34 ± 1.98 and 13.25 ± 0.94,

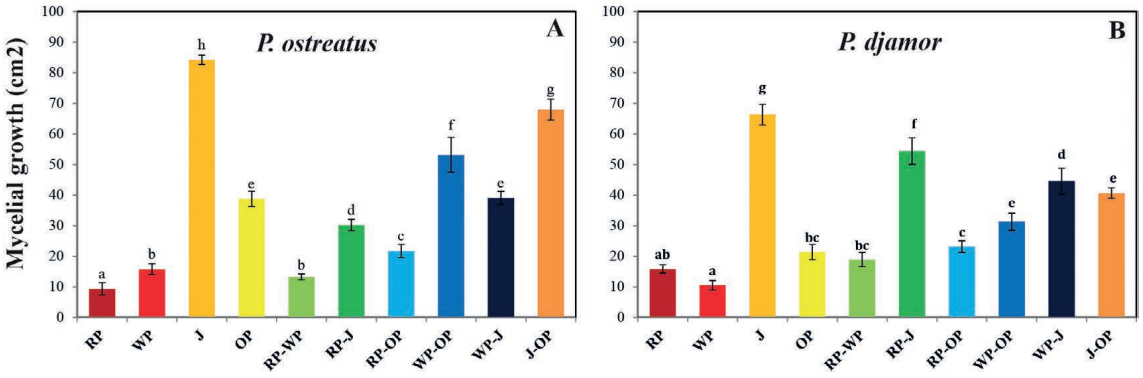

Fig. 1. Mycelial growth of *Pleurotus* strains grown on different lignocellulosic substrates and their combinations. A) *P. ostreatus*. B) *P. djamor.* Mean values within each picture sharing the same letters are not significantly different according to Tukey's test at P> 0.05.

Fig. 1. Crecimiento micelial de cepas de *Pleurotus* cultivadas sobre diferentes sustratos lignocelulósicos y sus combinaciones. A) *P. ostreatus*. B) *P. djamor*. Los valores medios dentro de cada imagen que comparte las mismas letras no presentan diferencias significativamente según la prueba de Tukey en P>0,05.

respectively, mean ± SD, see Fig. 1). Notably, the values for jojoba were nine times greater than those for red grape pomace. Regarding *P. djamor*, the treatments also showed significant differences in terms of colonization rate ($F = 211.79$, $df = 40$, $p < 0.0001$, see Fig. 1B). Similar to *P. ostreatus*, the substrate containing 100% jojoba exhibited the highest mycelial growth (66.29 ± 3.37 cm^2, mean ± SD, Fig. 1B), with a value six times greater than that for grape pomace (RP and WP). The latter demonstrated the lowest growth (15.83 ± 1.37 and 10.48 ± 1.54, respectively, mean ± SD in cm^2, Fig. 1B). Elevated values of growth were also observed for the RP-J and WP-J treatments (54.39 ± 4.33 and 44.54 ± 4-24, respectively, mean ± SD, Fig. 1B).

The time required for complete colonization varied depending on the species and substrate evaluated. For *P. ostreatus*, it took between 7 to 18 days, while for *P. djamor*, it ranged from 8 to 15 days (Table 1). Comparing both fungi, the treatments inoculated with *P. djamor* exhibited faster growth, while those corresponding to *P. ostreatus* required more incubation days. The mycelial densities for both *Pleurotus* species were mainly compact (+++), except for the treatments with jojoba which had a less compact mycelial density type (++). In general, there was longer colonization observed in both grape pomace substrates, with a tendency towards slower growth in the combinations containing these substrates, resulting in compact mycelial density (Table 1). On the other hand, the treatment with jojoba showed the fastest colonization for both fungi, with complete colonization achieved on day 7 for *P. ostreatus* and day 8 for *P. djamor*; however, it had a mycelial density type intermediate. A similar trend was observed in mixtures con-

Table 1. Duration of complete colonization in days for *Pleurotus* species and mycelium density after one week of inoculation on different substrates.

