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Ichu Valorization by *Pleurotus* spp. Cultivation and Potential of the Residual Substrate as a Biofertilizer

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Abstract

The high-Andean grass *Jarava ichu* (Poaceae) plays a vital role in water regulation and aquifer recharge. However, its limited use is often linked to forest fires, highlighting the need for sustainable alternatives. Therefore, this study aims to explore the valorization of ichu as a substrate for the cultivation of *Pleurotus* spp. (*P. citrinopileatus*, *P. djamor*, and *P. ostreatus*) and to evaluate the potential of the residual substrate as a biofertilizer, offering an ecological alternative to grassland burning in the Peruvian Andes. Samples of ichu from the district of Tomás (Lima, Peru) were used as culture substrate, analyzing productivity indicators such as crop cycle (CC), biological efficiency (BE), and production rate (PR), together with the nutritional profile of the fungi and the chemical properties of the residual substrate. The results showed an average biological efficiency of 19.8%, with no significant differences ($p > 0.05$) in CC, BE, or PR among the species, confirming the viability of ichu as a substrate. The fungi presented a high protein content (24.1–30.41% on a dry basis), highlighting its nutritional value. In addition, the residual substrate exhibited elevated levels of phosphorus (795.9–1296.9 ppm) and potassium (253.1–291.3 ppm) compared to raw ichu (0.11–7.77 ppm for both nutrients). Germination tests on radish seeds showed rates between 80% and 100%, without inhibition, supporting its potential as a biofertilizer. This study demonstrates the double potential of ichu as a substrate for the sustainable production of edible mushrooms of high nutritional value and as a source of biofertilizers.

Keywords: *Pleurotus* cultivation; sustainable substrate; valorization of Andean grasslands

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1. Introduction

High-Andean grasslands, which cover 14.2% of Peru's territory [1,2], are vital ecosystems of both economic and environmental significance. These regions support extensive livestock, primarily involving camelids, cattle, and sheep—an activity that constitutes a crucial income source for local communities. Additionally, these grasslands serve as the most cost-effective source of fodder, sustaining up to 84% of the country's high-Andean livestock production [1]. Beyond their economic role, high-Andean grasslands provide essential ecosystem services, including carbon sequestration—estimated at 0.5 Gt per hectare—and water regulation, which enhances soil infiltration and aquifer recharge during dry periods [3,4]. Furthermore, fodder vegetation coverage mitigates soil erosion in mountainous landscapes, while deep-rooted grasses contribute to soil structure stability and humidity retention [5]. These ecosystems also serve as habitats for endemic fauna and provide essential resources for farming communities, including construction materials and fuel for heating homes and cooking food (energy source) [1,6].

High-Andean grasslands provide essential economic and environmental benefits; however, 62% of these ecosystems are undergoing degradation [7] and face significant threats from traditional burning practices, overgrazing, and the impacts of climate change [8,9]. Among these threats, grassland burning—although a cost-effective and rapid method—results in biodiversity loss, soil fertility depletion, and exacerbation of climate change effects. This practice leads to a decline in bacteria and fungi essential for biogeochemical cycles, organic matter loss, and alterations in key soil properties, including pH and bulk density [10]. Given these challenges, adopting sustainable management strategies for high-Andean ecosystems is imperative.

One potential sustainable use of the Ichu (*Jarava ichu*) is as a substrate for cultivating edible mushrooms. *J. ichu*, a species of the Poaceae family, is characterized by its upright growth, forming dense tillers with stems reaching up to 180 cm in height. This grass thrives in high-Andean ecosystems from 3000 above sea level (masl) [11] and is predominant in the grasslands of the Jalca [12], puna, and paramo (Spanish term for high moorland) ecoregions. It is also distinguished by its easy availability, low harvesting cost (~0.15 USD$\cdot$kg^{-1}) [11], and high lignocellulose content [13]. As a native and wild-growing species, *J. ichu* does not require cultivation or compete with other crops for agricultural land use. Its annual production in Peru is approximately 76 kt [14]. These characteristics position the Ichu as a promising resource for various innovative local applications, including its potential use as a substrate for cultivating edible mushrooms, particularly the genus *Pleurotus* spp. This genus is widely recognized for its high nutritional value [15,16] and medicinal properties [17]. Additionally, *Pleurotus* species offer advantages such as rapid growth, minimal environmental requirements, heavy metal absorption capacity, and resistance to diseases and pests [18].

Mushroom cultivation not only provides direct benefits but also generates a valuable by-product: spent mushroom substrate (SMS) residue, which can be used as an organic amendment or biofertilizer to enhance soil fertility and quality [19–21]. Incorporating SMS as a soil amendment alters soil composition and porosity [22] while enriching its mineral profile [23]. Furthermore, substrate optimization studies have demonstrated how optimized SMS preparation can enhance soil quality and reduce nutrient losses, shedding light on the mechanisms through which SMS improves soil properties [24]. SMS is characterized by a high content of extracellular lignocellulolytic enzymes, including ligninases, cellulases, hemicellulases, and pectinases. These enzymes degrade the polysaccharides in the residual substrate's cell walls, breaking them into a wide range of organic compounds (such as carbohydrates, proteins, and lipids) and inorganic compounds, including ammonium nitrate [25]. This increased nutrient bioavailability facilitates the rapid propagation and colonization of fungal mycelium during mushroom production [26] and makes SMS a valuable resource for reuse.

Recent reviews, such as Kousar et al. (2024), emphasize the need to investigate the effectiveness of SMS across different soil types and crop systems, highlighting current gaps in knowledge and the potential for context-specific applications [27]. In this context, lignocellulosic SMS residues derived from *Jarava ichu* used as a substrate for *Pleurotus* cultivation, which would otherwise have no significant economic value, represent a promising resource for generating novel bio-inputs.

