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Original Article

Purple-skinned sweet potato as a substrate to produce hydrolytic enzymes by *Pleurotus eryngii* DPUA 1816

Batata-doce de casca roxa como substrato para produção de enzimas hidrolíticas por *Pleurotus eryngii* DPUA 1816

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Abstract

Fungi, particularly the edible mushrooms, are recognized as efficient producers of hydrolytic enzymes playing an essential role in various industrial processes, including food, beverage, leather, textile, and pharmaceutical industries. Despite its promising biotechnological potential, the enzymatic capacity of *Pleurotus eryngii* remains largely unexplored. Thus, this study investigated the production of hydrolytic enzymes by *P. eryngii* DPUA 1816 using purple-skinned sweet potato as a substrate under submerged fermentation. The fungus was grown in different culture media containing purple-skinned sweet potato under constant stirring at 150 rpm at 25 °C for 15 days. The enzymatic activities in the fermented broths were determined using specific substrates: *p*-nitrophenyl palmitate (lipases), starch 1% (amylases), carboxymethylcellulose 1% (cellulases), xylan 1% (xylanases), polygalacturonic acid 1% (pectinases) and azocasein 1% (proteases). The results showed the presence of hydrolytic enzymes, with highest proteolytic activity (60.84 U mL^{-1}), followed by pectinolytic activity (8.78 U mL^{-1}). Moderate activities were obtained for amylases, cellulases and lipases, with maximum means of 0.94 U mL^{-1}, 0.86 U mL^{-1} and 1.38 U mL^{-1}, respectively, and lower activity for xylanolytic enzymes (0.03 U mL^{-1}). The findings suggest that cultivation of *P. eryngii* on purple-skinned sweet potato represents a promising and sustainable strategy to produce hydrolytic enzymes with potential biotechnological applications.

Keywords: edible mushroom, *Ipomoea batatas*, hydrolases, submerged fermentation.

Resumo

Os fungos, especialmente os cogumelos comestíveis, são reconhecidos como produtores eficientes de enzimas hidrolíticas, desempenhando um papel essencial em diversos processos industriais, incluindo as indústrias de alimentos e bebidas, couro, têxtil e farmacêutica. Apesar de seu promissor potencial biotecnológico, a capacidade enzimática de *Pleurotus eryngii* permanece amplamente inexplorada. Assim, este estudo investigou a produção de enzimas hidrolíticas por *P. eryngii* DPUA 1816 utilizando batata-doce de casca roxa como substrato em fermentação submersa. O fungo foi cultivado em diferentes meios de cultura contendo batata-doce de casca roxa, sob agitação constante de 150 rpm, a 25 °C, durante 15 dias. As atividades enzimáticas nos caldos fermentados foram determinadas utilizando substratos específicos: *p*-nitrofenil palmitato (lipases), amido a 1% (amilases), carboximetilcelulose a 1% (celulases), xilano a 1% (xilanases), ácido poligalacturônico a 1% (pectinases) e azocaseína a 1% (proteases). Os resultados demonstraram a produção de enzimas hidrolíticas, com maior atividade proteolítica (60,84 U mL^{-1}), seguida pela atividade pectinolítica (8,78 U mL^{-1}). Atividades moderadas foram obtidas para amilases, celulases e lipases, com médias máximas de 0,94 U mL^{-1}, 0,86 U mL^{-1} e 1,38 U mL^{-1}, respectivamente, enquanto a atividade das xilanases foi menor (0,03 U mL^{-1}). Os achados sugerem que o cultivo de *P. eryngii* em batata-doce de casca roxa representa uma estratégia promissora e sustentável para a produção de enzimas hidrolíticas com potencial aplicação biotecnológica.

Palavras-chave: cogumelos comestíveis, *Ipomoea batatas*, hidrolases, fermentação submersa.

1. Introduction

Hydrolases represent one of the most important groups of enzymes in the global industrial enzyme market. These enzymes catalyze the hydrolysis of complex polymers into smaller molecules or monomers and include proteases, amylases, cellulases, pectinases, xylanases, and lipases, which are widely applied in diverse industrial processes (Farhan et al., 2025; Singh and Panesar, 2023).

Proteases are widely used in stain removal, leather processing and food industry (Naveed et al., 2021), while lipases are used in the production of biodiesel

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and biosurfactants (Chandra et al., 2020). Amylases have applications, notably in the pulp and paper industry (Ali et al., 2025), while xylanases are important for processes like pulp bleaching, as well as the pharmaceutical, textile and bio-refinery industries (Phuyal et al., 2023). Cellulases are employed in the extraction of flavoring materials, in the production of juices and wines, alongside applications in fiber processing, discoloration and modification and bioethanol production (Ejaz et al., 2021). Pectinases are essential for the maceration, liquefaction, clarification and filtration of beverages (Souza and Kawaguti, 2021).

