

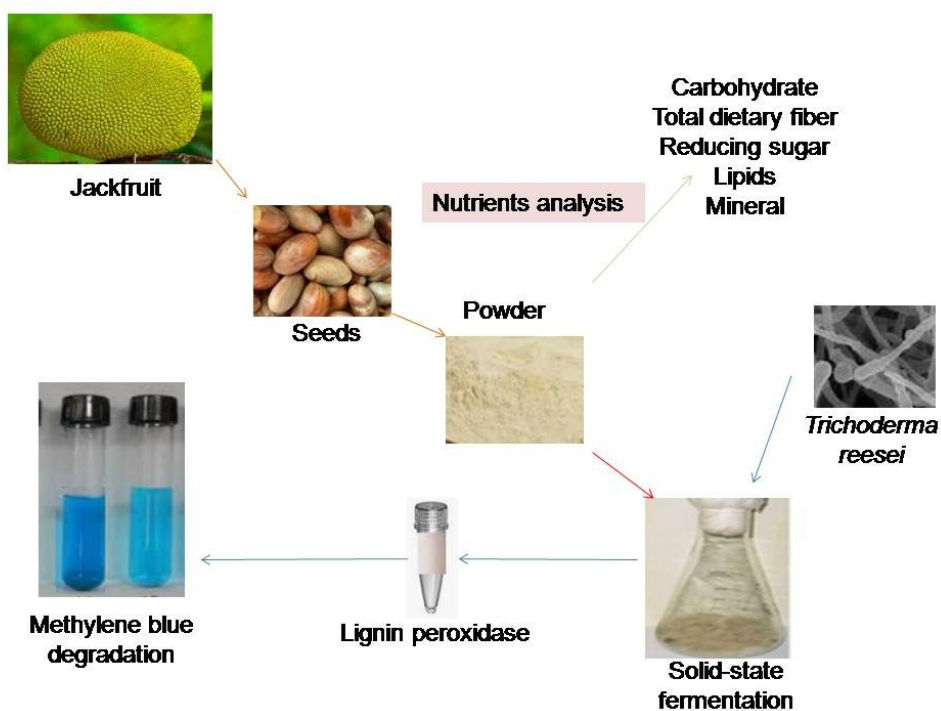
Chemical Composition and Nutrient Analysis of Jackfruit Seeds for Lignin Peroxidase Enzyme Production by *Trichoderma reesei* in Solid-State Fermentation for Environmental Applications

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GRAPHICAL ABSTRACT



Chemical Composition and Nutrient Analysis of Jackfruit Seeds for Lignin Peroxidase Enzyme Production by *Trichoderma reesei* in Solid-State Fermentation for Environmental Applications

Najla A. Alshaikh

This study aimed to analyze the chemical composition and nutrient analysis of jackfruit seeds and their use as substrates to produce lignin peroxidase by fungal fermentation for environmental applications. The jackfruit (*Artocarpus heterophyllus*) seeds presented $3.92 \pm 0.43\%$ ash, $69.4 \pm 2.5\%$ carbohydrate, $29.5 \pm 1.1\%$ total dietary fiber, $7.2 \pm 0.13\%$ reducing sugars, and $1.9 \pm 0.22\%$ lipids. The major mineral was magnesium (315.3 ± 10.4 mg%), followed by potassium (187.2 ± 9.3 mg%), phosphorus (87.3 ± 0.15 mg%), and calcium (73.21 ± 3.1 mg%). Water extraction was ideal for improving the total starch yield from jackfruit seeds ($84.1 \pm 3.4\%$), followed by alkaline extraction ($82.1 \pm 1.5\%$) and enzymatic extraction methods ($79.6 \pm 2.2\%$). Jackfruit seeds were used as a culture medium for lignin peroxidase production by *Trichoderma reesei* in solid-state fermentation. A central composite design was used to optimize enzyme production. Ligninolytic enzyme production varied from 3.1 to 360.2 U/g substrate, and the optimum culture conditions were an incubation temperature of 32.5°C , a pH of 6.0, and 0.5% yeast extract in jackfruit seed medium ($p < 0.001$). The crude lignin peroxidase decolorized methylene blue significantly ($p < 0.05$).

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Keywords: Jackfruit seed; Carbohydrates; Substrates; Enzymes; Optimization; Statistical methods

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INTRODUCTION

Jackfruit is an important fruit crop in India and Asian countries. It is cultivated in almost all states of India, and several wastes are produced from its ripe fruits. Ripe fruit waste is more palatable than raw fruit waste (Thanh *et al.* 2022). Jackfruit is rich in crude fiber (CF $\sim 14.1\%$), crude protein (CP $\sim 7.9\%$), and phosphorus (0.1%). The raw and ripe jackfruit wastes are applied for the production of nitrogen-free extracts (NFE $\sim 65\%$) and energy. The nonedible portion comprises $\sim 59.2\%$ of the whole jackfruit, including the rind, perianth meal, and core meal. Rinds are a major source of ripe fruit for cattle (Ranasinghe *et al.* 2019). Jackfruit waste (rind, perianth meal and core meal) contains carbohydrates (20% to 29%), crude fibers (12% to 17.3%), ash (6% to 7.5%), crude fat (1.7% to 7.3%), and crude protein (8% to 10.6%). Jackfruit peels are used for the production of biofuels, nutrient-enriched cattle feeds, and nonporous adsorbents. Nonporous adsorbents are used for the removal of inorganic dyes from the environment. In addition, the peels are suitable for the extraction of pectin (Bala *et al.* 2023). The seeds are rich in starch and protein and

are ideal for animal feed. The composition of nutrients in jackfruit seeds varies with fruit type and cultivation conditions. Unlike other fruits, due to their large size, significant amounts of peels, seeds, and pericarp are wasted despite their significant nutrient value (Thanh *et al.* 2022). Solid-state fermentation (SSF) offers several advantages for agricultural waste utilization, including higher volumetric productivity, lower water consumption, and similarly to the natural fungal habitat (Karimi *et al.* 2025).

