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Production of the "maguey mushroom" *Pleurotus agaves* on formulated substrates

Producción del "hongo del maguey" *Pleurotus agaves* en sustratos formulados

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RESUMEN

Antecedentes: *Pleurotus agaves* es un hongo comestible que crece silvestre en plantas de maguey y es consumido en zonas rurales de México.

Objetivo: comparar las eficiencias biológicas (EB´s) y tamaño de basidiomas de una cepa silvestre de *P. agaves* (IE-771) y otra comercial de *Pleurotus pulmonarius* (IE-115).

Método: las cepas se cultivaron en hojas de maguey mezcladas con paja de cebada (M) utilizando como control paja de cebada (S). Se analizó el contenido de fibra (lignina, celulosa, hemicelulosa) de los sustratos durante el ciclo de cultivo.

Resultados y conclusiones: las EB´s de la cepa IE-771 fueron 42.1 % (S) y 64.6 % (M) y para la IE-115 de 37.3 % (S) y 71.3 % (M), determinándose diferencias significativas entre los sustratos, pero no entre las cepas. Los ciclos de cultivo fueron más largos en la cepa de *P. agaves*. La IE-771 presentó diámetros de píleos de ≤ 4.9 a 19.9 cm en S y de ≤ 4.9 a ≥ 20 cm en el sustrato M; mientras que la IE-115 de ≤ 4.9 a 14.9 cm, en ambos sustratos. Una mayor disminución del contenido de polisacáridos en los sustratos estuvo asociada a la incubación. *Pleurotus agaves* podría ser cultivada comercialmente.

Palabras clave: cultivo de hongos, degradación de fibra, eficiencia biológica, hojas de maguey, setas

ABSTRACT

Background: *Pleurotus agaves* is a mushroom that grows wild on the agave plant and is consumed by rural populations in some regions of Mexico.

Objective: To compare biological efficiencies (BEs) and basidiome sizes between the wild strain of *P. agaves* (IE-771) and a commercial strain of *Pleurotus pulmonarius* (IE-115).

Method: The strains were cultivated in agave leaves mixed with barley straw (M) and barley straw alone (S), used as a control substrate. The fiber (lignin, cellulose, hemicellulose) content of the substrates during the crop cycles was analyzed.

Results and conclusions: The BEs were 42.1 % (S) and 64.6 % (M) for IE-771 and 37.3 % (S) and 71.3 (M) for IE-115, with statistical differences found between the substrates, but not between the strains. Crop cycles were longer in the *P. agaves* strain. IE-771 presented pileus sizes Ø from ≤ 4.9 to 19.9 cm on substrate S and from ≤ 4.9 to ≥ 20 cm on substrate M, whereas IE-115 presented sizes from ≤ 4.9 to 14.9 cm in both substrates. A greater decrease in polysaccharide compounds in the substrates was associated with the incubation stage. Due to its acceptable morphological characteristics, *P. agaves* strain could be suitable for commercial cultivation.

Keywords: biological efficiency, fiber degradation, maguey leaves, mushroom cultivation, oyster mushrooms

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INTRODUCTION

Members of the genus *Pleurotus* are known as "oyster mushrooms". They are xilophagous fungi with a pleurotoid habit, attached laterally to the substrate by a stem. Global production of oyster mushrooms has increased significantly in recent years and accounts for more than 19 % of the production of edible mushrooms worldwide (Royse and Sanchez 2017). Among the commercially cultivated species of *Pleurotus*, only *P. ostreatus* (Jacq.: Fr.) P. Kumm., *P. pulmonarius* (Fr.) Quél. and *P. djamor* (Rumph.: Fr.) Boedijn are cultivated in Mexico (Salmones *et al.* 2022). However, other wild species of the genus could potentially be incorporated into the national mushroom production (Salmones and Mata 2017). Among the wild species of *Pleurotus* that have long been consumed in Mexico is *P. agaves* Dennis, a mushroom from xerophytic temperate regions known as "the maguey mushroom", which grows among the fleshy and dead leaves of the agave plant (Camacho *et al.* 2012). These authors also considered that the correct species name should be *P. opuntiae* (Durieu & Lév.) Sacc. However, the cultivation of *Pleurotus* strains isolated from maguey plants has created discussion regarding the possible existence of at least two or three species: *P. dryinus* (Pers.) P. Kumm, *P. agaves,* or *P. opuntiae* (Huerta *et al.* 2010, González de la Tijera 2014, Salmones *et al.* 2017). *Pleurotus dryinus* differs from other species of the genus by presenting a membranous veil at the pileus margin or by forming an annular zone on the stem (Guzmán, 2000), whereas *P. opuntiae* is restricted to the Mediterranean area and *P. agaves* is a phylogenetically distinct species according to Zervakis *et al.* (2019).