Tabla 1. Duración de la colonización completa expresada en días para especies de *Pleurotus* y densidad del micelio después de una semana de inoculación en diferentes sustratos.

Substrate	*Pleurotus oestreatus*		*Pleurotus djamor*	
	Total colonization*	Mycelial density**	Total colonization*	Mycelial density**
RP	18	+++	15	+++
WP	14	+++	14	+++
J	7	++	8	++
OP	12	+++	13	+++
RP-WP	17	+++	13	+++
RP-J	12	+++	11	+++
RP-OP	14	+++	14	+++
WP-OP	10	+++	15	+++
WP-J	10	+++	9	+++
J-OP	9	+++	11	++

* Complete colonization time (days).

** Degree of mycelial density when the fungi fully colonize the plate: + indicates poor mycelial density, ++ indicates semi-compact growth of mycelium, and +++ indicates uniform and compact growth of mycelium.

taining jojoba litter, which demonstrated faster mycelial growth compared to grape pomace-based substrates.

During the mycelial growth assay, pH ranges between 3.69 and 5.48 were measured in the base substrates and their respective combinations. The highest pH value was observed in the substrate composed of 100% olive pomace, while the substrate composed entirely of red grape pomace had the lowest pH value. Regarding water retention capacity, values ranging from 51.95% to 71.46% were found for the evaluated substrates. The lowest value was observed in the mixture of red grape pomace and white grape pomace, while the highest value was recorded for jojoba litter.

Among all the treatments, J, J-OP, and WP-OP showed the most suitable mycelial growth in terms of both speed and density for *P. ostreatus*. While for *P. djamor*, the substrates J, RP-J, and WP-J exhibited the most favorable growth characteristics. These substrates were further evaluated during the production stage for their yield potential.

Basidiome production assay

When comparing both strains, *P. ostreatus* generally exhibited higher biological efficiency and production rates than *P. djamor*, showing significant differences between the treatments ($F= 18{,}41$ $df= 12$ P, $p < 0{,}05$, see Fig. 2). The treatment that exhibited the highest biological efficiency (BE) for *P. ostreatus* was that formulated using equal proportions of jojoba and olive pomace substrates, achieving a BE value of 52.49%. This was followed by the WP-OP treatment, which had a BE value of 45.91%. Conversely, the lowest BE value was observed in the treatment using jojoba litter, with a value of 33.91%. BE values of *P. djamor* strains showed variations, although not statistically significant ($F= 18{,}41$ $df= 12$ P, $p < 0{,}05$, see Fig. 2). The highest yields of *P. djamor* were observed in treatments using red and white

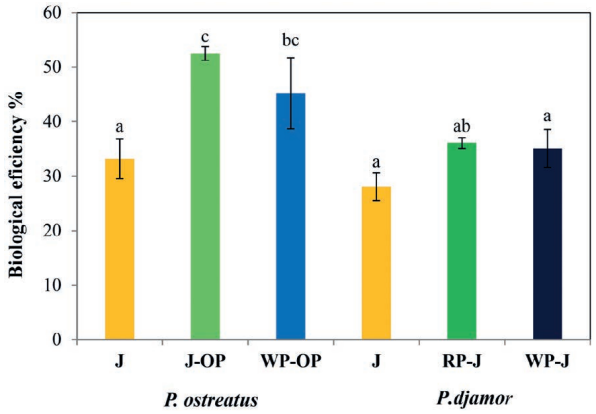

Fig. 2. Biological efficiency of *Pleurotus* strains grown on lignocellulosic substrates and their combinations. Values showed are mean ± S.D. ($p < 0.05$, Tukey).