These enzymes can be produced by a wide variety of biological sources, including plants, animals, and microorganisms, as well as through recombinant biotechnological processes (Razzaq et al., 2019). Among microorganisms, fungi are particularly notable due to their high capacity for extracellular enzyme production. Edible mushrooms, which belong predominantly to the macrofungi, represent a valuable resource for industrial enzyme production (Barua et al., 2024; Barbosa et al., 2025; Pimenta et al., 2025). Their industrial relevance is attributed to several advantages, such as low production costs and low production costs, ease of cultivation under submerged fermentation, and efficient recovery of extracellular enzymes. Moreover, enzyme production under controlled fermentation conditions is independent of seasonal and geographical constraints. (Liu and Kokare, 2017; Barua et al., 2024).

The genus *Pleurotus* is among the most extensively studied edible mushrooms due to its remarkable capacity to produce extracellular hydrolytic enzymes. Nevertheless, the enzymatic potential of *P. eryngii* remains underexplored. Since enzyme production is strongly influenced by substrate composition, the use of abundant plant-based agricultural substrates represents a sustainable and cost-effective strategy (Melanouri et al., 2022). Among these substrates, purple-skinned sweet potato (*Ipomoea batatas*) is particularly attractive because of its high carbohydrate content, structural polysaccharides, natural availability, and agricultural productivity. In the Amazon region, this crop reaches average yields of approximately 25 t ha^{-1} during a 150-day cultivation cycle and can be grown throughout the year (EMBRAPA, 2021).

Although various agricultural substrates and agro-industrial by-products have been investigated for hydrolytic enzyme production, information regarding the use of purple-skinned sweet potato for cultivating *P. eryngii* remains scarce. Owing to its high starch content, structural polysaccharides, natural availability, and bioactive composition, this substrate may simultaneously serve as a nutrient source and a natural inducer of enzyme production, supporting the simultaneous production of multiple hydrolytic enzymes in a single fermentation process (Bodjrenou et al., 2023; Ali et al., 2025).

Therefore, this study evaluated the potential of *Pleurotus eryngii* DPUA 1816 to simultaneously produce multiple hydrolytic enzymes by submerged fermentation using a previously unexplored natural substrate, purple-skinned sweet potato.

2. Materials and Methods

2.1. Submerged fermentation of Pleurotus eryngii DPUA 1816

Pleurotus eryngii DPUA 1816 was obtained from the DPUA Culture Collection at Universidade Federal do Amazonas. Mycelial fragments were inoculated in potato dextrose agar (PDA) supplemented with 0.5% yeast extract (YE) and incubated in the dark at 25 °C for 10 days. Different concentrations of glucose and yeast extract were evaluated to investigate the influence of carbon and nitrogen supplementation on hydrolytic enzyme production. Three 10-mm-diameter mycelial discs were transferred to Erlenmeyer flasks containing 100 mL of culture medium (Table 1), adjusted to pH 6.0 and prepared from an infusion of 200 g L^{-1} of freshly cut purple-skinned sweet potato (*Ipomoea batatas*), supplemented with glucose and yeast extract. The cultures were incubated in an orbital shaker (150 rpm) for 15 days at 25 °C. Fermented broths were recovered by vacuum filtration and stored at 4 °C until enzymatic analyses.

A second submerged fermentation was carried out over 18 days using the culture medium selected from treatment E4 (Table 1). The incubation conditions were identical to those described for the initial cultivation. For the time-course analysis, three independent Erlenmeyer flasks were randomly selected every two days for sampling throughout the cultivation period. Fermented broth samples were recovered and stored using the same procedure described for the initial experiment.

2.2. Lipolytic activity

Lipolytic activity was determined according to the method of Winkler and Stuckmann (1979). The reaction mixture consisted of 900 µL of substrate solution containing p-nitrophenyl palmitate and 100 µL of enzyme extract. The substrate solution was prepared by dissolving 30 mg of *p*-nitrophenyl palmitate in 10 mL of isopropanol and emulsifying it with 0.5 g of gum arabic and 2 g of Triton X-100 in 0.05 M Tris-HCl buffer (pH 8.0) at a 1:9 ratio. The reaction mixture was incubated at 37 °C for 15 min, and absorbance was measured at 410 nm. One unit of lipase activity was defined as the amount of enzyme required to release 1 µmol of *p*-nitrophenol per minute.

2.3. Amylolytic activity

Amylolytic activity was determined by mixing 50 µL of enzyme extract with 50 µL of 1% starch solution prepared in 1 M sodium acetate buffer (pH 6.0). The reaction mixture was incubated at 50 °C for 30 min and stopped by adding 100 µL of DNS reagent (3,5-dinitrosalicylic acid). The mixture was then heated at 100 °C for 5 min, diluted with 800 µL of distilled water, and the absorbance was measured at 540 nm using a glucose standard curve. One unit of amylase activity was defined as the amount of enzyme required to release 1 µmol of reducing sugars per minute (Miller, 1959).