Maguey or agave plants have been collected and used in Mexico since pre-Hispanic times, constituting a means of subsistence complementary to the cultivation of cereals (Álvarez-Ríos *et al.* 2020). Their more recognized uses include the production of alcoholic beverages such as tequila (*Agave tequilana* Weber), mezcal (*Agave* spp.), and pulque (*A. salmiana* Otto *ex* Salm.) (Zizumbo-Villarreal *et al.* 2013). Production of the latter beverage peaked in the 19th century (Hinke 2007). However, by the middle of the 20th century, the consumption of pulque had been replaced by beer, which reduced the number of hectares under cultivation from 30,000 (1930) to 11,000 (2018) (Álvarez-Ríos *et al.* 2020). For this reason, the cultivation of maguey for pulque practically ceased, causing the loss of the natural habitat of the maguey mushroom. Given this situation, the isolation and preservation of wild material in biological collections is a strategy for conserving its genetic resources (Mata and Salmones 2021). In addition, the cultivation of this fungus at an experimental level allows us to identify its biodegradative characteristics and determine the feasibility of its commercial production.

On the other hand, in a survey conducted among consumers of edible mushrooms, the maguey mushroom, cited as its synonym *P. opuntiae*, was recognized among the 16 most utilized species in Mexican gastronomy (Molina-Castillo *et al.* 2023), along with the genera *Ramaria, Boletus, Lyophyllum, Morchella, Lactarius, Amanita,* and *Russula* in agreement with Pérez- Moreno *et al.* (2021).

In the mushroom cultivation process, the stage of selection and preparation of the substrate is critical since it is carried out to provide the physical and chemical characteristics suitable for its colonization and biological selectivity that allow the mushroom to grow with an advantage over its various competitors. In the case of the *Pleurotus* genus, these mushrooms are easily adapted to different lignocellulosic materials, due to their ability to degrade them. This characteristic is based on the multienzyme system that they produce, including oxidases and hydrolases capable of attacking cellulose, hemicellulose, and lignin fractions (Adebayo and Martínez Carrera 2015, Mata *et al.* 2017).

The objective of this study is to utilize the cultivation of *P. agaves* in a conventional substrate (barley straw) mixed with maguey leaf residues to compare its biological efficiency (BE) with a commercial strain of *P. pulmonarius* cultivated under the same conditions and to identify productivity characteristics. Furthermore, the variation of cellulose, hemicellulose, and lignin contents in the substrates colonized by *P. agaves* was quantified to determine the biodegradative capacity of the strain.

MATERIALS AND METHODS

Strains

Two strains of *Pleurotus* were studied: one wild variety collected in a pulque maguey field in the region of Alchichica, Puebla, Mexico (19° 24' N y 97° 23' W), isolated in the laboratory, registered as IE-771 in the Strain Collection of the Institute of Ecology, Xalapa, Mexico, and identified as *P. agaves* (Mata *et al.* 2012). The other is a commercial *P. pulmonarius* strain widely used in Mexico and registered as IE-115 in the same mushroom collection.

For this study, strain IE-115 was cultured in a potato dextrose agar (PDA, Bioxon) culture medium, while IE-771 was cultivated in PDA supplemented with wheat extract (Mata and Salmones, 2021). Both strains were incubated at 28 °C in darkness.

Spawn production

The spawn was prepared using sorghum seeds (*Sorghum vulgare* Pers.). For strain IE-771, 50 g of sorghum seeds were placed in glass jars with 40 ml of water and sterilized for one hour at 121 °C. For strain IE-115, the sorghum grains were hydrated for 24 h, and then 200 g of seeds were placed in low-density plastic bags and sterilized for 1 h at 121 °C and 103.4 KPa (Guzmán *et al.* 2013). Following sterilization, 1 cm^2 of the mycelium of each strain, cultivated in culture medium, was placed on the sterile sorghum, and incubated at 28 °C in darkness until the mycelium completely covered the seeds (30 d for strain IE-771 and 15 d for IE-115). This spawn was called the primary spawn. The secondary spawn was prepared under identical conditions for both strains; placing 175 g of sorghum seeds hydrated in low-density plastic bags and sterilizing for 1 h at 121 °C, then inoculating each strain with 25 g of the primary spawn.