Jackfruit seeds are the major source of carbohydrates and protein and contain various nutrients, including isoflavones, lignans, and saponins, with potential antiaging, anticancer, and antioxidant activities. These phytochemical compounds have various health benefits, including antiulcer agents and anticancer agents, which are used to treat asthma and disorders (Swami *et al.* 2012; Hong *et al.* 2017). Agro-industrial wastes are valuable to produce lignocellulolytic enzymes in solid-state fermentation (Leite *et al.* 2021). Sawdust is considered an inexpensive substrate for ligninolytic enzymes production by white-rot fungi (Hultberg and Golovko 2024). Fungal protein is obtained from jackfruit seed waste *via* submerged fermentation (Chakraborty and Bhowal 2023). In addition, jackfruit seeds have been utilized for the production of pigments in solid-state fermentation (Babitha *et al.* 2007). Sharmila *et al.* (2013) employed jackfruit seed powder as a culture medium for the production of extracellular biopolymers by *Aureobasidium pullulans* MTCC 2195. Jackfruit seed powder serves as an excellent source to produce XG (Felicia Katherine *et al.* 2017). Response surface methodology was used for the optimized production of L-lactic acid using a jackfruit seed substrate with a central composite design, and optimization steps improved the product yield (Nair *et al.* 2016). Jackfruit waste is valuable for biogas, bioethanol, functional food additives, bioplastics, and other industrial products (Pathak *et al.* 2022). Fungi such as *Trichoderma viride*, *Aspergillus flavus*, and *Pleurotus ostreatus* produce lignocellulolytic enzymes *via* low-cost substrates (Singh *et al.* 2018). The response surface methodology approach has been widely used to improve enzyme yield. This method involves identifying the critical variables and optimizing the medium components to improve enzyme production (Pérez-Contreras *et al.* 2024). Jackfruit seeds are considered waste and are exploited for biochemical synthesis. Solid-state fermentation is useful for converting agricultural biomass into industrially useful products. The hypothesis of this work is that it is feasible to use low-cost agricultural wastes for the production of lignin peroxidase in SSF using fungus. The objective of this research was to utilize jackfruit seeds as a culture medium for the production of lignin peroxidase in SSF by *Trichoderma reesei*. In this research article, we optimized the culture conditions to determine the connection between the process variables and enzyme yield. In addition, crude lignin peroxidase was used to decolorize methylene blue.

EXPERIMENTAL

Jackfruit Seeds

Jackfruit was collected from the market, and jackfruit seeds were obtained from ripened fruit. The whole jackfruit weight was approximately 12 kg, and 0.8 kg of seed was obtained (wet weight). The collected seeds were dried, and a powdered and fine powder was obtained. It was further packed in a plastic container and stored at -20 °C for further analysis.

Proximate Composition of Jackfruit Seeds

The compositions of ash, proteins, moisture, lipids, reducing sugars, and total carbohydrates were determined. An enzymatic-gravimetric method was used for the determination of total dietary fiber. Briefly, one gram of defatted powder was mixed with α -amylase and incubated for 20 min at 60 °C (pH 7.0). Then, it was digested with proteolytic enzyme for 30 min at 60 °C (pH 7.0) and further treated with amyloglucosidase for 30 min (pH 4.5). The mixture was filtered and washed with 95% ethanol and acetone. The filtrate was dried and ash, the protein content was determined, and the TDF content was calculated. The ash content of the sample was assayed according to AOAC Method 940.26 (AOAC 2000). The total protein content of the samples was evaluated using Kjeldahl method (984.13-1994) (AOAC 1996). The lipid content of the jackfruit seeds was estimated *via* the Soxhlet extraction method. Ether was used as the reflux solvent and was extracted for 4 h. After 4 h of extraction, the mixture was concentrated, and the oil was recovered in a rotary evaporator. The moisture content of each sample was removed using sodium sulfate, and the lipid yield was estimated. The carbohydrate content of the sample was determined according to Djordjević *et al.* (2018).

Extraction of Starch from Jackfruit Seed

Water extraction method

Jackfruit starch was extracted as described previously by Perez *et al.* (1993), with slight modifications. Briefly, jackfruit seed powder (25 g) was mixed with 100 mL of double distilled water. Then, it was filtered using a muslin cloth. The sample was stored at 4 °C for 24 h, and the clear supernatant was removed. The floating particles were continuously removed, and the starch was collected. It was lyophilized and further sieved through 200 mesh sieves.

Alkali method

Jackfruit seed powder was soaked with 0.5% NaOH for 6 hat ambient temperature. The mixture was stirred every 5 min. The final slurry was filtered through a muslin cloth, and the sediment samples were washed with double distilled water. The filtrates were combined and stored at 4 to 6 °C. The clear supernatant was removed, and the starch was suspended in double distilled water. This process was repeated three to four times. The sample was subsequently dried and powdered *via* a mortar and pestle (Ascheriet *al.* 2014).

Enzymatic method

Ten grams of jackfruit seed powder was mixed with 100 mL of 0.1 M sodium phosphate buffer. Then, 200 U of α -amylase was added, and the mixture was incubated for 5 h at ambient temperature (29 ± 1 °C). It was placed on an orbital shaker incubator and filtered through a muslin cloth. The filtrate was combined and stored at 4 °C. The clear supernatant was removed, and the mixture was washed with double distilled water several times. Then, the starch cake was placed in an oven at 40 °C for 24 h. The starch cake was subsequently ground into a powder using a mortar and pestle (Bello-Pérez *et al.* 2006).

Mineral Composition

The mineral content of each sample was tested as described by the Adolfo Lutz Institute (AOAC, 2012).

Fungi and Culture Conditions

The fungal strain *Trichoderma reesei* (ATCC 26921) was cultivated on potato dextrose agar (PDA) media (Himedia, Mumbai, India) and incubated for one week at 30 °C until sporulation. Five milliliters of sterile 0.01% (v/v) Tween 80 (polysorbate 80) was loaded onto the plates, and the spore concentration was determined through a Neubauer cytometer.

Solid-State Fermentation

Solid-state fermentation was carried out using 10 g of jackfruit seed powder mixed with medium in a 100 mL Erlenmeyer flask at pH 6.5, and the initial moisture content was 60%. The moisture and pH of the substrate were selected on the basis of previous experiments. The culture medium was sterilized by autoclaving for 25 mm at 121 °C. The mixture was subsequently cooled and inoculated with 1×10^6 spores/g substrate. The mixture was incubated for 7 days, after which enzyme production was assayed (Kalaiyarasi *et al.* 2020).