Fig. 2. Eficiencia biológica de cepas de *Pleurotus* cultivadas sobre sustratos lignocelulósicos y sus combinaciones. Los valores mostrados son media ± D.E. ($p < 0.05$, Tukey).

grape pomaces combined with jojoba litter, reaching BE values of 36.06 ± 0.99 and 35.08 ± 3.5, respectively. Similarly to *P. ostreatus*, the lowest yield in this species was obtained from jojoba litter.

The incubation period for treatments inoculated with *P. ostreatus* significantly varied from 18 to 24 days, while for *P. djamor*, colonization times were similar approximately 20 days for all substrates analyzed (Table 2). Regarding the production period, the experimental cultures had a maximum duration of 62 days for the fungus *P. ostreatus* and 63 for *P. djamor*, three well-defined harvests were obtained in all treatments. Production rates for *P. ostreatus* ranged between 0.65 and 0.84 gr of fungus per day and for *P. djamor* reached values of 0.62 (gr/day). Despite the occurrence of specific contaminations with *Trichoderma* fungi in some treatments, these were not significant and all repetitions were considered in the production evaluations.

The basidiomes were categorized into three size groups. The majority of the production fell into category G1, with percentages ranging from 62-51% for *P. ostreatus* and 75-60% for *P. djamor* (Table 3). The least represented size category was G3, with maximum percentages varying from 5 to 7%. The G2 category ranged from 24 to 49% for combinations of jojoba litter inoculated with *P. djamor* and white grape pomace and olive pomace inoculated with *P. ostreatus*, respectively. The development and morphology of

Table 2. Incubation, time and rate production by *Pleurotus* species.
Tabla 2. Incubación, tiempo y tasa de producción de las especies de *Pleurotus*.

Strain	Substrate	Incubation period*	Total production period**	PR***
P. ostreatus	J	18.53 ± 2.06a	51.04 ± 3.67a	0.65 ± 0.06
P. djamor	WP-J	20 ± 0ab	63.12 ± 0.58d	0.56 ± 0.02
P. djamor	RP-J	20 ± ab	58.6 ± 0.41cd	0.62 ± 0.1
P. djamor	J	20 ± ab	57.8 ± 0.75bc	0.55 ± 0.04
P. ostreatus	WP-OP	22.53 ± 1.65bc	53.66 ± 2.81ab	0.84 ± 0.01
P. ostreatus	J-OP	24.2 ± 3.19c	62.73 ± 3.42d	0.84 ± 0.05

* Incubation period (days required for the complete colonization of substrates).
** Production period (starts with the fungal inoculation and ends with the third harvest).
*** Production rate (gr of fungus per day).

Table 3. Pileus sizes (%) for *Pleurotus* strains.
Tabla 3. Tamaño de píleo (%) de cepas de *Pleurotus*.

Strain	Substrate	Incubation period*		
		G1	G2	G3
P. ostreatus	J	62	38	–
P. djamor	WP-J	64	29	7
P. djamor	RP-J	60	38	2
P. djamor	J	75	25	–
P. ostreatus	WP-OP	52	43	5
P. ostreatus	J-OP	51	49	–

*G1: <5cm, G2: between 5 and 9cm, and G3: >10cm.

the basidiomes obtained in the treatments were normal, and mushrooms exhibited desirable aroma, taste, and overall appearance; a significant characteristic from a commercial standpoint. The experimental cultures showed adequate conditions, of aeration, humidity, and texture, for the complete development of the basidiomes of both *Pleurotus* strains tested.

Chemical characterization of the substrates

The results of the chemical analysis showed that the total carbohydrate content was highest in the combination WP-OP substrate, with a value of 83.5%, while it was lowest in the jojoba litter substrate, with a value of 56% (see, Table 4). The nitrogen content ranged between 1.46% and 1.08%, indicating relatively homogeneous values among the substrates. The C/N ratio was highest in the J-OP (37.5%), followed by WP-OP (35.7%) and jojoba litter (29.5%). These ratios suggest that jojoba litter may be less favorable for *Pleurotus* strains compared to treatments with olive pomaces. The moisture and pH values were similar in the WP-OP and J-OP combinations, while jojoba litter had higher moisture content (71.5%) and pH value (6.4). Overall, these chemical parameters provided insights into the potential degradation capacity of *Pleurotus* strains on different substrates, highlighting their preferences for certain nutrient compositions and physical conditions during cultivation.