These include biofertilizers for agriculture, reusable substrates for edible mushroom cultivation, and high-nutritional-value feed for livestock [23,26]. In this regard, long-term evaluations, such as the study by Iglesias et al. (2025), highlight the environmental and economic implications of SMS application, offering a broader perspective on its sustainability and potential trade-offs [28]. Furthermore, Leong et al. (2022) emphasized the need to optimize SMS application methods to enhance its agronomic benefits and maximize its efficiency in sustainable agricultural practices [29].

While various lignocellulosic agricultural by-products have been evaluated as substrates for edible mushroom cultivation [30,31], the Ichu—despite its high lignocellulose content and wide availability—has not yet been studied for this purpose in Peru. Its utilization could mitigate the need for pasture burning and provide a novel source of functional foods, given its nutraceutical properties and bioactive compounds, which serve as natural sources of antioxidants, proteins, and amino acids [32]. Additionally, *J. ichu* holds potential for biofertilizer production, contributing to the sustainability of high-Andean agricultural systems. Although previous studies have examined the cultivation of *Pleurotus* spp. using lignocellulosic substrates, there is currently no scientific record of using *Jarava ichu*, a native grass of high-Andean ecosystems, for such purposes. This study introduces an integrative and novel approach by assessing *J. ichu* not only as a sustainable substrate for edible mushroom cultivation but also as a potential source of organic biofertilizers. The combination of these two high-value applications within a single experimental system constitutes an original contribution to current scientific knowledge. In this context, the objective of this work is to evaluate the dual potential of *Jarava ichu* as an alternative substrate for the production of edible mushrooms of high nutritional value and as an organic input for the source of biofertilizer production, thus fostering sustainability strategies for the valorization of native species in high-altitude agroecological systems.

2. Materials and Methods

2.1. Sample Collection and Selection of Biological Material

Ichu samples (6 kg) were randomly collected from a 2 m × 2 m (4 m^2) plot with homogeneous characteristics, including vegetation coverage, age, and height. Within this designated plot, stems were extracted exclusively from tillers in the senescence stage—a phenological phase characterized by the lowest humidity levels in the species' growth cycle. The sample collection site is situated at an altitude of 4050 masl, with geographical coordinates 418,914.20 East and 8,647,138.14 North, in the district of Tomas, province of Yauyos, department of Lima, Peru.

The fungal species *Pleurotus citrinopileatus*, *Pleurotus djamor*, and *Pleurotus ostreatus*, known for their ability to degrade lignocellulosic materials, were used to assess the Ichu's suitability as a potential substrate for its cultivation. These species were supplied by a company specializing in research, product development, and innovative solutions based on edible and medicinal mushrooms.

Raphanus sativus var. Crimson Giant was used to assess the use of spent mushroom substrate as a biofertilizer derived from the cultivation of *Pleurotus* species.

2.2. Proximal Analysis of the Ichu-Based Substrate

The Ichu samples were transported to the Food Analysis Laboratory of the Nutritional Research Institute (IIN) for proximal analysis and determination of their main components. Humidity, ash, fat, protein, and crude fiber contents were measured following AOAC (2023) standard procedures [33]. Carbohydrate content, total energy, and kilocalorie values were calculated using the MS-INN method described by Collazos (1993) [34].

2.3. Cellulose and Lignin Analysis in the Ichu-Based Substrate

The cellulose and lignin analyses in the Ichu samples followed the official ANKOM reference methods [35] for their quantification: cellulose (ANKOM, 2005; Method N° 5) and lignin (ANKOM, 2005; Method N° 8). These analyses were performed at the Food Nutritional Evaluation Laboratory of the Universidad Nacional Agraria La Molina, Peru.

2.4. Pleurotus spp. Cultivation

The fungal cultivation process was conducted following the procedure described by Akcay et al. (2023) [36], with some modifications. Initially, Ichu was chopped into 3–4 cm fragments, and a total of 3600 g was weighed and evenly distributed into nine perforated polypropylene bags, each containing 400 g. The sealed bags were then immersed in drinking water for 12 h. To remove excess humidity, the bags were drained for 1 h before transferring the material to new polypropylene bags for autoclaving at 121 °C for 15 min. After sterilization, the substrate was allowed to cool to room temperature. The fungal species *P. citrinopileatus*, *P. djamor*, and *P. ostreatus* were then inoculated separately using wheat seeds colonized by the mycelium of each species, following the modified method of Rodríguez et al. (2021) [37]. To prepare the mycelium-colonized seeds, 100 g of shelled wheat seeds were washed with detergent and rinsed thrice. The seeds were placed in 250 mL Erlenmeyer flasks and autoclaved at 121 °C for 15 min. Once cooled to room temperature, the seeds were inoculated with a 10 mm diameter agar disc containing mycelium previously grown on PDA agar. The inoculated seeds were incubated in the dark at 28 °C for five days until fully colonized by the mycelium. Contamination absence was assessed through visual inspection, and any samples exhibiting abnormal coloration or lacking mycelial growth were discarded. The wheat seed inoculum accounted for 8.1% of the dry weight of the substrate in each *Pleurotus* culture bag.

The experiment was conducted with three replicates for each *Pleurotus* species. Substrate-filled bags were maintained in an incubation chamber at 25 °C with 70–80% relative humidity for 21 days. Once mycelial growth became abundant and/or primordia had formed, the bags 0077ere transferred to the fruiting chamber for 7 to 10 days. At this stage, a 2 cm incision was made in two central areas on the lateral sides of each bag to facilitate the development of fruiting bodies or basidiocarps [38], as shown in Figure 1. Relative humidity and temperature in the incubation chamber were monitored using a Giardino HTC-2 digital thermo-hygrometer to ensure stable conditions within the recommended parameters.