Substrate and culture preparation

The maguey leaves were collected in Alchichica, Puebla, Mexico, and sun-dried for transportation to and storage in the laboratory. To obtain the maguey substrate, sun-dried maguey leaves were rewetted for 24 h and maintained for 7 d forming a pile of 70 cm in height covered with plastic to maintain values of temperature and humidity that favored the fermentation process. To promote homogeneous conditions in the pile, the materials were removed daily (Mata and Torres-Hernández 2008). Once the fiber of the maguey leaves had softened, these were chopped into fragments of 5 to 10 cm in length using a fodder cutter. Barley straw was previously chopped, rewetted for 24 h, and fermented for 3 d at ambient temperature. This substrate, defined as S, presented a moisture content of 75 %.

Fermented maguey leaves and barley straw were mixed at a 1:1 proportion (w/w) to produce substrate M. This substrate reached a final moisture content of 80 %. The selection of days of fermentation and the combination ratio between maguey leaves and barley straw were determined by preliminary tests (data not presented). Both substrates were pasteurized using a steam tunnel of 3.5 m^3, with a constant steam flow of 276 Kpa, maintaining a constant compost temperature of 65 °C for 6 h (Velázquez-Cedeño *et al.* 2006), then left to cool to ambient temperature. Pasteurized substrates were then placed in polythene bags (60 x 40 cm), manually mixing the substrate with the secondary spawn at 5 % (w/w), until reaching a moist weight of 4 kg in each bag. Inoculated samples were incubated in darkness at 26±2 °C. On the second day of incubation, the plastic bags were perforated to allow gaseous exchange, 12 cross perforations (2 cm in length) were made per bag, 4 on each face and 2 on each side. At the end of the incubation period, which was marked by the appearance of the primordia, the samples were transferred to a production room under conditions of periods of light (350 Lux) and darkness of 12/12 h, with a temperature of 21±2 °C, following the recommendation of Barrales and Mata (2016), and a relative humidity of 80-85 %. In the case of samples corresponding to the strain IE-771, it was necessary to carefully fragment the mycelium after the first harvest to make a hole in the center of the samples, without reaching the base of the bag, which allowed improved internal hydration of the samples to obtain the following harvests.

The harvested sporophores were weighed and measured manually. They were categorized into the following pileus diameter size groups: G1= ≤ 4.9 cm; G2 = 5.0-9.9 cm; G3 = 10.0-14.9 cm; G4 = 15.0-19.9 cm and G5 ≥ 20 cm (Salmones *et al.* 2020). Percentages per size group were obtained by dividing the

number of sporophores in each size group by the total number in all size groups and multiplying by 100, while the average weight of the mushrooms in each group was obtained by dividing the total weight in each group by the number of sporophores in that group. Productivity was evaluated over three flushes in each treatment and expressed as biological efficiency (BE %) = percentage ratio of the fresh weight of harvested mushrooms over the dry weight of the substrate (Royse *et al*. 2004).

Experimental design

The following treatments were used: a 1:1 mixture of barley straw and maguey leaves (M) and barley straw alone (S). Both substrates were inoculated with the strains IE-115 and IE-771, with 10 replicates utilized per strain. The arrangement in the production area was a completely random design, with 40 units placed at three levels on three shelves.

Data analysis

Data related to pileus size group and treatment was converted to proportions and the values arcsin square-root transformed (Zar 1999). These data were analyzed with a nested analysis of variance using the General Linear Model (GLM) process, the declaration LSMEANS Statement, and the PDIFF option of SAS, version 2003. The BE data obtained were subjected to an analysis of variance with a completely random design. When significant differences were detected, means were compared using Tukey´s multiple range test with a significance level of 0.05 %.