Lignin Peroxidase Assay

The lignin peroxidase activity of each sample was tested. This assay method was based on the oxidation of veratryl alcohol (3,4-dimethoxybenzyl alcohol) to veratraldehyde with the help of H₂O₂. The reaction mixture consisted of 0.200 mL of 1 mM veratryl alcohol, 0.5 mL of 0.1 M citrate buffer, and 0.2 mM H₂O₂. The absorbance of each sample was read at 310 nm against a reagent blank (Tien and Kirk 1983).

Optimization of Lignin Peroxidase Production by a Single-factor Analysis

A single factor experiment was employed to screen the effect of each selected parameter. These include temperature (20 to 45 °C), pH (4 to 8), and inoculum (2% to 8%). Fermentation period (1 to 8 days), carbon source (1% of glucose, maltose, trehalose, lactose, starch), and nitrogen source (1% of beef extract, yeast extract, peptone, urea, and casein) were selected. Approximately 2 g of substrate was mixed with 1.5 mL of buffer and sterilized. After sterilization, the experiment was performed on the basis of culture conditions, and an enzyme assay was carried out.

Central Composite Design and Response Surface Methodology

To test the major factors affecting lignin peroxidase activity, a central composite design experiment was performed to optimize enzyme production. The response surface methodology-based experiment was designed *via* the statistical software Stat-Ease, Inc. (Minneapolis, MN, USA) (Mishra and Kumar 2007; Arockiasamy *et al.* 2008). This experimental design comprised three variables (temperature, inoculum, and yeast extract). To analyze the interactive effect of the selected variables, a CCD with five levels was employed under SSF. It has been previously reported that inoculum and temperature significantly influence enzyme bioprocesses; thus, these factors were selected (Gao *et al.*, 2009; Shi *et al.* 2009). The levels of the variables were selected on the basis of preliminary experimental trials. A multifactor experiment was performed to study the interaction between the three selected independent factors and the yield (response) of the selected variables at various levels. The fitness of the model was tested, and the fitness of the linear terms, intercept terms, and quadratic terms were analyzed.

Dye Decoloration Efficacy of Crude Enzyme

The decolorizing ability of methylene blue dye by crude lignin peroxidase was determined in a 3 mL mixture containing methylene blue at a concentration of 200 mM. The absorbance value was not greater than 1.5 at 630 nm, and the sample was stored at 4 °C in the dark until use. The experiment involved mixing 2.9 mL of dye solution and 0.1 mL of crude sample (50 U/mL). The mixture was incubated for 90 min, and the absorbance of the sample was read between 300 and 800 nm. For the control reaction, deactivated enzyme was used, and the rest of the experimental conditions were the same. The experiments were performed in triplicate, and the mean percentage of dye decolorization was determined.

Statistical Analysis

Response surface methodology-based experiments were designed using Design Expert statistical software (Stat-Ease Inc., Minneapolis, MN, USA), and the results were analyzed *via* statistical software. Single-factor optimization results and the dye degradation potential of lignin peroxidase were analyzed by one-way analysis of variance. The p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Proximate Composition of Jackfruit Seeds

The chemical composition of the jackfruit seeds is described in Table 1. The ash content was found to be $3.92 \pm 0.43\%$, and the mean moisture level of the seeds was $7 \pm 0.02\%$. The carbohydrate content was $69.4 \pm 2.5\%$, and the increase in carbohydrate content in the jackfruit seeds was similar to that reported previously (Ocloo *et al.* 2010; Eke-Ejiofor *et al.* 2014; Lima *et al.* 2014). The total protein content of the jackfruit seeds was $13.5 \pm 1.4\%$, which was similar to the results of previous studies (Eke-Ejiofor *et al.* 2014; Lima *et al.* 2014). The total protein content of jackfruit flour is greater than that of wheat flour (Lima *et al.* 2014). Therefore, it can be used for the preparation of diets to replace wheat flour. The lipid content of the jackfruit seeds was $1.9 \pm 0.22\%$, which was consistent with a previous report of Ocloo *et al.* (2010). The dietary fiber content was $29.5 \pm 1.1\%$, which revealed the suitability of this seed for the preparation of functional food. The dietary fiber content observed in this study was greater than that reported previously (Juárez-Barrientos *et al.* 2017; Hager *et al.* 2012). Jackfruit seeds have relatively high percentages of carbohydrates and fiber and relatively low percentages of lipids (Uwanta *et al.* 2024).

Table 1. Proximate Composition of Jackfruit Seeds

Composition (%)	Results (mean±standard deviation)
Ash	3.92 ± 0.43
Moisture	7 ± 0.02
Carbohydrates	69.4 ± 2.5
Total Dietary Fiber	29.5 ± 1.1
Reducing Sugars	7.2 ± 0.13
Total Protein	13.5 ± 1.4
Lipids	1.9 ± 0.22

Total Starch

Three extraction procedures were used for the extraction of total starch from the jackfruit seeds. The water extract improved the total starch yield to $84.1 \pm 3.4\%$, followed by the alkaline ($82.1 \pm 1.5\%$) and enzymatic methods ($79.6 \pm 2.2\%$) ($p < 0.05$) (Fig. 1). The higher yield of starch in the distilled water observed in this study might be attributed to its preserving capacity of the granular structure, and improving its physical performance compared to aggressive chemical and enzymatic methods. The nutritional profile of jackfruit seeds varies in terms of variety and harvesting season and shows diverse compositions of macro- and micro-nutrients. Jackfruit seeds are considered to be a cheap source of carbohydrates, and the seeds are rich in starch, accounting for approximately 8% to 15% of the fruit weight (Suzihaque *et al.* 2022). Starch is widely used in various industries, including the nonfood and food industries. The starch content of the jackfruit seeds was 75.5% of the dry weight of the jackfruit seeds, which was consistent with a previous report on the starch content of other jackfruit seeds (Tulyathan *et al.* 2002). It has been reported that jackfruit seed starch contains more protein than most commercially modified starches do. The chemical composition of the seed starches varies from $18.92 \pm 0.34\%$ to $22.86 \pm 0.46\%$ among five jackfruit cultivars (Olaire *et al.* 2020).