2.5. Productivity Indicators of Pleurotus Cultivation

Mushroom productivity was assessed using the following indicators: the cultivation cycle (CC), biological efficiency (BE), and production rate (PR). The CC was defined as the number of days from sowing to the last harvest. The BE was calculated as the ratio between the mushroom's fresh weight (g) and the dry weight of the substrate (g), as stated by Salmones et al. (1997) [39]. The PR was determined as the quotient of BE and the total time elapsed from inoculation to the last harvest [40].

2.6. Proximal Analysis of Pleurotus

The proximal analysis of the fruiting bodies of *P. citrinopileatus*, *P. djamor*, and *P. ostreatus* was conducted following established procedures in official reference methods for the quantification of key parameters: protein (AOAC 2001.11.2023) [33], humidity (NTP-ISO 1442.2006) [41], ash (NTP 201.022.2002) [42], total fat content (NTP 201.016.2002) [43], and crude fiber (AOCS-BA 6-84.2017) [44]. Carbohydrate content was determined using the MS-INN method described by Collazos (1993) [34]. These analyses were performed at the Food Analysis Laboratory of the Nutritional Research Institute of Peru.

Figure 1. Progressive development of *Pleurotus* spp. cultivated on Ichu (*J. ichu*) substrate: (**A**) primordia formation of fruiting bodies, (**B**) growth of *P. djamor* fruiting bodies, (**C**) growth of *P. ostreatus* fruiting bodies, and (**D**) mature *P. citrinopileatus* fruiting bodies ready for harvest.

2.7. Potential of Pleurotus SMS Spent Substrate as Biofertilizer

The effect of the post-culture substrate from *P. citrinopileatus, P. djamor*, and *P. ostreatus* on the germination of *Raphanus sativus* var. Crimson Giant was evaluated. The experimental design included three treatments (T1, T2, and T3), in which 50 mL of spent substrate from *P. citrinopileatus*, *P. djamor*, and *P. ostreatus* was mixed with 450 mL of sterile soil. The control treatment (Tc) consisted of 500 mL of sterile soil, with fertilization provided by a hydroponic solution sourced by Universidad Nacional Agraria La Molina. This solution was prepared by diluting 0.5 mL of Solution A and 0.2 mL of Solution B in 100 mL of irrigation water. A completely randomized design was used, with each treatment replicated five times. In each replicate, two seeds of *Raphanus sativus* var. Crimson Giant were sown. The germination percentage was recorded for each treatment.

In addition, the chemical characteristics of the residual substrate from each of the three *Pleurotus* species were analyzed following official AOAC standard procedures [33,45]. The assessed parameters included humidity (AOAC 930.04, 2023) [46], nitrogen (AOAC 2001.11, 2023) [33], pH (AOAC 981.12, 2023) [47], phosphorus (AOAC 970.39, 2019) [48], and potassium (AOAC 975.03, 2019) [49]. All analyses were conducted at the Food Analysis Laboratory of the Nutritional Research Institute of Peru.

2.8. Economic Analysis or Valorization of Pleurotus spp. Cultivation on Ichu

The economic viability of cultivating *Pleurotus* spp. using *J. ichu* as a substrate was assessed through a methodological framework encompassing mushroom production, the residual substrate's valorization, and the production system's financial analysis. A 24 m^2 production area was established for a small-scale, resource-limited producer. The processing capacity was 150 kg of dried Ichu per production cycle. Fixed and variable costs were determined, considering infrastructure, inputs, labor, transportation, and energy. Fresh mushroom production was estimated at 490 kg annually, costing 3.56 USD kg^{-1}. A benchmark selling price of 6.58 USD·kg^{-1} was established, enabling the net profitability of the system's calculation. Additionally, the input–output ratio and rate of return on investment were estimated to evaluate the financial feasibility of the system.

2.9. Data Analysis

Treatment comparisons were conducted using analysis of variance (ANOVA) for a completely randomized design (CRD) with SPSS software (version 26) at a 95% confidence level. Significant differences between treatments were identified through multiple comparisons using the Tukey HSD test as a post hoc analysis. Additionally, ANOVA assumptions, including residual normality and homogeneity of variances, were assessed using the Kolmogorov–Smirnov and Levene tests to ensure the statistical validity of the results.

3. Results and Discussion

3.1. Proximal Composition of Ichu

The nutrient composition of the substrate directly influences the chemical composition and biological characteristics of *Pleurotus* spp. [50]; therefore, the proximal composition of Ichu was determined. The results indicated that crude fiber (38.90 g·100 g^{-1}) and carbohydrates (40.26 g·100 g^{-1}) were the primary components of this material (Table 1). The protein content (5.53 g·100 g^{-1}) was also recorded, which falls within the range reported for *J. ichu.* (3.43–7.17%) across different seasons, as described by Mamani-Linares and Cayo-Rojas (2021) [51]. Furthermore, the humidity content of the analyzed samples at the senescence phenological stage aligned with the values reported by Mori et al. (2019) [14]. During this stage, the species undergoes loss of green pigmentation, wilting of plant structures, and the plant's progressive drying, explaining the reduction in humidity levels compared to other phenological stages. For instance, in previous stages, humidity values as high as 57% on a dry basis have been observed [52].

Regarding energy content, the majority of the available energy in Ichu is derived from carbohydrates (161 kcal·100 g^{-1}), followed by protein (22 kcal·100 g^{-1}) and fat (6 kcal·100 g^{-1}). This composition profile makes Ichu a suitable substrate for mushroom mycelium growth, providing an optimal energy balance and essential nutrients.