Substrate fiber analysis

Variation in the cellulose, hemicellulose, and lignin contents was determined in the substrates (S and M) used for the fructification of *Pleurotus agaves* before inoculation of the fungus (T1), at the end of the incubation stage (T2), and after the final flush of mushrooms (T3). Samples of approximately 250 g were extracted from each bag (T1, T2, and T3), dried at 65 °C for 24 h, and ground in a blender. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined, as well as acid detergent lignin (with sulfuric acid at 72 %), according to Van Soest *et al*. (1991), using the Ankom Fiber Analyzer, model 200/220. NDF fraction was determined by adding ND solution (water/sodium lauril, sulfate/EDTA/triethylene, glycol/sodium, borate/sodium phosphate) and ADF with water/sulfuric acid/cetyltrimethylammonium bromide solution. Cellulose content was directly estimated from ADF-lignin. Hemicellulose was calculated as NDF-ADF. All samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Production of carpophores

During the incubation period, mycelia covered the entire surface of the substrates between 21 to 39 days, and the first flushes were harvested between 27.9 (IE-115, S substrate) to 43.1 (IE-771, M substrate) days of cultivation. Two or three flushes were obtained from different treatments (Table 1). During the cultivation of *P. agaves* in both substrates and after the first flush, the samples gave no indication of a second flush. In an attempt to activate production, the mycelium in the bags was partially broken for better rehydration of the samples. Rodríguez-Estrada *et al*. (2009) reported that certain substrates must be fragmented following the first flush and Guzmán *et al*. (2013) cited the formation of a pseudosclerotium in some strains of *Pleurotus*. This is the case with *P. agaves*, after the manipulations of the mycelium and substrate carried out in this study, primordia of the second and third flushes were obtained. The development and morphology of the mature fruiting bodies produced by both strains on the different flushes were typical characteristics of the genus *Pleurotus* (Figure 1).

Average fresh mushroom production varied from 302.9 g (IE-115, S) to 555.6 g (IE-115, M) per sample. The strains cultivated on the mixture of maguey leaves and barley straw had the highest production, reaching BE averages of 71.3 % (IE-115) and 64.6 % (IE-771) (Table 1). The BE of the three flushes reported for *P. pulmonarius* in barley straw (S) is within the range of values recorded by Gaitán-Hernández *et al*. (2009) in four flushes, even in the supplemented substrates (Vogel and Salmones 2000) (in two flushes). The BE values found in this study did not differ from those reported for *P. pulmonarius* in other substrates (Philippoussis 2009, De Siqueira *et al*. 2012, Adebayo *et al*. 2014, Vega and Franco 2013, Abdulgani *et al*. 2017,

Picornell *et al*. 2017). Sánchez *et al.* (2002) reported a BE of 38.3 for the strain IE-115 (one flush) in grape skins, which is comparable to our finding in barley straw for the same strain. Nevertheless, our results are lower than those reported by Salmones *et al*. (2020) in barley straw and Bernabé-González *et al*. (2004), who reported three flushes using maguey bagasse from *Agave cupreata* (sourced from the mezcal industry) with and without rice straw as a substrate. Regarding the cultivation of *P. agaves*, Barrales and Mata (2016) reported biological efficiencies of *P. agaves* (~ *P. opuntiae*) strains in barley straw combined with sugarcane bagasse, with and without a 7-day fermentation process, ranging from 86.1 to 105.2 %, and of *P. pulmonarius* at greater than 230 %. Portilla-Segura *et al*. (2019) cultivated *P. agaves* (cited as *P. opuntiae*) in maguey leaves alone or combined with wheat straw, corn cobs, and bean straw, reaching biological efficiencies of between 47.92 to 89.81 % and observing that formulations containing maguey residues favored higher yields. This has been reported for other commercial species of the genus. *Pleurotus ostreatus* cultivated in *A. salmiana* residues has presented biological efficiencies from 11 to 70 % (Heredia Solís *et al*. 2014, España Rodríguez *et al.* 2021) and *P. djamor*, a pantropical species, from 58.3 to 70.56 % (Velázquez de Lucio *et al*. 2022).

Table 1 shows that BE values were greater in the 1:1 maguey leaves-barley straw mixture than those reported in barley straw alone, in both *P. pulmonarius* and *P. agaves*, and that no significant differences exist between the BE values of these species in substrate M. Several studies have demonstrated that BE is directly associated with the strain used, the species, substrates, and environmental conditions of the cultivation (Lechner and Albertó 2011, Dundar *et al*. 2009, Philippoussis 2009).

In addition to the BE, time of production is an important parameter for the producers since they face problems such as limited space in cultivation chambers and the risk of pests and contaminants for prolonged periods (Gaitán-Hernández *et al*. 2009). The time from inoculation to the first flush was 27.9-30.6 d for *P. pulmonarius* and there was approximately a two-week period between the first and second flushes and between the second and third flushes, which is within the range reported for this species (Salmones *et al*. 2020, Bernabé-González *et al*. 2004) (Table 1). More time was required to produce *P. agaves* than for the commercial strain. From inoculation to the first flush took 43.1-44.5 d (~13 d more than in the commercial strain), and the second flush was produced 80 d after the first flush, a period that was sufficient to produce three flushes in *P. ostreatus*. Between the first and second flush, to activate the production of *P. agaves*, the substrate and mycelium were manipulated once, as described previously, thereafter the third flush occurred after 11 d in S and 51 d in M.