2.4. Cellulolytic activity

Cellulolytic activity was determined by mixing 50 µL of enzyme extract with 50 µL of a 1% carboxymethylcellulose

Table 1. Composition of the culture media used for submerged cultivation of *Pleurotus eryngii* for 15 days at 25 °C and 150 rpm.

Composition	Formulation								
	E0	E1	E2	E3	E4	E5	E6	E7	E8
Glucose (g L^{-1})	-	10	20	30	40	10	20	30	40
Yeast extract (g L^{-1})	-	2	2	2	2	-	-	-	-
Sweet potato (g L^{-1})	200	200	200	200	200	200	200	200	200

(CMC) solution prepared in 50 mM acetate-phosphate buffer (pH 5.5). The reaction mixture was incubated at 50 °C for 30 min and stopped by adding 100 µL of DNS reagent. The mixture was then heated at 100 °C for 5 min, diluted with 800 µL of distilled water, and absorbance was measured at 540 nm using a glucose standard curve (Miller, 1959). One unit of cellulase activity was defined as the amount of enzyme required to release 1 µmol of reducing sugars per minute.

2.5. Proteolytic activity

Proteolytic activity was determined according to Leighton et al. (1973). The reaction mixture consisted of 150 µL of enzyme extract and 250 µL of 1% azocasein solution prepared in 0.2 M Tris-HCl buffer (pH 7.2). The mixture was incubated at 25 °C in the dark for 60 min. The reaction was stopped by adding 1.2 mL of 10% (w/v) trichloroacetic acid, followed by centrifugation at 10,000 rpm for 10 min. An 800-µL aliquot of the supernatant was mixed with 1.4 mL of 1 M sodium hydroxide, and absorbance was measured at 440 nm. One unit of protease activity was defined as the amount of enzyme producing an increase of 0.01 absorbance units after 1 h.

2.6. Xylanolytic activity

Xylanolytic activity was measured by incubating 50 µL of enzyme extract with 75 µL of 1% xylan prepared in 50 mM sodium acetate buffer (pH 6.5) at 50 °C for 10 min. The reaction was stopped by adding 125 µL of DNS reagent, followed by heating at 100 °C for 5 min. After dilution with 1.0 mL of distilled water, absorbance was measured at 540 nm. Reducing sugars were quantified using a xylose standard curve according to Miller (1959). One unit of xylanase activity was defined as the amount of enzyme required to release 1 µmol of xylose per minute per mL.

2.7. Evaluation of pectinolytic activity

Pectinolytic activity was measured by mixing 125 µL of the substrate (1% polygalacturonic acid prepared in 0.052 M sodium acetate buffer pH 4.4) with 125 µL of the enzyme extract. The reaction was incubated for 40 minutes at 37 °C, followed by the addition of 250 µL of DNS reagent. The reaction mixture was heated at 100 °C for 15 min, diluted with 500 µL of distilled water, and absorbance was measured at 540 nm using a standard curve prepared with polygalacturonic acid (1 g L^{-1}). One unit of pectinase activity was determined as the amount of polygalacturonic acid released per milliliter per minute (µmol mL^{-1} min^{-1}).

2.8. Statistical analysis

All experiments were performed in triplicate. The enzymatic activity data were subjected to one-way analysis of variance (one-way ANOVA). Differences among means were evaluated using Tukey's multiple comparison test at a significance level of $p < 0.05$. All statistical analyses were performed using Minitab® 17 software.

3. Results and Discussion

Figure 1 shows the production of hydrolytic enzymes by *P. eryngii* DPUA 1816 cultivated in different culture media containing purple-skinned sweet potato. Lipolytic activity was detected in all culture media tested (Figure 1A). Medium E1 yielded the highest activity (1.38 U mL^{-1}), followed by E2 (0.97 U mL^{-1}) and E3 (0.96 U mL^{-1}). However, no statistically significant difference was observed between E2 and E3.

Although direct comparisons should be interpreted with caution because of methodological differences, the lipolytic activities obtained in this study were comparable to or higher than those reported for *Pleurotus* spp. For example, Almeida (2016) reported a lipolytic activity of 0.0011 U mL^{-1} for *P. ostreatus*, whereas Zorn et al. (2003) reported 0.312 U mL^{-1} for *P. sapidus*. Although a different methodology was used by Zorn et al. (2003), the higher activity observed in the present study supports the potential of *P. eryngii* DPUA 1816 cultivated on purple-skinned sweet potato for lipase production.