Analyzing the changes that occur in the substrate during cultivation is important for understanding the fungal requirements and optimizing production times from a commercial perspective. Before the fungal culture, the total sugar content varied highly among the different treatments, in the J-OP was observed with the highest value (66.7 glu/gr.), and WP-OP was the lowest value (17.1 mg of glucose per gram, see Fig. 3A). In contrast, protein values were generally low across all treatments, with a range oscillating between 5.01% and 9.47% (Fig. 3B). Total lipid content showed more homogeneous values between 10.52% and 13.02% (Fig. 3C).

After cultivating *P. ostreatus* and harvesting of basidiomes, all nutrient contents decreased in the three treatments tested (see, Fig. 3). The

Table 4. Chemical composition of the lignocellulosic substrates before their use for the fungal culture, expressed in gr/100gr.

Tabla 4. Composición química de los sustratos lignocelulósicos antes de su utilización para el cultivo de hongos, expresada en gr/100gr.

Substrate	Total carbohydrate	N	C/N	pH	Moisture %
J	56.14	1.10	29.54	6.39	71.50
WP-OP	83.5	1.46	35.78	5.52	57.00
J-OP	69.99	1.08	37.55	5.81	63.00

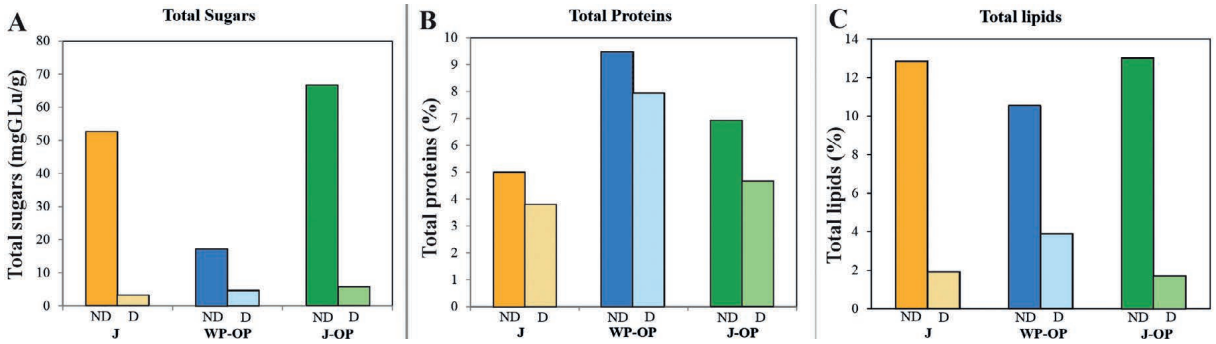

Fig. 3. Nutrient composition of the non-degraded (ND) and degraded (D) treatments by *P. ostreatus* strain. A) Total sugars (mgGLu/g). B) Total proteins (%). C) Total lipids expressed (%).

Fig. 3. Composición nutricional de los tratamientos no degradados (ND) y degradados (D) por la cepa de *P. ostreatus.* A) Azúcares totales (mgGLu/g). B) Proteínas totales (%). C) Lípidos totales expresados (%).

total sugar content showed the greatest reduction, ranging from 72.51% to 93.92% compared to the non-degraded substrate. Protein content also decreased but to a lesser extent for all treatments. Lipid content diminished by an average of 75%, with jojoba-olive pomace litter treatment showing the largest decrease in this component.