The combination and/or supplementation of substrates with C and N sources is recommended to increase productivity in the *Pleurotus* spp. cultivation process (Picornell *et al*. 2017). The mixture of maguey leaves and barley straw may cause an increased C/N ratio on the substrate, and this increases the BEs (Dundar *et al*. 2009). In addition to its nutritional contribution, adding barley straw improves the rheological characteristics of the maguey leaves, avoiding compression and bonding. Barley straw also increases water retention and provides enough space for the spawn.

Pileus size is a quality parameter used by mushroom growers to classify their products. Figure 2 shows the percentage per size category recorded in the two strains used in this study. The best-represented group was G2, corresponding to between 49.4 (IE-771, M substrate) and 58.7 % (IE-115, S substrate) of the total harvested production. The second position corresponded to G1 for IE-115 and to G3 for IE-771. The latter strain also produced basidiomes of groups 4 (S and M) and 5 (S). In general, both strains cultivated on M substrate developed the largest pileus sizes. These results are comparable to those of Lechner and Albertó (2011), who studied the pileus diameter of two species of *Pleurotus* (*P. pulmonarius* and *P. albidus*), using the same classification criteria utilized in this study. Moreover, Gaitán-Hernández *et al*. (2009) reported that the most frequent size in *P. pulmonarius* cultivated in fermented and non-fermented barley straw was G2, which agrees with the findings of this study, and reported that G3 was the second most frequent size class. These results also coincide with Vega and Franco (2013), who cultivated *P. pulmonarius* in corn stover and corncob. On the other hand, Salmones *et al*. (2020), who cultivated three parental strains and forty-eight progeny strains of *P. pulmonarius* in barley straw, reported that the largest group was G1 (62.5 and 58.3 %, respectively), followed by G2 (22.6.

and 23.1 %, respectively) and G3 (9.9 and 15.4 %, respectively). In the present study, the second most frequent size class was G1 in *P. pulmonarius* and G3 in *P. agaves*, and this is the first time that cap diameter sizes have been reported for a cultivated strain of this species.

Regarding the analysis of the weight of the mushrooms grouped by pileus size, it was observed that the variation between the values depended more on the strain than on the substrate (Table 2). In general, the strain IE-771 presented fruiting bodies with a higher average weight than IE-115 in the different culture conditions, although only the samples of *P. agaves* (G3) cultivated in the substrate M were statistically different from those of *P. pulmonarius* (G3), in both substrates. It is important to note that the recorded weights of the fructifications included the stipe so the variability of the weight also depended on robustness. This characteristic of IE-771 of presenting a higher average weight of basidiomes in the different size groups should be corroborated with greater variability of strains and/or substrates since it could be of interest for its commercial cultivation.

Although the results of the statistical analysis do not indicate significant differences between the substrates, the values in Table 2 show an increase in the average weights of the fruiting bodies of strain IE-771 when grown in the M substrate.

Table 1. Comparison of *P. pulmonarius* (IE-115) and *P. agaves* (IE-771) production parameters in the different treatments

Treatments		Average of fresh mushroom weight (g)					Average of days since inoculation		
Strain	Substrate	Flushes			Total	BE %	Flushes		
		1st	2nd	3rd			1st	2nd	3rd
IE-115	S	209.6*	57.1	36.2	302.9	37.3 a**	27.9	40	61
	M	385.4	106.3	63.9	555.6	71.3 b	30.6	49.7	65.6
IE-771	S	191.0	121.2	75.0	342.2	42.1 a	44.5	124.9	135.2
	M	186.9	201.7	191.6	503.6	64.6 b	43.1	122.4	175.4

*$\bar{X}$ ten samples.

**Values followed by different letters within a column are significantly different at $P \leq 0.05$ (HSD Tukey's test). S: barley straw. M: maguey leaves-barley straw 1:1.