In mushroom cultivation, the nitrogen content of the substrate plays a crucial role in mycelial development, as it is essential for cell wall formation, as well as protein, nucleic acid, and polysaccharide synthesis. Furthermore, nitrogen directly influences the growth and yield of *Pleurotus* spp. [50]. For instance, Rizki and Tamai (2011) reported that a substrate composed of oil palm residues with a nitrogen content of 2.09% facilitated complete mycelial colonization and enhanced the formation of initial structures (pinheads) in *P. ostreatus* [53]. However, excessive nitrogen can inhibit mycelial growth and slow its development (slower spawn run), as Baysal et al. (2003) observed [54]. Previous studies suggest that an optimal nitrogen range of 0.5–1.5% in the substrate enhances the biological efficiency of *Pleurotus* spp. cultivation [55]. In this study, Ichu exhibited a nitrogen content of 0.88%, indicating that it falls within the optimal range for efficient *Pleurotus* spp. cultivation.

Table 1. Proximal composition of Ichu (*J. ichu*).

Parameter	Unit	Result
Humidity	$g \cdot 100\ g^{-1}$	8.8
Ashes	$g \cdot 100\ g^{-1}$	5.75
Fat	$g \cdot 100\ g^{-1}$	0.68
Proteins	$g \cdot 100\ g^{-1} N \times 6.25$	5.53
Crude fiber	$g \cdot 100\ g^{-1}$	38.90
Carbohydrates	$g \cdot 100\ g^{-1}$	40.26
Total energy	$kcal \cdot 100\ g^{-1}$	189
Carbohydrates	$kcal \cdot 100\ g^{-1}$	161
Proteins	$kcal \cdot 100\ g^{-1}$	22

3.2. Cellulose and Lignin Content of Ichu

Lignin is a complex, recalcitrant polymer that forms a physical barrier around structural polysaccharides in plant biomass, making it difficult for fungi to access the cellulose and hemicellulose necessary for their growth. Pleurotus fungi possess ligninolytic enzymes, such as laccases, lignin peroxidases, and manganese peroxidases, which allow them to degrade lignin and access fermentable carbohydrates. However, the efficiency of this process may vary according to the specific composition and structure of the lignin present in the substrate [56].

The cellulose and lignin contents in Ichu (*J. ichu*) were found to be 34% and 9.58%, respectively. These values are comparable and close to those reported for other species within the *Jarava* genus (synonymous with Stipa), such as *Stipa obtusa*, which has been documented to contain 38.07% cellulose and 15.56% lignin [14]. Variations in these values may be attributed to intraspecific differences, variations in environmental conditions, or the phenological stage of the plants at the time of sampling. According to Albarracín et al. (2019), the chemical composition of grasses undergoes significant changes throughout their life cycle, with cellulose and lignin concentrations progressively increasing as the species's senescence advances [57]. Additionally, studies have demonstrated that factors such as altitude and soil type can influence lignin content in *Jarava* species and other seasonal grasses [51].

3.3. Performance of Pleurotus Cultivation Based on Productivity Indicators

The productivity of three *Pleurotus* species cultivated using Ichu as a substrate was evaluated using the following indicators: cultivation cycle (CC), biological efficiency (BE), and production rate (PR), as shown in Figure 2. ANOVA results for each productivity indicator yielded a p-value > 0.05, indicating no significant differences in CC, BE, or PR among the three *Pleurotus* species grown on Ichu as a substrate. These findings suggest that, despite species differences, their yields were similar. Consequently, the average BE of the three species, 19.8%, will be used for further analysis. This BE value is higher than that reported by Puliga et al. (2022), who found BE values of 0.8%, 11%, and 10.8% for *G. lucidum*, *L. edodes*, and *P. cornucopiae*, respectively, using hazelnut husks as a substrate [58]. However, the 19.8% BE observed in this study is lower than the values reported in other studies. Nieto-Juárez (2021) reported a BE of approximately 60% using coffee husk residue as a substrate [27]. Obadai et al. (2003) found a BE of 50.64% for *P. ostreatus* cultivated on a rice straw-based substrate [59].

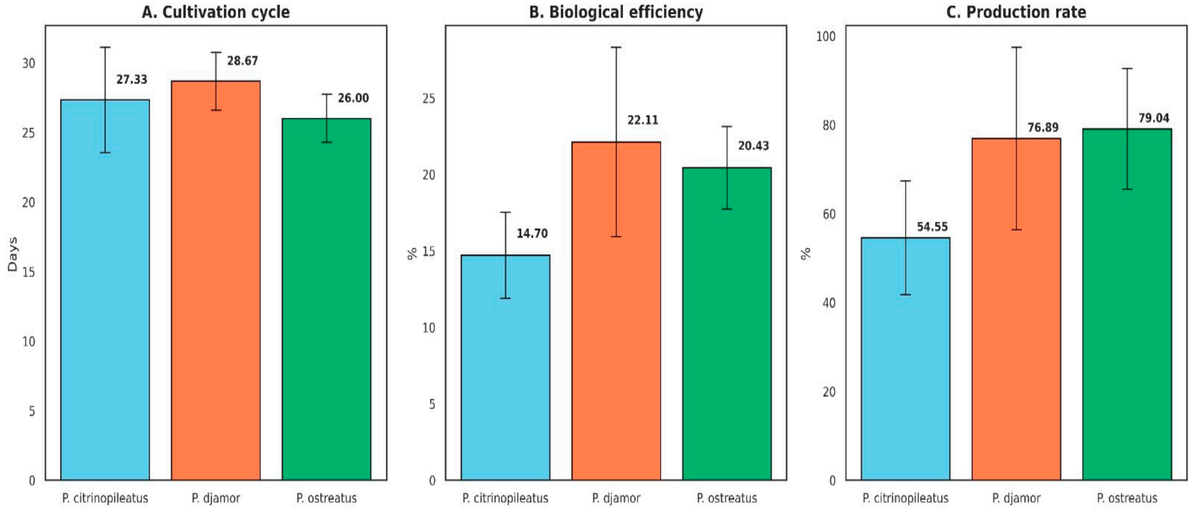

Figure 2. Comparative analysis of productivity indicators in three Pleurotus species cultivated on *Jarava ichu* substrate: (**A**) cultivation cycle, (**B**) biological efficiency (%), and (**C**) production rate. No significant differences were observed among species ($p > 0.05$, ANOVA).