In addition, lipolytic activity was consistently higher in culture media supplemented with yeast extract (YE) (E1–E4) than in media lacking this nitrogen source. This difference may be attributed to the rich nutritional composition of YE, which provides nitrogen-containing compounds, vitamins, and growth factors that promote fungal metabolism and extracellular enzyme biosynthesis. In contrast, media without YE contained only glucose and purple-skinned sweet potato, suggesting that their more limited nutrient composition may have reduced lipase production. These findings indicate that the nutritional composition of the culture medium plays an important role in regulating lipase production by *P. eryngii*.

Proteolytic activity was also detected in media lacking yeast extract (E0, E7, and E8), suggesting that purple-skinned sweet potato itself supplied nutrients capable of sustaining fungal metabolism. In addition to its high carbohydrate content, this substrate contains proteins, amino acids, minerals, and other bioactive compounds that may partially satisfy the nutritional requirements of *P. eryngii*. Therefore, the absence of yeast extract does not

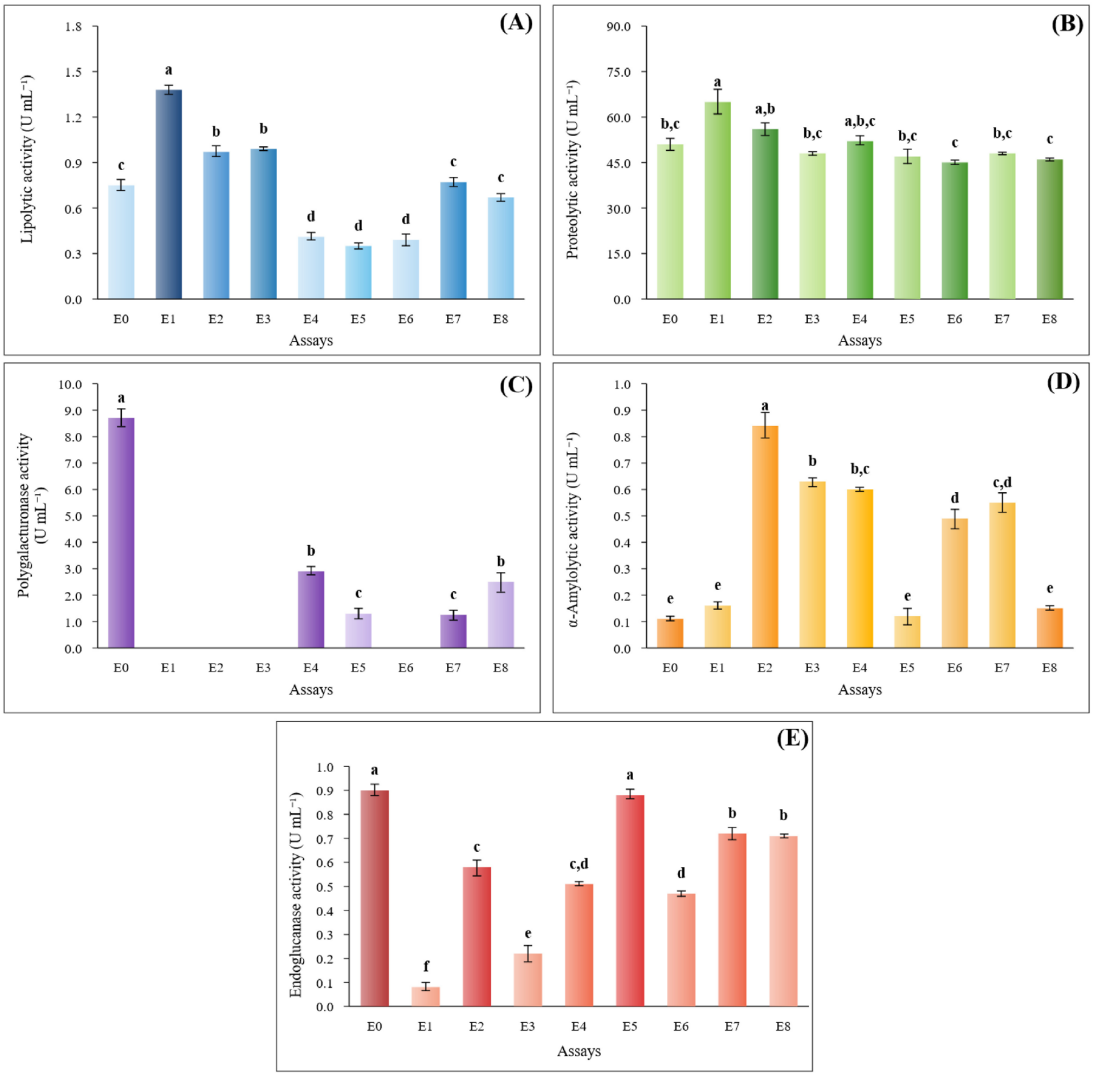

Figure 1. Lipolytic (A), proteolytic (B), polygalacturonase (C), amylolytic (D), and endoglucanase (E) activities of *P. eryngii* cultivated in different culture media for 15 days at 25 °C and 150 rpm. Means followed by the same letter do not differ significantly according to Tukey's test ($p < 0.05$).