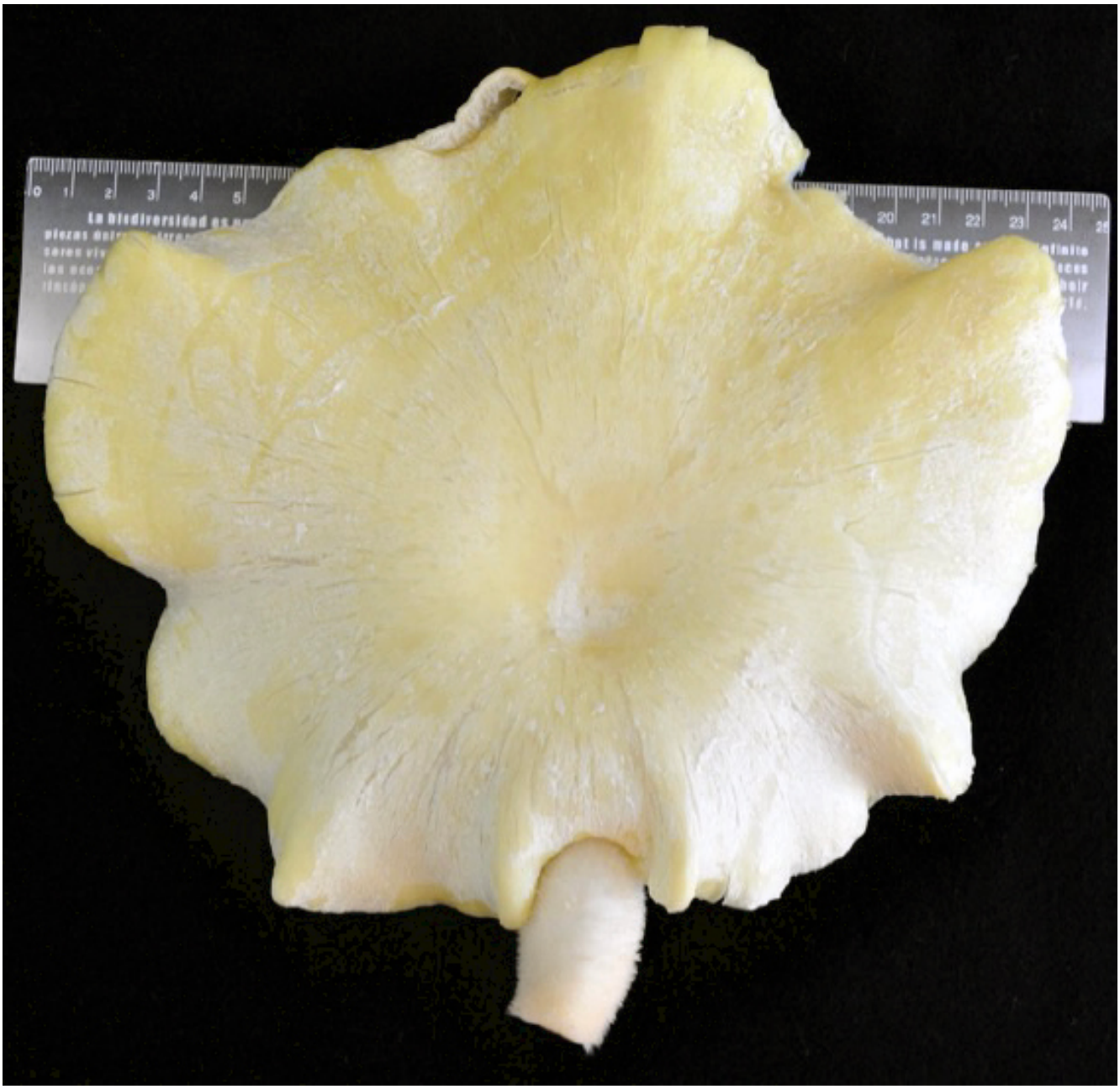

Figure 1. Sporophore of *Pleurotus agaves*, class G5 (more than 20 cm), obtained in agave leaves mixed with barley straw (substrate M).