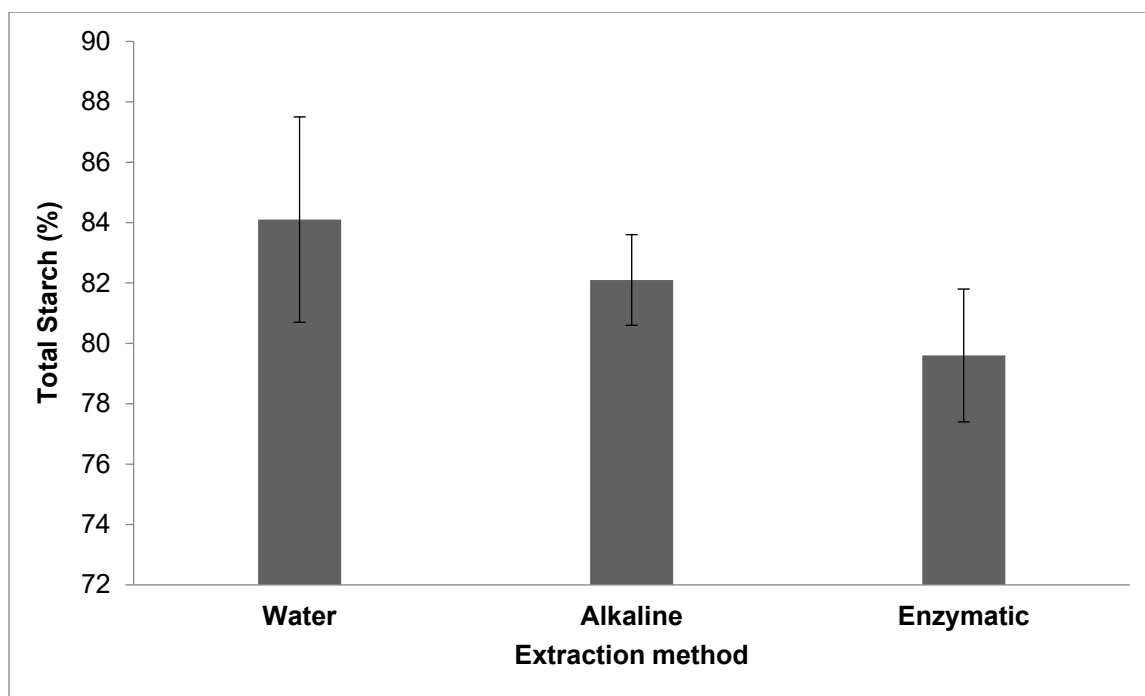


Fig. 1. Total starch content of jackfruit seeds extracted via three different methods

Mineral Content

The mineral content of the jackfruit seed powder was estimated, and the results are shown in Table 2. The major mineral was magnesium, followed by potassium, phosphorus, and calcium (Table 2). The potassium content of the jackfruit seeds was lower than that in previous reports (Mohamad *et al.* 2019). The amount of phosphorus in the jackfruit seeds was much lower in this study than in previous report by Leite *et al.* (2019) (171 mg%). The iron level observed in this study was 1.81 ± 0.15 mg%, which was 12.6 mg% in an earlier study (Borgis and Bharati 2020). The range of zinc and sodium contents observed in this study was similar to that reported in previous studies (Mohamad *et al.* 2019). The variation

in the mineral content of jackfruit seeds may be related to the jackfruit variety, season, topography, and environmental conditions. Jackfruit seeds contain 0.9 to 1.2 g of total minerals, including 50 mg of calcium, 54 mg of magnesium, 38 mg of phosphorus, 246 mg of potassium, 63.2 mg of sodium, 1.5 mg of iron, 10 IU of vitamin A, 0.25 mg of thiamine, 0.11 mg of riboflavin, and 11 mg of vitamin C per 100 g of seed (Swami *et al.* 2012). Jackfruit seed starch is a potential source of resistant starch (75%), has good swelling properties, good water holding capacity, and good gelatinization temperature (76 °C to 88 °C) (Kushwaha *et al.* 2023).

Table 2. Mineral Content of Jackfruit Seed Power

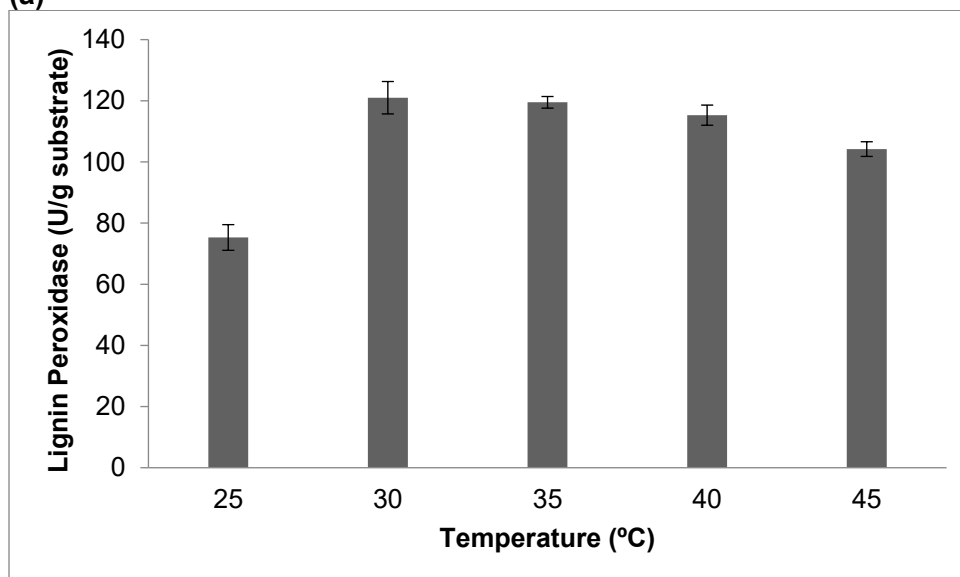
Minerals (mg%)	Results
Calcium	73.21±3.1
Copper	2.4±0.22
Iron	1.81±0.15
Manganese	0.92±0.011
Magnesium	315.3±10.4
Potassium	187.2±9.3
Phosphorus	87.3±0.15
Sodium	2.92±0.22
Zinc	1.52±0.15