DISCUSSION

Our findings indicated that the tested lignocellulosic biomass provided optimal conditions for the development of both *Pleurotus* strains in the mycelial growth and productive phases. However, it should be noted that the production of these fungal species on different agricultural residues depends on several factors, which will be further discussed below.

Regarding the growth of fungal mycelia, *P. ostreatus* displayed a superior growth rate compared to *P. djamor*. This aligns with the results reported by Hoa and Wang (2015) when these mushroom species were cultivated using cereal substrates. The treatment containing 100 % jojoba litter exhibited the fastest mycelial growth for both fungi, albeit with an intermediate mycelial density (Table 1). Likewise previous research on *Pleurotus* strains also observed this trend (Girmay *et al*., 2016; Shrestha *et al*., 2006). The notable growth observed in jojoba litter can be attributed to its nutritional and physical properties (Tinoco *et al*., 2001). The larger particle size and pores of this substrate may facilitate gas exchange around the mycelium, thereby enhancing fungal growth rate (Nguyen & Ranamukhaarachchi, 2020). Total colonization time and the density degree of the treatments were adequate and close to other tested *Pleurotus* strains (Badu *et al*., 2011; Martínez, 2015).

At the production level, the substrates jojoba litter, olive and grape pomace, as well as their combinations, successfully promoted both *P. ost-*

reatus and *P. djamor* production, although evidencing variability in certain parameters. The highest BE values were achieved for *P. ostreatus* cultivated on treatments combining jojoba with olive and grape pomace. These productive yields can be attributed to the optimal C/N ratio, the nutritional composition, and favorable physical properties such as pH and moisture content measured for these substrates, in agreement with previous studies (Li *et al*., 2017; Xu *et al*., 2016; Khan *et al*., 2013). In addition, our results of the nutritional analysis suggest that *P. ostreatus* largely degraded nutrients present in these substrates, particularly sugars and lipids, while protein content remained more stable, this partially matches with the previously observed by Sánchez *et al.* (2008) and Martínez *et al*. (2015). Similar production values were reported by Rugolo *et al*. (2016) who evaluated *F. velutipes* on a substrate consisting of 60% olive pomace. On the contrary, other studies detected lower BE values ranging from 26% to 33% when using substrates combined with olive pomace for *P. ostreatus* (Mansour-Benamar *et al*., 2013; Ananbeh and Almomany, 2005). Likewise, low yields were observed for *Pleurotus* species produced on grape pomace (Sánchez *et al*., 2002; Papadaki *et al*., 2019). Numerous studies have focused on cultivating *Pleurotus* strains using a wide range of agro-industrial waste and have recorded much higher yield values (Fracchia *et al*., 2009; Sanjel *et al*., 2021; Zakil *et al*., 2019; Tavarwisa *et al*., 2021). However, our production values can be improved by varying the proportion of substrates, or supplementing with compounds such as soy flour, beer bagasse, wheat, or rice bran, and adjusting physical variables, among other factors (Jafarpour *et al*., 2010; Luque *et al*., 2021; Rizki and Tamai, 2011).

Few studies have reported the incorporation of jojoba residues in edible mushroom production. In a recent work by Elsakhawy *et al*. (2022), the potential of pure and combined jojoba bagasse for *P. ostreatus* production was evaluated, showing promising average yields. Although our treatments with 100% jojoba litter showed the lowest BE values for both strains, the mixtures containing this residue led to enhanced in cultivation results. Das and Mukherjee (2007) and Vega *et al*., (2022) also found that the mixtures improved production when evaluating *Pleurotus* cultures in pure and combined substrates of vegetable residues and coffee pulp. This lower productive efficiency of jojoba can be attributed to its low content of soluble nitrogen and carbon compounds, as well as its higher content of structural compounds such as lignin, similar to sawdust-based substrates (Badu *et al*., 2011; Shah *et al*., 2004). Although *Pleurotus* fungi are capable of degrading lignin, they also require other easily assimilable carbon sources like glucose or cellulose (Hernández Domínguez *et al*., 2017). Fracchia *et al*. (2009) also examined the use of jojoba expeller as an effective supplement to improve BE in *Pleurotus* species combined with *Jatropha macrocarpa* residues. Additionally, Fracchia *et al*. (2022) reported slightly higher BE yields for *Pleurotus* species on jojoba wastes. Based on our findings, we suggest that due to its physical nature and high porosity, jojoba litter is susceptible

to desiccation used as a sole substrate, and we recommend its use albeit combining it with other agricultural waste to enhance cultivation yields.