represent a complete nutritional limitation, which may explain the considerable proteolytic activity observed under these conditions. Furthermore, moderate nutrient limitation may stimulate the secretion of extracellular hydrolytic enzymes as an adaptive strategy for nutrient acquisition, thereby explaining the maintenance of protease production even in the absence of yeast extract (Sinsabaugh, 1994; Baldrian, 2008).

In addition to its nutritional contribution, purple-skinned sweet potato represents a complex plant-derived substrate rich in starch and structural polysaccharides, containing starch, pectin, cellulose, hemicellulose, soluble sugars, and minor amounts of proteins and phenolic compounds (Alam, 2021). This heterogeneous composition likely provided multiple natural signals and substrates capable of promoting the simultaneous production of different hydrolytic enzymes (Liguori et al., 2015; Melanouri et al., 2022; Østby et al., 2020; Baig, 2020). Unlike defined synthetic media, the substrate offers diverse carbon sources that can sustain fungal metabolism and enzyme production according to the physiological requirements of the fungus. Such metabolic versatility has been reported as one of the main adaptive characteristics of *Pleurotus* species growing on plant-derived substrates (Mata et al., 2016; Zhang et al., 2020; Melanouri et al., 2022).

A similar pattern was observed for proteolytic activity across all culture media tested (Figure 1B). The highest proteolytic activity was recorded in medium E1 (57.11 U mL^{-1}), followed by media E2 (51.56 U mL^{-1}) and E4 (49.56 U mL^{-1}), which did not differ significantly from each other. The proteolytic activities obtained in this study were comparable to or higher than those reported in previous studies. Machado et al. (2017) cultivated *P. ostreatoroseus* on tropical tubers and reported comparable proteolytic

activities in *Dioscorea trifida* (51.04 U mL^{-1}) and *Manihot esculenta* (31.26 U mL^{-1}), whereas cultivation on *Dioscorea alata* resulted in a substantially higher activity (142 U mL^{-1}). Furthermore, the maximum proteolytic activity obtained in the present study (57.11 U mL^{-1}) exceeded the 50.03 U mL^{-1} reported for *Tricholoma saponaceum* PKSR8 (Khaund and Joshi, 2014).

The slight reduction in proteolytic activity observed under the highest glucose concentration (40 g L^{-1}; E4) compared with E1 may be associated with carbon catabolite repression (CCR), a regulatory mechanism commonly observed in filamentous fungi. Under conditions of excess readily metabolizable carbon, fungal cells preferentially utilize glucose as an energy source, potentially reducing the expression of genes involved in the production of extracellular hydrolytic enzymes, including proteases. Consequently, the physiological demand for degrading complex protein substrates decreases, leading to lower extracellular protease production. Nevertheless, the proteolytic activity detected in E4 remained considerable, suggesting that the nutritional complexity of purple-skinned sweet potato may have partially mitigated this regulatory effect (Ruijter and Visser, 1997; Liu and Kokare, 2017; Alfaro et al., 2020; Assis et al., 2021; Noël et al., 2021).

These findings suggest that hydrolytic enzyme production by *P. eryngii* depends on a balanced nutritional environment, in which moderate availability of carbon and nitrogen sources supports fungal growth and enzyme secretion, whereas excessive glucose concentrations may induce carbon catabolite repression, while limited availability of specific nutrients may activate adaptive mechanisms that promote extracellular enzyme production (Mata et al., 2016; Melanouri et al., 2022).

Pectinolytic activity (Figure 1C) was detected in five culture media formulations used for the cultivation of *P. eryngii* DPUA 1816. The highest activity was observed in medium E0 (8.78 U mL^{-1}), followed by E4 (2.76 U mL^{-1}) and E8 (2.60 U mL^{-1}). The absence of significant differences between E4 and E8 may be related to their identical carbon source composition (40 g L^{-1} glucose and 200 g L^{-1} purple-skinned sweet potato). The high pectinolytic activity observed in E0, together with enzyme production in five different media, suggests the potential of purple-skinned sweet potato as a suitable substrate for pectinase production by *P. eryngii*. The maximum pectinolytic activity obtained in the present study (8.78 U mL^{-1}) was comparable to the 9.40 U mL^{-1} reported for *P. pulmonarius* cultivated in orange juice (Inácio et al., 2015) and exceeded the activities reported for *P. ostreatus* cultivated on tomato pomace (2.18 U mL^{-1}), *Pleurotus* sp. cultivated on sisal residues (3.31 U mL^{-1}), and *P. pulmonarius* cultivated on a glucose-based synthetic medium (0.70 U mL^{-1}) (Freixo et al., 2008; Raymond et al., 2015; Díaz-Godinez et al., 2016).