Optimization of Enzyme Production via a Single-Factor Optimization Approach

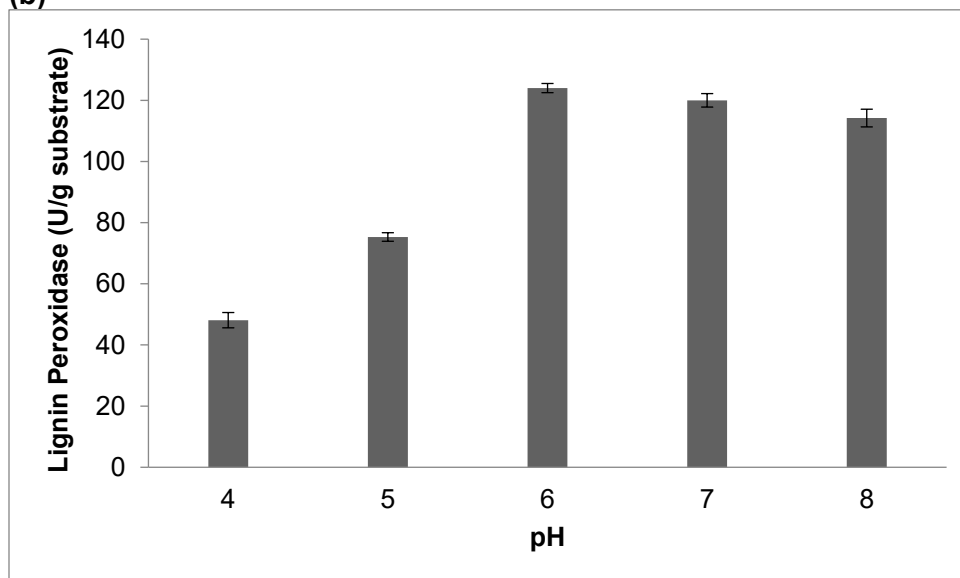
To determine the impact of temperature on ligninolytic enzyme production by *T. reesei*, incubation temperatures ranging from 25 °C to 35 °C were used. SSF was carried out for a period of 72 h, and maximum ligninolytic enzyme production was observed at 35 °C (121±5.3 U/g substrate) ($p < 0.05$) (Fig. 1a). As shown in Fig. 2b, the maximum enzyme activity was observed at pH 6.0 (124±1.5 U/g substrate). Ligninolytic enzyme production reached a maximum at 7% inoculum (135±1.2 U/g substrate); however, it decreased slowly to 8% inoculum (128.5±1.5 U/g substrate) ($p < 0.05$) (Fig. 2c). Ligninolytic enzyme synthesis reached a maximum after day 6 (149.4±1.1 U/g substrate), after which it decreased further ($p < 0.05$) (Fig. 2d). The effects of nutrient sources on enzyme production were evaluated by supplementation with various nitrogen and carbon sources. Compared with the control, the incorporation of glucose significantly improved ligninolytic enzyme activity (157±14.2 U/g substrate) ($p < 0.05$) (Fig. 2e). Different nitrogen sources improved ligninolytic enzyme production, and beef extract significantly improved enzyme production (174.2±5.5 U/g substrate) compared with the control (without a nitrogen source) ($p < 0.05$) (124.8±2.5 U/g substrate) (Fig. 2f). Agro-industrial waste has been widely utilized for the production of lignin-modifying enzymes from several fungal species, including white rot fungi. Spent tea leaves and corn cobs were used as substrates for manganese peroxidase production by cultivating *Inonotus* sp. Sp2 in static flask culture, and the crude enzyme activity was 31.3 and 18.5 U/g substrate, respectively. Manganese peroxidase production reached a maximum after 30 days of incubation, and the initial moisture content was 70% (Contreras *et al.* 2023). The optimum pH for lignin peroxidase production was observed to be pH 6.0, which was higher than that reported previously. Flanetraj *et al.* (2025) utilized a banana pseudostem substrate for the production of

ligninolytic enzymes, and the maximum enzyme production was obtained at pH 4.5 and 60% moisture. Velásquez-Quintero *et al.* (2022) utilized corncob residue for the production of laccases from *Pleurotus ostreatus*, and the maximum enzyme production was detected in the culture medium containing malt extract. The addition of yeast extract improved the enzyme yield, which was consistent with the results of the present study. Laccase (LaC) and lignin peroxidase (LiP) production were optimized in solid-state fermentation using the *Trichoderma hamatum* strain NGL1. Enzyme production was improved by 0.42% glucose, in addition to 0.64% malt extract, and 0.084 mM copper sulfate (Arokiyaraj *et al.* 2025). The present findings and those of previous investigations revealed that glucose is an important nutrient for laccase production in microbial fermentation.

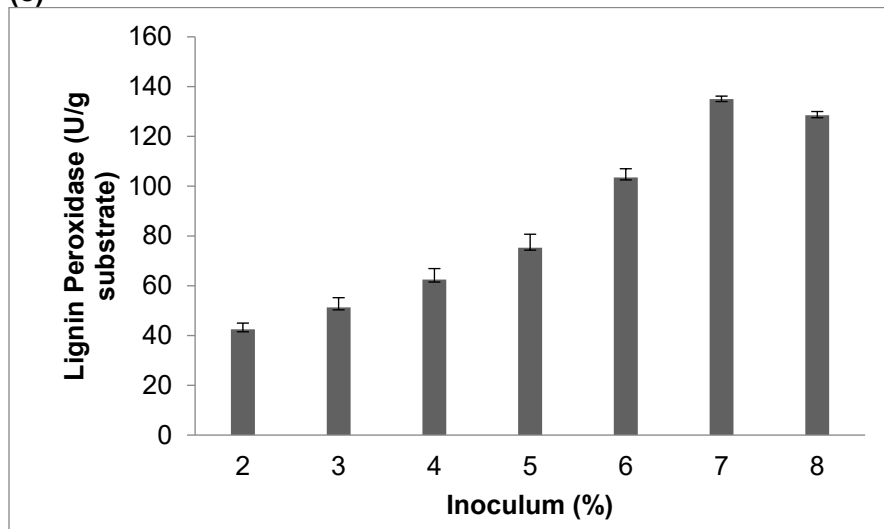
(a)