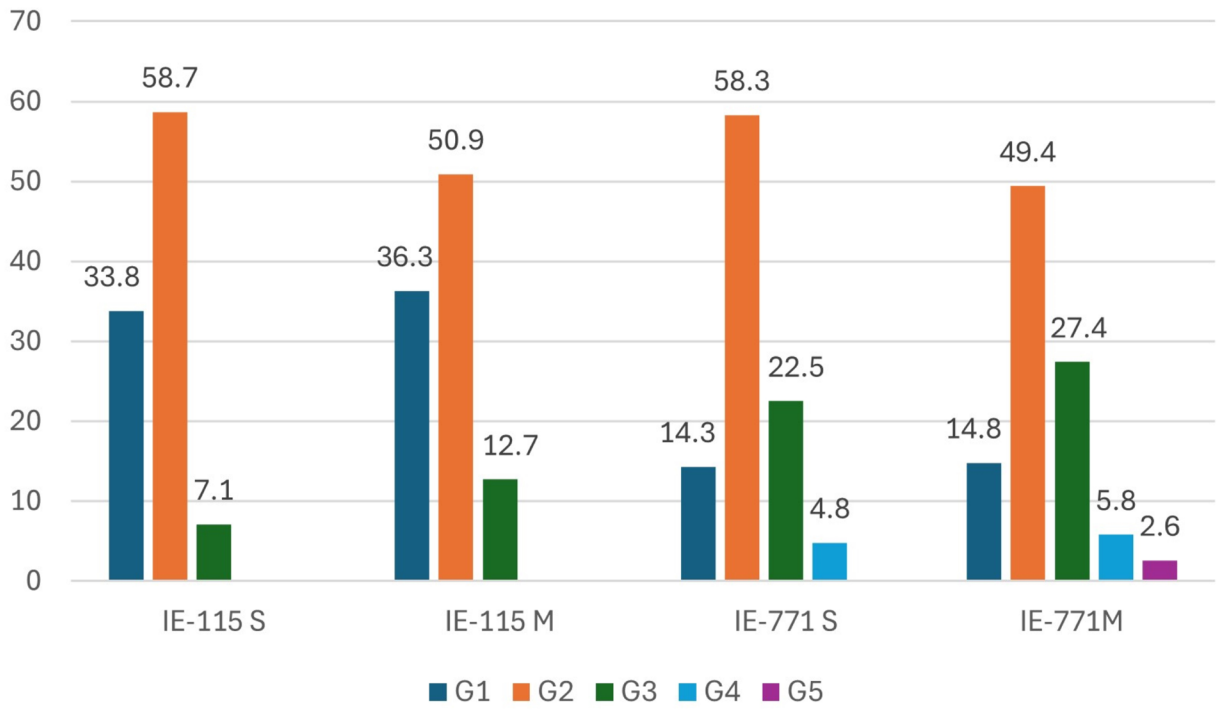

Figure 2. Percentage of sporophores by size category obtained in both formulations, with the strains IE-115 (*Pleurotus pulmonarius*) and IE-771 (*P. agaves*).

Table 2. Average weight (g) of *Pleurotus pulmonarius* and *P. agaves* basidiomes grouped by pileus diameter

		Size categories				
Strain	Substrate	G1*	G2	G3	G4	G5
IE-115	S**	3.5(0.9)ab***	16.3(3.2)abc	40.5(11.1)de		
	M	3.6(0.7)a	14.9(2.9)abc	39.6(5.7)d		
IE-771	S	7.5(5.2)ab	20.2(6.5)bc	54.4(16.3)def	97.3(26.2)g	
	M	9.2(5.6)ab	25.6(4.0)cde	58.5(13.1)f	105.6(27.6)g	181.1(25.6)h

*Pileus diameter groups: G1 = ≤ 4.9 cm. G2 = 5 to 9.9 cm. G3 = 10 to 14.9 cm. G4 = 15 to 19.9 cm and G5 = ≥ 20 cm.
**S: Barely straw. M: Maguey leaves-barley straw 1:1.
***Values followed by different letters within a column are significantly different at P ≤ 0.05 (HSD Tukey's test).

Substrate fiber content

Consumption of the main fiber components of the two substrates during *P. agaves* cultivation is presented in Table 3. By the end of the incubation period, the polysaccharide content of the substrates had been consumed by the mycelia, and the consumption was noticeable after the third harvest. Degradation varied between times and substrates. In particular, IE-771 cultivated in barley straw (S) efficiently degraded cellulose and lignin throughout the incubation period and hemicellulose after the third harvest, while the samples grown on M substrate were characterized by high consumption of all fractions (cellulose, hemicellulose and lignin) during the incubation period. In general, a greater decrease in lignocellulosic compounds was observed for barley straw samples alone than when barley straw was combined with maguey leaves.