The differences observed in BE could be attributed to variations in the lignin content of the substrates. According to Albarracín et al. (2015), Ichu contains 45.9% cellulose and 18.2% lignin [52]. Similarly, Sims et al. (2008) reported that cellulose constitutes 40–50% of the dry weight of plant material, while lignin accounts for 15–20% [60]. In contrast, hazelnut husks have a lignin content of 40–50% (Puliga et al., 2022) [58]. These findings suggest that a high lignin content in substrates negatively affects fungal BE. Badu et al. (2011) emphasized the importance of substrate composition, particularly lignin content, in the cultivation performance of *P. ostreatus* [61]. Lignin acts as a protective barrier, limiting cellulose and hemicellulose degradation and thereby reducing sugar availability for fungal metabolism. Additionally, it is essential to acknowledge that BE is influenced by multiple factors, including fungal species, substrate composition, and environmental conditions [62,63].

This study explores the potential use of Ichu as a substrate for mushroom cultivation. The results demonstrate that all three tested species successfully grew on this substrate, which has not previously been utilized for *Pleurotus* cultivation. Furthermore, the findings suggest that Ichu is as effective as other lignocellulosic substrates in supporting *Pleurotus* production. However, it is important to note that biological efficiencies above 50% are generally considered economically viable for mushroom cultivation, according to Ríos et al., 2010 [64]. Therefore, to optimize mushroom production, it is necessary to enhance the Ichu-based substrate by supplementing it with alternative substrates rich in easily metabolized carbohydrate sources to facilitate lignin degradation.

Applicability of pretreatment methods such as chemical [56,65], biological [61], and combined pretreatments, considering the lignocellulosic composition of *Jarava ichu*, could improve its suitability as a substrate for *Pleurotus* cultivation. However, it is essential to evaluate the economic and environmental feasibility of these methods in the specific context of *Jarava ichu* and local production conditions. In future research, it is recommended to explore and optimize these pretreatments to determine their effectiveness in improving the BE and sustainability of the *Pleurotus* cultivation process.

3.4. Proximal Composition of *Pleurotus* spp.

Mushrooms of the *Pleurotus* genus are widely recognized for their high nutritional value and beneficial medicinal properties [66–68], which, according to previous studies, are influenced by cultivation conditions [69]. Table 2 presents the proximal analysis results for the main components of *P. citrinopileatus*, *P. djamor*, and *P. ostreatus* cultivated on Ichu substrate. The data indicate that humidity is the predominant component in all three species, ranging from 82.29% to 84.03%, followed by carbohydrates, which vary between 49.9% and 56.1%. Protein content was determined using a conversion factor of 4.38, a recommended value for fungi due to their high levels of non-protein nitrogenous compounds such as chitin [70–73]. Accordingly, protein content ranged from 24.1% to 30.41%. Fiber, ash, and fat were present in lower proportions, with values ranging from 1.50% to 13.59%. These results fall within the ranges reported in previous studies for *Pleurotus* species cultivated on various types of substrates [31,74–79]. Residual normality and homogeneity of variances were confirmed by Kolmogorov–Smirnov and Levene tests, respectively. Both tests indicated that the data met the assumptions of normal distribution and equality of variances ($p > 0.05$). Moreover, ANOVA revealed that no significant differences ($p > 0.05$) were observed among the three *Pleurotus* species cultivated on Ichu substrate, so no post hoc tests were performed, in accordance with standard statistical procedures.

Table 2. Proximal composition of *P.* citrinopileatus, *P.* djamor, and *P.* ostreatus.

Parameter	Species	Wet Basis (%)	Dry Basis (%)	SD
Humidity (g·100 g^{-1})	*P. citrinopileatus*	82.69	-	1.03
	P. djamor	84.03	-	3.83
	P. ostreatus	82.29	-	6.41
Ash (g·100 g^{-1})	*P. citrinopileatus*	1.24	7.16	0.18
	P. djamor	0.99	6.2	0.22
	P. ostreatus	0.98	5.53	0.3
Fat (g·100 g^{-1})	*P. citrinopileatus*	0.32	1.85	0.08
	P. djamor	0.24	1.5	0.24
	P. ostreatus	0.69	3.9	0.3
Protein (g·100 g^{-1}N × 4.38)	*P. citrinopileatus*	5.26	30.41	0.6
	P. djamor	4.19	26.18	0.96
	P. ostreatus	4.27	24.1	0.92
Crude fiber (g·100 g^{-1})	*P. citrinopileatus*	1.83	10.57	0.33
	P. djamor	2.17	13.59	0.93
	P. ostreatus	1.9	10.73	1.09
Carbohydrates (kcal·100 g^{-1})	*P. citrinopileatus*	8.65	49.97	0.04
	P. djamor	8.08	50.59	1.83
	P. ostreatus	9.95	56.18	4.59
Total energy (kcal·100 g^{-1})	*P. citrinopileatus*	67.67	-	4.04
	P. djamor	59	-	14.53
	P. ostreatus	70	-	25.94
Carbohydrates (kcal·100 g^{-1})	*P. citrinopileatus*	34.67	-	0.58
	P. djamor	32.33	-	7.51
	P. ostreatus	40	-	18.33
Protein (kcal·100 g^{-1})	*P. citrinopileatus*	30	-	3.46
	P. djamor	24	-	5.29
	P. ostreatus	24	-	5.2
Fat (kcal·100 g^{-1})	*P. citrinopileatus*	3	-	1
	P. djamor	2.67	-	2.08
	P. ostreatus	6	-	2.65