Figures 1D and 1E present the amylolytic and cellulolytic activities produced by *P. eryngii* DPUA 1816, with the highest activities observed in media E2 (0.76 U mL^{-1}) and E5 (0.71 U mL^{-1}), respectively. It is worth noting that both enzymatic activities were detectable in all different culture media formulations tested. The amylolytic activity was expected due to the presence of glucose and the starchy nature of the sweet potato residue (potato broth) in the culture media, acting as effective carbon sources and inducers. However, cellulolytic activity yielded better overall results, a trend that was maintained in both the initial screening and the second time-course experiment using medium E4 (Figures 2D and 2E).

Although direct comparisons should be interpreted with caution because of differences in fungal species, cultivation conditions, substrates, and assay methodologies, the cellulolytic and amylolytic activities obtained in the present study were lower than those reported in some previous studies. For example, a cellulolytic activity of 3.19 U mL^{-1} was reported for *P. ostreatus* after 9 days of cultivation (Liguori et al., 2015). Similarly, amylolytic activities ranging from 1.10 to 3.10 U mL^{-1} were reported for the edible mushroom *Neurospora intermedia* (Shahryari et al., 2019). Higher amylolytic (40.29 U mL^{-1}) and cellulolytic (20.26 U mL^{-1}) activities were reported for *Inocybe* sp. PKSR10 by Khaund and Joshi (2014). These differences are likely associated with variations in fungal species, substrates, cultivation conditions, and enzymatic assay protocols.

The moderate amylase production observed in this study may be explained by carbon catabolite repression. During submerged cultivation, the starch present in purple-skinned sweet potato likely promoted amylase production, while its hydrolysis gradually released glucose into the culture medium. As glucose accumulated, it may have repressed further amylase synthesis through carbon catabolite repression, reducing enzyme production despite the continued availability of starch (Fernandes et al., 2007).

Figure 2 shows the time-course production of hydrolytic enzymes by *P. eryngii* over 18 days in medium E4. The highest lipolytic activity (0.97 U mL^{-1}) was recorded on the fourth day of cultivation (Figure 2A), followed by a second peak of similar magnitude on the 14th day. This biphasic profile suggests temporal regulation of lipase production during distinct stages of fungal growth. A similar trend was observed for proteolytic activity (Figure 2B), with the maximum activity (60.84 U mL^{-1}) also occurring on the 4th day. Following this initial peak, proteolytic activity declined but remained relatively stable until the 18th day.

Our finding of maximum production early in the cultivation phase is supported by Bano et al. (2016), who observed optimal protease production for *P. eryngii* at 4 days (0.546 U mL^{-1}), using glucose, and peptone. Similarly, Benmrad et al. (2019) reported optimal activity for *P. sajor-caju* CTM10057 after just 3 days (10,500 U mL^{-1}), highlighting that optimal yields often occur rapidly in submerged fungal cultures.

Time-course analysis of pectinolytic activity showed that the highest activity (6.84 U mL^{-1}) occurred on the 14th day of cultivation. Although the activity measured on the 16th day (6.28 U mL^{-1}) did not differ significantly from that observed on the 14th day, the highest activity recorded during the 18-day cultivation period occurred on the 14th day (Figure 2C). Interestingly, the activity profile exhibited a pattern similar to that observed for the other hydrolytic enzymes, characterized by pronounced peaks in enzyme production followed by substantial declines, occasionally reaching undetectable levels. Moreover, peaks of pectinolytic activity appeared to recur at approximately 6-day intervals, suggesting temporal regulation of pectinase production.