The degradation of lignocellulosic fractions by the genus *Pleurotus* depends on the production and secretion of hydrolytic and oxidative enzymes such as cellulases, hemicellulases, and ligninases (Mata *et al.* 2017). Comparing the initial composition (T1) of the formulations used in our study, Table 3 shows a higher proportion of cellulose and hemicellulose in substrate S, while lignin was present in a higher proportion in substrate M. We found in both substrates that the degradation of cellulose is greater during the incubation period (T2) than during fructification. This aligns with that reported by Mata and Torres-Hernández (2008) for *P. ostreatus* in barley straw supplemented with coffee pulp or peat. However, contrary to the findings of these authors, we found that degradation of hemicellulose was greater during fructification in fermented barley straw. This finding has also been reported by Salmones *et al*. (2005), who cultivated *P. ostreatus* and *P. pulmonarius* in wheat straw and coffee pulp; among the three fractions evaluated, cellulose and hemicellulose were the most degraded during the selective growth of the fruiting bodies since they provide the necessary nutrients for their development and the variation in the decrease depends on the sugars and other carbon sources available. Regarding lignin, species of the genus *Pleurotus* are known to selectively degrade lignin and acquire other readily available sources of carbon (Mata *et al*. 2017). The consumption of lignin recorded during the incubation period (T2) in our study, reached approximately 50 % in both substrates but was practically zero during fructification (T3). These results align with those reported by Pandey and Singh (2014), who found a high degradation of lignin from the incubation period to the formation of primordia. However, the degradation of lignocellulose can vary during the different stages of the crop cycle since it is affected by various factors such as the enzymatic activity of the fungus, availability of O_2 in the substrate, composition, and condition of the substrate, interaction between mycelium and substrate, strain genotype, environmental conditions, and the morphogenetic status of the cultivation (Baldrian *et al.* 2005, Sherief *et al*. 2010).

Table 3. Variations of the content (%) of cellulose, hemicellulose and lignin in the substrates during the cultivation of *Pleurotus agaves*

	Barley straw (S)			Maguey leaves-barley straw 1:1(M)		
Stage	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
T1*	29.95(0.78)**	39.83(0.69)	4.10(0.22)	17.69(0.62)	23.91(0.40)	6.77(0.74)
T2	20.78(0.28)	32.46(0.42)	2.09(0.26)	14.58(0.23)	20.47(0.19)	3.47(0.31)
T3	15.17(0.54)	17.18(0.21)	2.67(0.48)	13.28(0.56)	21.52(0.57)	3.33(0.26)

*T1: prior to inoculation. T2: ending incubation period. T3: after the last flush of mushrooms.
**: $\bar{X}$(SD)

The chemical composition of a substrate can influence the nutritional and organoleptic properties of the mushroom (Rodríguez-Estrada *et al.* 2009; Vlasenko and Kuznetsova 2020). Aerobic fermentation of the straw for short periods improves adequate mycelial colonization since fungistatic bacterial communities increase the competitive ability of *Pleurotus* against its main antagonist *Trichoderma* spp. (Velázquez-Cedeño *et al.* 2006). Regarding the maguey leaves, fermentation allowed softening of the fiber, resulting in a better and more manageable consistency for mixing with the fungus spawn (Mata and Torres-Hernández 2008). Guzmán-Dávalos *et al.* (1987) used the "piña" or heart of the *Agave tequilana* (a waste product of the tequila industry) to cultivate *P. ostreatus*, obtaining a BE of 60.2 %. This value increased to 96.4 % when the tequila industry waste was mixed with straw. These authors concluded that a more selective substrate had been obtained and that contamination by molds had been avoided (Soto-Velazco *et al.* 1989). This was observed in our study since no mold contamination was detected in the samples and only one sample had to be discarded due to insect larvae contamination.

In conclusion, maguey mushrooms are highly appreciated in rural areas of central Mexico, and they have been consumed in various traditional dishes such as soups, "quesadillas" or "guisados" (Minerva Mendoza, personal communication). The *P. agaves* strain cultivated in this study presented size and color characteristics that made it suitable for introduction to the market.

This study demonstrated that maguey leaves and barley straw, alone or mixed, could be used as substrates in the cultivation of *P. agaves*. The selection of the former substrate was based on the origin of the strain studied, but the most viable option is to utilize grass straw. Given its morphological and cultivation characteristics, *P. agaves* could compete in the market with other commercial species from temperate environments, such as *P. ostreatus* and *P. pulmonarius*. The isolation and cultivation of wild genetic material from various regions of the country should therefore be continued and a program of selection and improvement of the commercial characteristics of the species should be initiated.

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