The humidity observed in this study for *P. citrinopileatus* (82.69%), *P. djamor* (84.03%), and *P. ostreatus* (82.29%) falls within the range reported for *P. eryngii* (80.7%) (Ryu et al., 2015) [74] and *P. ostreatus* (88.2%) [27,76]. High humidity content is a common characteristic of edible mushrooms, making them highly susceptible to microbial growth and enzymatic activity, negatively impacting their shelf life and texture [76,80]. However, various preservation techniques for *Pleurotus* mushrooms have been reported for commercial applications [81–83].

The carbohydrate content on a dry basis in *P. citrinopileatus* (49.97%), *P. djamor* (50.59%), and *P. ostreatus* (56.18%) is comparable to values reported for *Pleurotus* spp., cultivated on substrates derived from various agricultural waste sources [77,78,84]. In mushrooms, carbohydrates, including monosaccharides and polysaccharides—serve as an energy source and are stored primarily as glycogen rather than starch, as in plants. Additionally, carbohydrates contribute to the characteristic taste of mushrooms (Kalač, 2016) [80].

The values of protein content in this study for *P. citrinopileatus* (30.41%), *P. djamor* (26.18%), and *P. ostreatus* (24.1%) are higher or similar to those reported by previous studies for different *Pleurotus* species, in particular for *P. ostreatus*, *P. sajor-caju*, *P. columbinus*, and *P. sapidus*, whose values ranged from 16.07% to 25.15% [79]. The values of *P. ostreatus* cultivated on sawdust substrate and different organic fertilizers were between 19.27% and 33.41% [85]. Also, *P. ostreatus* and *P. djamor* reported values ranging from 17% to 35% and from 14.02% to 19.68%, respectively, using agricultural wastes (barley stubble and maize husk) as substrate [86]. Compared to the values obtained in this study, these are lower for *P. djamar* and *P. citronopileatus*, while for *P. ostreatus,* they are within the reported range, although below the maximum value of 35%.

Silva et al. (2020) reported crude protein values ranging from 22.4% to 27.5% in *P. ostreatus* cultivated on agro-industrial residues from oil palm fruits and cocoa almonds as a substrate [78]. Additionally, previous studies indicate that nitrogen content in the substrate enhances protein accumulation in the fungus [77,78,87]. However, it must be considered that nitrogen concentrations equal to 1.5% or higher inhibit fungal growth [88]. Based on the present study's findings, Ichu is a suitable substrate for cultivating *Pleurotus* species with high protein content, enhancing their nutritional value.

The fiber content observed in this study was 10.57% for *P. citrinopileatus* and 10.7% for *P. ostreatus*, while *P. djamor* exhibited a higher value of 13.59%. These results exceed those reported by Peter et al. (2019), who found fiber levels ranging from 8.2% to 9.1% in *P. ostreatus* cultivated on agricultural residues such as cassava peels, banana leaves, and sawdust [76]. However, the obtained values in our study are lower than those reported for *P. citrinopileatus* (16.20–21.6%) and *P. ostreatus* (13.0–19.89%) grown on agro-industrial and forest residues [89,90]. Overall, mushrooms are an important source of dietary fiber, which, although indigestible by the human digestive system, provides numerous nutritional and physiological benefits [83].

Edible mushrooms have low lipid content (less than 5%), primarily composed of unsaturated fatty acids, contributing to their characteristic aroma [76,88]. In this study, the fat content determined for *P. citrinopileatus* (1.85%), *P. djamor* (1.50%), and *P. ostreatus* (3.9%) falls within this range. The obtained values for *P. citrinopileatus* and *P. djamor* are lower than those reported by İnci et al. (2023) [84], who found levels ranging from 2.0% to 2.3% using wheat straw and quinoa stalks as substrates. For *P. ostreatus*, the obtained fat content in this study (3.9%) is consistent with previously reported values, which range from 1.64% to 4.62% [76,91]. These differences underscore the lipid content variability depending on the fungal species and the substrate used.

The findings of this study indicate that the Ichu-based substrate is effective for cultivating *Pleurotus* spp., with notable protein, fiber, and carbohydrate content compared to previous studies. The three analyzed species exhibited superior nutritional profiles, particularly the protein content of *P. citrinopileatus* (43.39%) and *P. djamor* (37.35%), suggesting that Ichu could be a viable substrate source for producing mushrooms with high nutritional value.