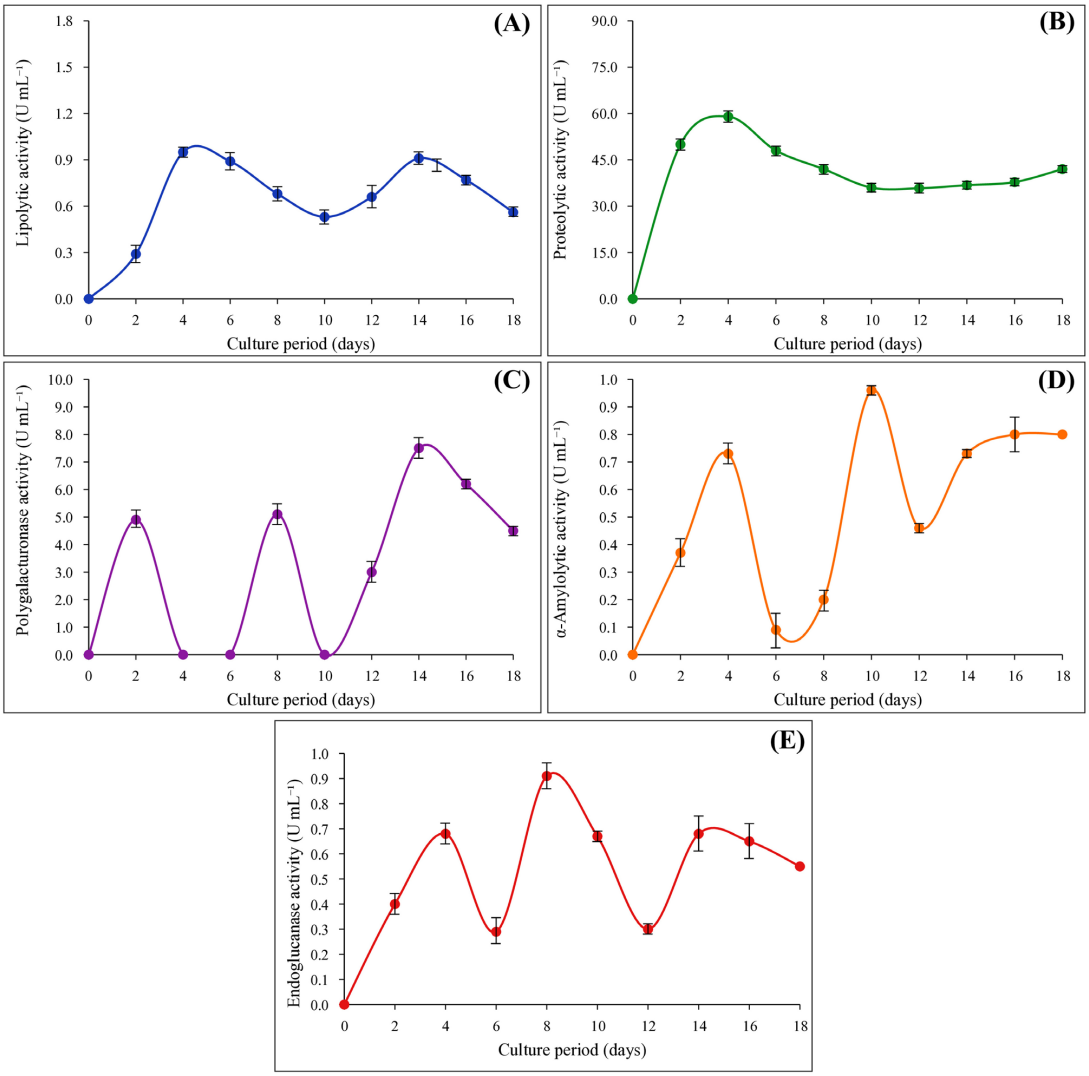

Figure 2. Lipolytic (A), proteolytic (B), polygalacturonase (C), amylolytic (D), and endoglucanase (E) activities of *P. eryngii* cultivated in the E4 culture medium over an 18-day cultivation period at 25 °C and 150 rpm. Means followed by the same letter do not differ significantly according to Tukey's test ($p < 0.05$).

The maximum amylolytic activity (0.94 U mL^{-1}) was observed on the 10th day of cultivation (Figures 2D and 2E). The cellulolytic profile showed a similar trend, with its peak (0.86 U mL^{-1}) occurring slightly earlier, on the 8th day. Although amylolytic and cellulolytic activities were successfully detected in *P. eryngii* DPUA 1816 cultivated on purple-skinned sweet potato, the values obtained were lower than those reported in some previous studies. For instance, Daba et al. (2011) achieved higher cellulolytic activity (3.13 U mL^{-1} and 4.0 U mL^{-1}) after 6 and 8 days, respectively, when cultivating *P. ostreatus* NRRL-0366.

Conversely, the cellulolytic activity obtained in the present study (0.86 U mL^{-1} on the eighth day of cultivation) was higher than that reported for several *Pleurotus* species cultivated in defined media. Goyal and Soni (2011) evaluated cellulolytic activity in three *Pleurotus* species cultivated in Czapek–Dox medium and reported lower activities after 12 days: 0.42 U mL^{-1} for *P. ostreatus*, 0.48 U mL^{-1} for *P. florida*, and 0.45 U mL^{-1} for *P. sajor-caju*. Even when the medium was supplemented with carboxymethyl cellulose (CMC, 1%) and malt extract (ME), the reported cellulolytic activities remained relatively low. For example, *P. florida* showed maximum activities of 0.46 U mL^{-1} with 0.5% ME after 12 days, 0.34 U mL^{-1} with 1.0% ME after 5 and 7 days, and 0.38 U mL^{-1} with 0.1% ME after 15 days.