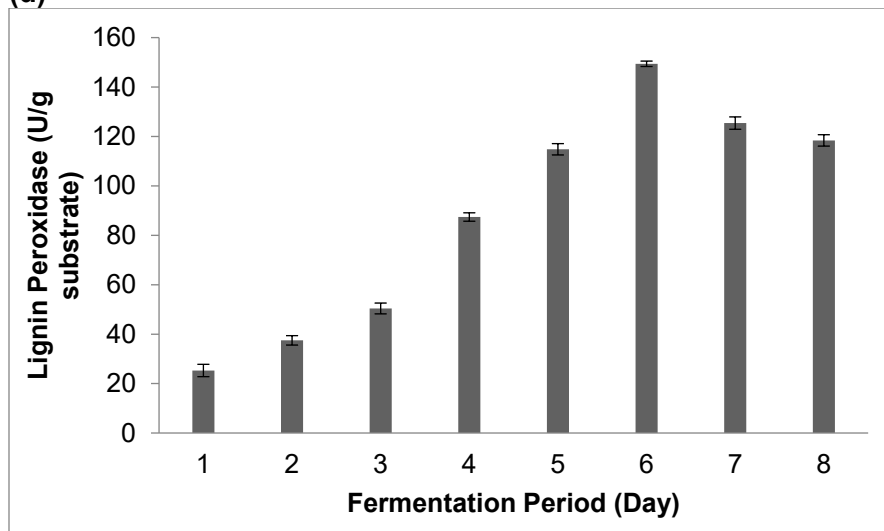
(b)



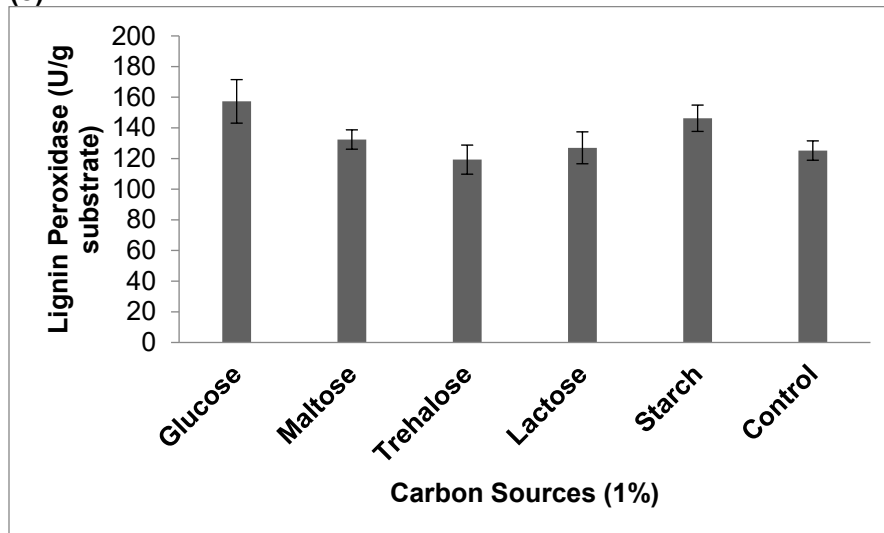
(c)



(d)



(e)



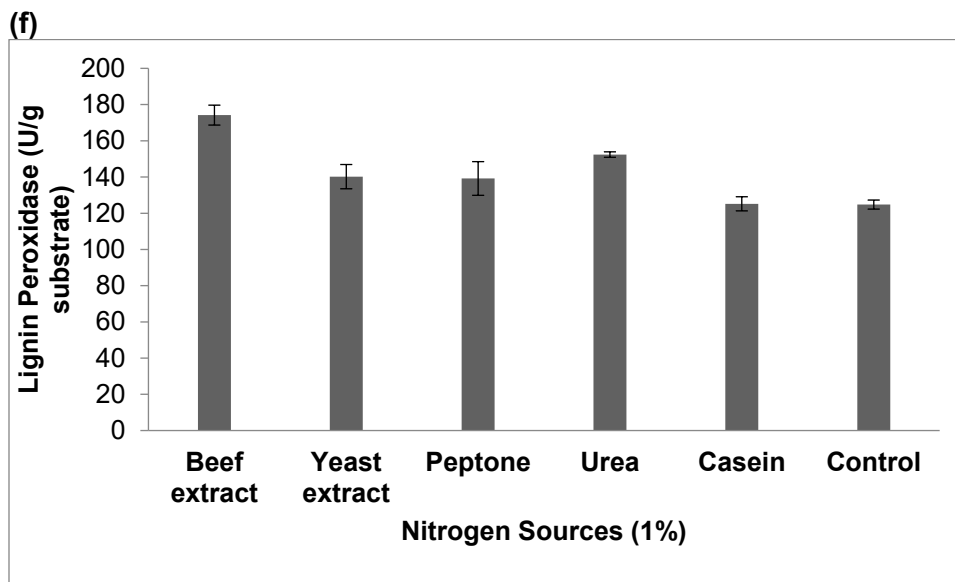


Fig. 2. Effects of temperature (a); pH (b); inoculum (c); fermentation period (d); carbon source (e); and nitrogen source (f) on lignin peroxidase production

Optimization of Enzyme Production by Central Composite Design and Response Surface Methodology