3.5. Potential of Spent Mushroom Substrate as a Biofertilizer

Table 3 presents the chemical composition of the spent mushroom substrate (SMS) obtained from cultivating three *Pleurotus* species—*P. ostreatus*, *P. citrinopileatus*, *P. djamor*, and the Ichu substrate without mycelium. These results indicate that SMS is rich in essential nutrients such as phosphorus, potassium, and nitrogen, supporting its potential use as a biofertilizer. These findings align with previous studies, highlighting the agronomic value of SMS [92,93]. Specifically, the humidity content of SMS ranged from 45.80 to 51.04 g·100 g^{-1}, indicating a high water retention capacity, which is beneficial for soil hydration. This is consistent with the findings of Lipiec et al. (2021) [94], who demonstrated that SMS application increases field water retention capacity (at −100 h·Pa) within a range of 42.4% to 48.5% at depths of 0–40 cm. Nitrogen levels in SMS ranged from 0.47 to 0.59 g·100 g^{-1}, with *P. ostreatus* exhibiting the highest value. However, these nitrogen levels were lower than those found in Ichu without mycelium (4.02 g·100 g^{-1}). The lower nitrogen levels in *Pleurotus* spp. post-culture substrate (SMS) than in uninoculated ichu can be explained by several factors related to fungal metabolism and substrate composition. During growth, *Pleurotus* spp. use the nitrogen available in the substrate to synthesize proteins and other essential compounds. This consumption reduces the amount of nitrogen present in the residual substrate after the mushrooms are harvested [95]. The process of decomposition of organic matter by the fungus can alter the availability of nutrients in the substrate. Although uninoculated ichu has a higher nitrogen content initially, fungal activity during cultivation can mobilize and consume this nitrogen, decreasing its concentration in the SMS. As a lignocellulosic material, ichu has a chemical composition that can influence nitrogen availability. The carbon-to-nitrogen (C/N) ratio of the substrate is a determining factor in the efficiency of colonization and growth of the fungus. Substrates with a high C/N ratio may require nitrogen supplementation to optimize fungal growth and fruiting body production [50].

Table 3. Chemical composition associated with the biofertilization potential of residual substrates from *Pleurotus* spp. cultivation.

Cultivated Fungus	Humidity (g·100 g^{-1})	Nitrogen (g·100 g^{-1})	pH	Phosphorus (ppm)	Potassium (ppm)
P. ostreatus	46.89	0.59	5.2	795.9	253.1
P. citrinopileatus	45.80	0.47	5.6	989.4	291.3
P. djamor	51.04	0.54	5.6	1296.9	262.6
J. ichu (sin cultivo)	5.40	4.02	5.75	0.11	7.77

Additionally, phosphorus content was significantly high in SMS, particularly in *P. djamor* (1296.9 ppm), followed by *P. citrinopileatus* (989.4 ppm) and *P. ostreatus* (795.9 ppm). These values were considerably higher than Ichu's (0.11 ppm), underscoring SMS as a rich source of this essential nutrient. Potassium levels in SMS ranged from 253.1 to 291.3 ppm, with *P. citrinopileatus* exhibiting the highest concentration. These values were substantially higher than those found in Ichu without mycelium (7.77 ppm). Similarly, Uzun (2004) reported that SMS is a good source of potassium and phosphorus but is relatively low in nitrogen [96]. However, when applied as a fertilizer, its nutrients are released gradually over an extended period, allowing for more efficient plant uptake. Furthermore, its use provides additional benefits, such as improving the soil's physical, chemical, and biological properties. Additionally, the pH of SMS ranged from 5.2 to 5.6, indicating a slightly acidic nature, which is optimal for nutrient availability in most agricultural soils.

The spent mushroom substrate (SMS) effect on seed germination was evaluated using SMS derived from *P. citrinopileatus*, *P. djamor*, and *P. ostreatus* alongside a chemical fertilizer control (Table 4). Germination rates were measured to assess SMS's biofertilizer potential. The application of spent mushroom substrate (SMS) as a biofertilizer can influence seed germination through several mechanisms. One of the primary mechanisms is the supply of essential nutrients: SMS enriched with nitrogen, phosphorus, and potassium can improve the availability of these elements to developing plants, thereby promoting more efficient germination [97]. In addition, the incorporation of SMS into soil can increase electrical conductivity, potentially altering the osmotic balance of the medium. This change in osmolarity may affect the seed's ability to absorb water, thereby influencing germination rates. Moreover, during the decomposition of SMS, bioactive compounds such as humic and fulvic acids are released, which may stimulate physiological processes in seeds and further enhance germination [98].

Table 4. Effect of SMS on radish germination.

Coding	Treatment	Dose (mL)	Germination Percentage (%)
T1	Ichu with *P. citrinopileatus* mycelium	50 ml	90
T2	Ichu with *P. djamor* mycelium	50 mL	100
T3	Ichu with *P. ostreatus* mycelium	50 mL	80
T4 *-control	Chemical fertilizer	50 mL	90

* 0.5 mL of solution A and 0.2 mL of solution B (diluted in 100 mL of water).

Treatment T1, which utilized SMS from *P. citrinopileatus*, achieved a germination rate of 90%, indicating its effectiveness in promoting seed germination. Treatment T2, incorporating SMS from *P. djamor*, recorded the highest germination rate (100%), likely due to its elevated phosphorus (1296.9 ppm) and potassium (262.6 ppm) content. Treatment T3, using SMS from *P. ostreatus*, resulted in an 80% germination rate, demonstrating its efficacy, albeit to a lesser extent than the other SMS treatments. Treatment T4, the chemical fertilizer control, also achieved a 90% germination rate, comparable to T1, suggesting that SMS from *P. citrinopileatus* and *P. djamor* can be as effective as conventional fertilizers. These findings align with previous studies indicating that SMS can enhance the nutrient content of the germination substrate by supplying essential elements such as nitrogen, phosphorus, and potassium, which are crucial for seedling development [99,100].

Previous studies have shown that residual substrate from fungal cultures can be beneficial for different plant species. For example, research has indicated that the use of residual substrates of *Pleurotus ostreatus* improved the development of tomato (*Lycopersicum esculentum*) seedlings when mixed with fertilized soil, providing the nutrients necessary for optimal growth (Fontalvo et al., 2013) [101]. In addition, it has been observed that the incorporation of residual fungal substrates into the soil can increase nutrient availability and improve microbial activity, potentially benefiting the growth of various plant species (Martín et al., 2023) [102]. Its agronomic applicability needs to be evaluated more broadly, it is essential to conduct field trials with various plant species and to analyze the long-term effects on soil health and crop yields.