This observation is further supported by the findings of Asgher, Khan and Bilal (2016), who reported peak cellulolytic activity for *P. eryngii* WC888 cultivated on other agro-industrial residues. Specifically, they reported cellulolytic activities of only 0.20 U mL^{-1} after 6 days using banana stem residue and 0.13 U mL^{-1} after 4 days using corn straw residue. These comparisons support the potential of purple-skinned sweet potato as an effective and low-cost substrate for cellulase production by *P. eryngii*

DPUA 1816, yielding cellulolytic activities comparable to or higher than those obtained using defined media and conventional inducing substrates.

In contrast to the other enzymes investigated, *P. eryngii* DPUA 1816 produced only low levels of xylanase in the culture media. This low activity is consistent with the findings of Álvarez-Cervantes et al. (2016), who reported similarly low xylanase activity (0.18 U mL^{-1}) for *P. ostreatus* cultivated in a defined medium containing only glucose (10 g L^{-1}) and yeast extract (5 g L^{-1}). These results suggest that the available glucose in the culture medium may have promoted carbon catabolite repression, as xylanase production is typically inducible. This hypothesis is supported by Altaf et al. (2016), who reported high xylanolytic activity (0.46–0.92 U mL^{-1} after 4–8 days) for *P. eryngii* cultivated in the presence of xylose, a well-established inducer of xylanase production. Thus, the high content of readily metabolizable sugars in the purple-skinned sweet potato-based medium may have limited xylanase induction, resulting in reduced enzyme production.

The temporal production curves for most enzymes in medium E4, with the notable exception of proteases and xylanases, displayed characteristic peaks of high activity followed by rapid declines. This kinetic behavior is commonly attributed to several factors in submerged cultivation, including product inhibition, enzyme instability or denaturation, and the inhibitory action of secondary metabolites released during fungal growth (Lakshmi et al., 2014; Cui et al., 2015; Bonomini et al., 2017). Furthermore, the observation of multiple activity peaks may suggest the differential production of isozymes within the same enzyme class (Bano et al., 2018). The ability of *P. eryngii* to produce a diverse range of hydrolytic enzymes on purple-skinned sweet potato likely reflects its metabolic adaptability to utilize the complex polysaccharides and other nutrients present in this substrate (Mata et al., 2016).

The literature demonstrates considerable variability in enzymatic profiles among *Pleurotus* species. Studies on *P. djamor* var. *roseus* (Hernandez-Nava et al., 2016) and *P. tuber-regium* (Jonathan and Adeoyo, 2011) reported low xylanolytic, cellulolytic and pectinolytic activities (<0.15 U mL^{-1}), even when standard inducers such as carboxymethyl cellulose (CMC) and maltose were used (<0.64 U mL^{-1}). Similarly, Bonomini et al. (2017) reported moderate amylolytic (0.35 and 0.45 U mL^{-1}) and pectinolytic (0.29 U mL^{-1}) activities in *P. sajor-caju* CCB019 and *P. djamor* UNIVILLE01, while no detectable cellulolytic, proteolytic or lipolytic activities were observed. In contrast, *P. eryngii* DPUA 1816 exhibited activity for all of these enzymes, demonstrating a broader hydrolytic profile under the cultivation conditions employed in the present study.

Although some enzymatic activities were lower than those reported in the literature, these results were obtained using a simple culture medium without the addition of conventional enzyme-inducing substrates. Under the cultivation conditions employed, the synthetic carbon source (glucose) and the purple-skinned sweet potato were the only potential inducers of enzyme production.

Overall, the findings demonstrate that purple-skinned sweet potato represents more than a simple carbon source. Its complex nutritional composition supported the simultaneous production of several hydrolytic enzymes by *Pleurotus eryngii*, highlighting its potential as an inexpensive and renewable substrate for enzyme production. This strategy may contribute to reducing production costs while adding value to agricultural resources, thereby supporting a more sustainable enzyme production system.

4. Conclusion

This study demonstrated that purple-skinned sweet potato is a promising alternative natural substrate for hydrolytic enzyme production by *P. eryngii* DPUA 1816 under submerged fermentation. The cultivation system supported the production of a broad hydrolytic enzyme profile, including proteases, pectinases, lipases, amylases and cellulases, without the need for specific synthetic inducers. These findings highlight the potential of this underutilized agricultural resource as a sustainable substrate for fungal enzyme production. Further studies on enzyme characterization and process optimization may contribute to the development of future biotechnological applications.

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Data Availability Statement

The data set analyzed or produced in this study can be requested from the corresponding author.

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