After initial screening for ligninolytic enzyme production, the three most important variables were selected for optimization studies *via* central composite design (CCD). Ligninolytic enzyme production ranged from 3.1 to 360.2 U/g substrate (Table 3). In this study, the CCD was selected for analyzing the relationships between the response (enzyme yield) and the three individual variables (temperature, inoculum, and yeast extract). Each factor for a desired response is analyzed at different levels, and the behavior of the system is explained by means of a fitted quadratic model equation. Analysis of variance was conducted, as depicted in Table 4, to validate the results. The F-value was high for the fitted quadratic model ($F=6.843$), and the P value was <0.01 . The P value was used to check the significance of each variable, and a P value <0.05 was considered to indicate statistical significance. The R-square value of the model was 0.86, and a value >0.75 designated a good statistical model, indicating the aptness of the designed CCD model. Temperature and inoculum had insignificant effects on ligninolytic enzyme production, whereas yeast extract significantly influenced the enzyme production ($P=0.0083$) (Table 4). From the CCD analysis, the optimal fermentation conditions were found to be an incubation temperature of 32.5 °C, a pH of 6.0, and a 0.5% yeast extract concentration in the jackfruit seed substrate medium. Under these optimal culture conditions, the predicted ligninolytic enzyme activity was 369.5 U/g substrate. To validate this model, an experiment was performed under optimized conditions, and the predicted values were in good agreement with the experimental results. The 3D response surface plots of temperature, inoculum, and yeast extract are shown in Fig. 3. Enzyme production was significantly affected by the yeast extract concentration in the culture medium (jackfruit seed medium) compared with the other tested physical factors (temperature and inoculums). Ligninolytic enzyme production was found to increase when the temperature or inoculum was fixed at its optimal value and when the concentration of yeast extract was increased. The statistical-assisted optimization strategy improved lignin peroxidase, and this approach has been used previously to improve the production of enzymes, such as cellulolytic enzymes using

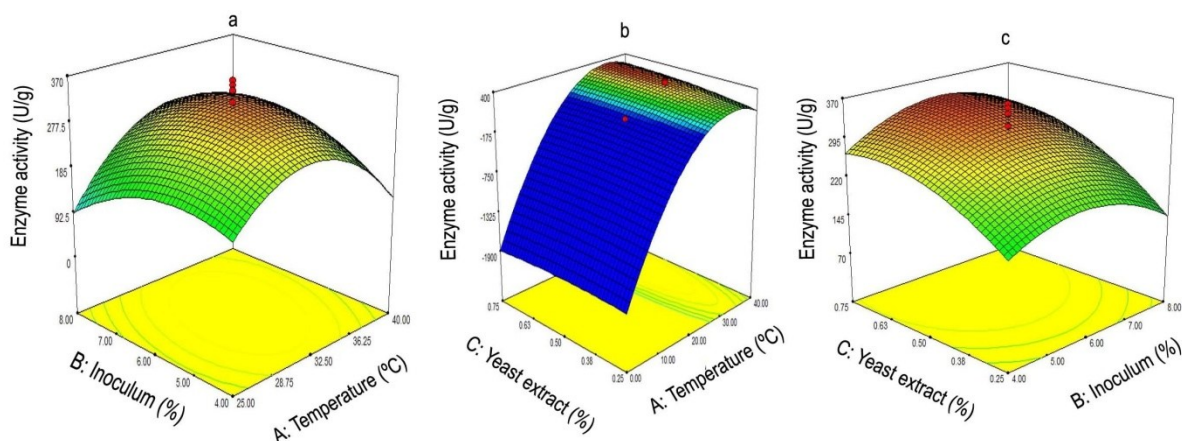
Trichoderma harzianum and *Aspergillus fumigates* (Teo *et al.* 2025), fungal xylanase (Azzouz *et al.* 2022), endoglucanase synthesis *Trichoderma harzianum* (Teo and Wahab 2023), phenolic-associated enzymes by *Trichoderma reesei* (Abdel-Atyet *et al.* 2022), and laccase production by SSF using *Penicillium commune* s6 (Abdelraof *et al.* 2026). In the present study, RSM improved lignin peroxidase production 3-fold and was comparable with the results of a previous report (Arokiyara *et al.* 2024). In this research, a 3D response surface plot was used to identify the optimum response of the variables, and yeast extract was a significant factor influencing lignin peroxidase activity (Fig. 3). Statistical optimization of laccase production leads to the elimination of insignificant medium components in the initial steps and the utilization of the most significant factors to determine the optimum medium components. The optimized yield of lignin peroxidase obtained in this study was greater than that of laccases produced by various fungi cultivated in the SSF, such as laccases from *Pleurotus ostreatus* (65.4 U/g) (Mishra and Kumar 2007), *Trametes hirsuta* (68.4 U/g), *Trametes hirsuta* (68.4 U/g) (Couto *et al.* 2004), and *Coriolus rigida* (108 U/g) (Gómez *et al.* 2005). SSF has been frequently employed for the production of ligninolytic enzymes by microorganisms, such as *Pleurotus ostreatus* (Karp *et al.* 2015), *Penicillium commune* (Abdelraof *et al.* 2026), *Trametes troglia* (Levin *et al.* 2008), *Phoma herbarum* isolate KU4 (Debnath *et al.* 2021), and *Marasmiellus palmivorus* LA1 (Chenthamarakshan *et al.* 2017).

Table 3. Central Composite Design Matrix for the Optimization of Enzyme Production by *T. reesei* in Solid-State Fermentation

Run	A: Temperature (°C)	B: Inoculum (%)	C: Yeast extract (%)	Enzyme Activity (U/g)
1	25	4	0.75	165.9
2	25	4	0.25	60.1
3	32.5	6	0.5	160.4
4	25	8	0.75	49.6
5	32.5	2.6	0.5	177.2
6	32.5	6	0.079	77.4
7	40	8	0.25	57.2
8	32.5	6	0.5	339.4
9	32.5	6	0.92	340.2
10	19.8	6	0.5	3.1
11	32.5	9.3	0.5	150.2
12	40	4	0.75	101.4
13	32.5	6	0.5	340.2
14	32.5	6	0.5	351.4
15	32.5	6	0.5	360.2
16	40	8	0.75	287.4
17	40	4	0.25	73.1
18	32.5	6	0.5	317.4
19	25	8	0.25	38.4
20	45.1	6	0.5	5.3

Table 4. Analysis of Variance for Ligninolytic Enzyme Production by *T. reesei* via CCD Matrix