3.6. Economic Analysis of Pleurotus spp. Production

The cultivation of *Pleurotus* spp. using Ichu as a substrate presents a profitable alternative within edible mushroom production systems. To evaluate its economic viability on an artisanal scale, a processing capacity of 150 kg of Ichu was established within a 24 m^2 area, designed for management by an artisanal producer with limited resources.

The annual production cost is estimated at USD 1746.32. With a projected yield of 490 kg of fresh mushrooms per year, the production cost is 3.56 USD kg^{-1}. Given a selling price of 6.58 USD·kg^{-1}, the net profit per kilogram is USD 3.02. These values indicate a profitability of 84.6%, demonstrating that the cultivation of *Pleurotus* spp. using Ichu as a substrate is viable and competitive, even on a small scale.

In addition to mushroom production, the residual substrate can be reused as a biofertilizer, adding further value to the production system, which consists of four key components: facilities, energy, transport, and labor [100]. In this study, SMS derived from Ichu demonstrated a superior input–output ratio compared to traditional organic fertilizers and has the potential to generate additional income by being sold as a soil conditioner, particularly for phosphorus and potassium-rich soils [103–105].

In the future, recycling SMS as a fertilizer presents a valuable opportunity for its application in family farming, enabling production cost reductions through improved substrate management and enhanced crop yields. To maximize these benefits, optimizing the *Pleurotus* cultivation production process is recommended. Commercializing the residual substrate as a biofertilizer would also support investment recovery and enhance the system's sustainability. Finally, expanding the market for edible mushrooms in high-Andean communities is suggested as a viable alternative source of plant-based protein. The use of *J. ichu* as a substrate for *Pleurotus* spp. in artisanal systems is not only profitable but also capitalizes on an underutilized resource, reduces environmental pollution, and provides a sustainable alternative to grassland burning. Furthermore, the residual substrate's potential as a biofertilizer enhances the system's economic and environmental viability, fostering more efficient and sustainable agricultural production.

It is important to note that the profitability estimates may be optimistic due to the lack of a specific and up-to-date price analysis tailored to the regional context. In this regard, it is considered necessary to recommend conducting updated market studies to obtain accurate data on mushroom commercialization prices in the target regions, which would strengthen the economic projections of the present study.

3.7. Research Limitations and Perspectives for Future Exploration

Although the objective of the study was to preliminarily evaluate ichu as a substrate for *Pleurotus* spp., the absence of a comparison group with other commonly used substrates limits the possibility of establishing its relative efficiency. Future studies could include comparisons with traditional substrates, such as agricultural residues or wood, to better contextualize the results.

Although the properties of ichu were analyzed prior to the experiment, no post-culture analysis was performed to determine the extent to which *Pleurotus* spp. degraded the lignocellulosic components. Future research could include degradation trials at different stages of cultivation to better understand the dynamics of ichu decomposition.

This study was conducted under controlled conditions, so its applicability in large-scale production systems has not yet been validated. Future studies should address the viability of ichu as a substrate in different environmental conditions and its impact on mushroom productivity in the field.

Although the residual substrate was tested on radish germination, its long-term effects on soil fertility and other crops were not evaluated. Further studies could explore its impact on soil fertility and other crops.

The practical application of mushroom post-crop substrate (SMS) as a biofertilizer presents several limitations that must be considered, such as variability in the composition of SMS, possible presence of pathogens or contaminants, limited availability and logistics of application, and interactions with other agricultural inputs.

4. Conclusions

This study demonstrates the feasibility of using *J. ichu*, a largely underutilized Andean resource, as a substrate for cultivating *Pleurotus* spp. and its subsequent application as a biofertilizer. The research presents an innovative production model that enhances sustainability and contributes to the valorization of Ichu in the high-Andean regions of Peru.

J. ichu possesses a nutritional composition well-suited for cultivating *Pleurotus* spp. It has high levels of crude fiber (38.90 $g\cdot 100\ g^{-1}$), carbohydrates (40.26 $g\cdot 100\ g^{-1}$), and nitrogen (0.88%), which support mycelial development and enhance the fungus's biological efficiency. The study confirmed that *Jarava ichu* is a viable substrate for the cultivation of *Pleurotus* spp., as evidenced by an average biological efficiency of 19.8%.

The results demonstrated increased phosphorus and potassium content in the residual substrate of the mushroom cultivation compared to the proximal analysis of unprocessed *Jarava ichu*, suggesting its potential as a biofertilizer. This dual-use approach maximizes the utilization of Ichu, offering both a nutritious, protein-rich food source and a valuable agricultural input.

The findings of this study extend beyond the specific case of Ichu, highlighting the potential of other underutilized lignocellulosic resources in fragile ecosystems for similar applications. This approach could be replicated in other regions to enhance sustainability, reduce grassland burning, and mitigate environmental degradation.

This research proposes an integrated model that combines edible mushroom production with biofertilizer generation. This model provides a sustainable solution for the valorization of Ichu, supports agricultural development, and enhances food security in high-Andean communities. Highlighting the notable protein content of the mushrooms cultivated in this substrate, which ranges from 24.1% to 30.41% on a dry basis, indicating their significant nutritional value.

Further research is needed to optimize the cultivation process and assess the long-term impact of the residual substrate on different soil types and crops. Additionally, evaluating the economic feasibility of the model at a community scale and its social acceptance in high-Andean communities would provide valuable insights for its broader implementation.

These results provide unprecedented knowledge on the valorization of *Jarava ichu* in agricultural biotechnology, opening new avenues for its sustainable use in high-Andean ecosystems. Researchers, policymakers, and local communities are encouraged to consider this production model as a sustainable strategy for valorizing underutilized natural resources and addressing environmental, social, and economic challenges in the high-Andean regions of Peru and beyond.

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