Similar to other studies, we did not find direct relationship between mycelial growth and the productive behavior of the strains (Jin *et al*., 2018; Liang *et al*., 2011). Nevertheless, we detected productive difference between the evaluated strains (Guzmán, 2000; Stajic *et al*., 2005). In general, treatments inoculated with *P. djamor* did not show significant differences and yielded lower biological efficiency values. Similarly, Ashraf *et al*. (2013) detected production yields of around 40% for *P. ostreatus* and 33% for *P. djamor* on cotton residues. The lower BE values found for *P. djamor* can be attributed to its poor ability to degrade recalcitrant compounds present in the used substrates (Atikpo *et al*., 2008; Yingyue *et al*., 2014). However, *P. djamor* growth requires higher temperatures, which is an advantage due to the high temperatures recorded in the region under study (Kibar and Peksen, 2008; Miranda *et al*., 2019). The observed times of primordial initiation between treatments inoculated with *P. ostreatus* presented differences. On the contrary, other works show similar times of around 23-24 days for *P. djamor* (Oseni *et al*., 2012; Jegadeesh *et al*., 2018). In all treatments, good-looking basidiomes were harvested, with consistency, smell and flavor similar to those produced in standard substrates (Suguimoto *et al*., 2001; Jayachandran *et al*., 2017). For both strains, the proportion of fruiting sizes that predominated was the G2 category, with G3 sizes being observed more frequently for *P. ostreatus* (Merlo and Mata, 2005). A decreasing trend in the size of the basidiomes, and in the productive yield was detected, from the first to the third harvest (Ejigu *et al*., 2022; Rizki and Tamai, 2011). Currently, edible mushroom cultivation is a feasible alternative to improve access to quality foods and establish a sustainable agriculture system in rural and impoverished communities (Bandara *et al*., 2021). CRILAR has been developing strategies to introduce mushroom cultivation through workshops and community projects in the region. Our community faces challenges related to its sociocultural context, including traditional gastronomy and long-standing food production practices, which are major obstacles to the cultivation process. However, through collaborative projects with secondary schools, small producers, and cooperatives, mushroom production is successfully incorporated, using locally sourced raw materials as substrates. In the future, we consider it crucial to include other government institutions such as INTA, the municipal and provincial government, and waste-generating companies (wineries San Humberto and Aminga) to expand production on a larger scale. This activity could be developed by generating a cooperative of associated producers, as a sustainable solution to transform residual biomass into quality nutritious food and additional income, contributing thus to rural development.

CONCLUSION

In conclusion, all the evaluated substrates showed potential for edible mushrooms production of *Pleurotus* species. The main results of this research are summarized in Figure 4. The jojoba litter-olive pomace and grape pomace-olive pomace treatments for the *P. ostreatus* strain were the most successful, with optimal biological efficiency values and desirable physicochemical properties. Particularly, these treatments of higher BE values were formulated with the problematic residues of olive trees and grape, which are generated in enormous quantities in the province. This ensures a continuous supply of lignocellulosic biomass for cultivating these fungal species over an extended period. To promote this activity in the region, it is crucial to improve yields by utilizing mixed substrates with appropriate