Source	Sum of Squares	df	Mean Square	F-Value	P value Prob > F
Model	280368.5	9	31152.06	6.843162	0.0030
A-Temperature	3192.347	1	3192.347	0.701262	0.4219
B-Inoculum	12.96885	1	12.96885	0.002849	0.9585
C-Yeast extract	48932.62	1	48932.62	10.74901	0.0083
AB	11865.7	1	11865.7	2.606535	0.1375
AC	2502.781	1	2502.781	0.549785	0.4755
BC	1439.161	1	1439.161	0.31614	0.5863
A ²	176886.9	1	176886.9	38.85669	< 0.0001
B ²	42643.77	1	42643.77	9.367543	0.0120
C ²	21307.81	1	21307.81	4.68068	0.0558
Residual	45522.89	10	4552.289		
Lack of Fit	17091.07	5	3418.215	0.601125	0.7050
Pure Error	28431.82	5	5686.364		
Cor Total	325891.4	19			

**Fig. 3.** 3D response surface plots of the interactions between inoculums and temperature: (a) yeast extract and temperature, (b) yeast extract and inoculums, and (c) a fixed concentration of yeast extract, inoculums, and temperature

Methylene Blue Decoloration by Lignin Peroxidase

The decoloration ability of laccases varies with source (Forootanfar *et al.* 2011; Othman *et al.* 2018). Microbial peroxidases are cost-effective and efficient biocatalysts that catalyze the oxidation of Mn^{2+} to Mn^{3+} ions and can oxidize phenolic substrates. Dye decolorization is a highly complex process, and enzymes such as heme-dependent peroxidases, lignin peroxidase, and dye-decolorizing peroxidases generate free radicals, and hydrogen peroxide that may be responsible for the dye degradation process. Ligninolytic enzymes from *Bacillus thuringiensis* (Wu *et al.* 2022), *Bacillus albus* MW407057 (Kishor *et al.* 2021), *Phanerochaete chrysosporium* (Sosa-Martínez *et al.* 2020), and the endophyte *Trichoderma harzianum* AUMC14897 (Salem *et al.* 2024)

presented dye-degrading activity. The decolorization potential has been increased with increasing incubation time (Thampraphaphon *et al.* 2022). The decolorization percentages were 0%, 7.1%, 12.4%, 19.4%, 24.3%, 33.2%, 40.6%, 51.7%, 64.3%, and 79.4% after 0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 min of incubation, respectively (Fig. 4). The decoloration ability of fungal ligninolytic enzymes varies with the source, reaction time, dye, and enzyme concentration (Pham *et al.* 2022; Panda *et al.* 2026). The lignin peroxidase reacts with the methylene blue dye, and electrons from the catalysts are transferred to the conjugate bonds. This transfer reduces the color intensity and changes the configuration of the dye (Dinh Giap *et al.* 2022). The present findings reveal that lignin peroxidase from *T. reesei* is involved in the color removal of synthetic methylene blue dye and has the ability to degrade lignocellulosic biomass. Moreover, for dye degradation, lignin peroxidase should be combined with other enzymes, including versatile peroxidases, manganese peroxidases, and laccases (Yadav and Yadav 2015).

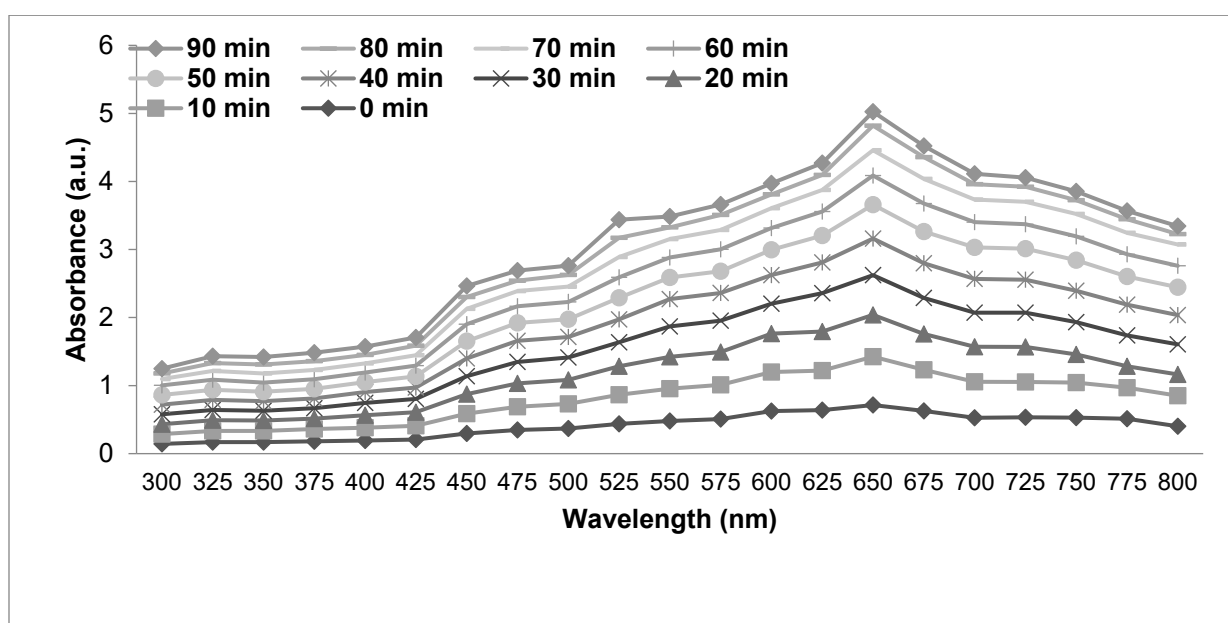


Fig. 4. *Trichoderma reesei* crude lignin peroxidase enzyme on methylene blue dye decolorization, treated with 200 mM methylene blue for 90 min

CONCLUSIONS

1. Jackfruit seed could be an effective medium for the production of lignin peroxidase in SSF by *Trichoderma reesei* via solid state fermentation (SSF). The carbohydrate content of the jackfruit seed was approximately 70%, which favors microbial growth and product formation.
2. The central composite design (CCD) was used to improve enzyme production and achieved more than 2-fold enzyme production. The physical factors (inoculums and temperature) and nutrients (yeast extract) were influenced on lignin peroxidase production.

3. The fungal lignin peroxidase exhibited photocatalytic activity and decolorized methylene blue. Crude lignin peroxidase is cost-effective biocatalyst used for the decolorization of synthetic dyes.

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Conflict of Interest

Authors do not have any conflict of interest in publication of this research article.

Use of Generative AI

Authors did not use AI tools in the preparation of text, data analysis, and collection of references.

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