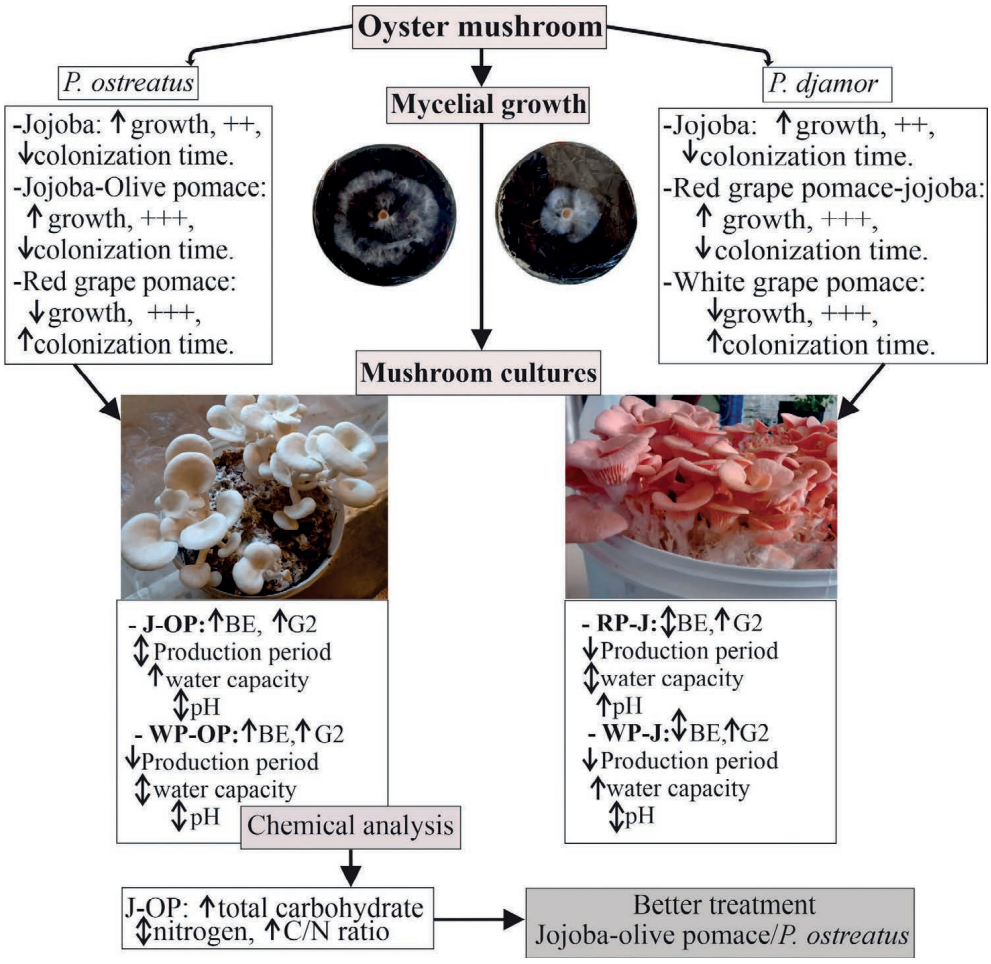

Fig. 4. Schematic representation of the key findings obtained in this study. The diagram depicts three types of arrows that indicate varying levels among the measured parameters. The arrow ↑ denotes elevated values, whereas ↕ represents intermediate values, and ↓ signifies low values. The plus symbol + represents the degree of mycelial density.

Fig. 4. Representación esquemática de los resultados clave obtenidos en este estudio. La figura muestra tres tipos de flechas que indican niveles variables entre los parámetros medidos. La flecha ↑ indica valores elevados, mientras que ↕ representa valores intermedios y ↓ significa valores bajos. El símbolo más + representa el grado de densidad micelial.

supplementation and using strains with higher productivity potential. In this context, mushroom production can become a biotechnological alternative for mitigating environmental impact and generating healthy foods from agricultural wastes.

CONFLICT OF INTEREST

Authors have no conflict of interests.

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