RESEARCH PAPER

Valorization of Banana Pseudostem Extract and Jackfruit Seed Waste on a Coco-Bed Matrix for Protein-Enriched Mycelial Biomass of *Pleurotus pulmonarius*

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ABSTRACT

Fungal biomass grown on farm and food-industry residues is gaining attention as a low-footprint protein source. This work examined whether banana pseudostem extract and jackfruit seed powder, mixed into a coconut-coir (coco-bed) matrix, can raise the protein level of *Pleurotus pulmonarius* mycelial biomass. The pseudostem extract served as a slowly released carbon supply, the seed powder as a fast starch-derived energy source, and the coir as a porous support. Substrates were sterilized at 121 °C for 15 min, inoculated at several points with actively growing PDA culture discs, and incubated at 25–30 °C and 70–85% relative humidity. Process variables (carbon-to-nitrogen ratio, temperature, humidity, pH and moisture) were screened and optimized with a Plackett–Burman-based quadratic model ($F = 28.34$, $p < 0.0001$). The model pointed to a 50:50 pseudostem-to-jackfruit-seed mix, 26.8 °C, 88.5% humidity, pH 6.32 and 67.5% moisture, with a predicted protein content of 44.8% and biological efficiency of 89.4%; the matching single experimental run recorded identical values (not replicated). Biuret colour intensity rose from the coir-only control through single-supplement treatments to the combined treatment, and the Lowry calibration was linear over 20–100 µg/mL. Kjeldahl analysis of one separate sample gave 2.128% nitrogen (about 13.3% crude protein with the 6.25 factor), a lower figure that is reported alongside, not merged with, the optimization values. Together, the results indicate that combining a lignocellulosic and a starch-rich residue on coir is a workable, waste-reducing route to protein-enriched mushroom mycelium. Larger-scale trials, amino-acid profiling and food-safety evaluation are the next steps.

Keywords: *Pleurotus pulmonarius*, Banana pseudostem, Jackfruit seed, Coco-bed substrate, Protein enrichment, Agro-waste valorization.

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INTRODUCTION

As populations grow and diets shift toward protein-dense foods, livestock-based supply chains are under pressure from their land, water and energy demands and their greenhouse gas output[1]. Microbial and fungal protein offers an alternative that needs far fewer inputs. Edible mushrooms in particular supply good-quality protein, including essential amino acids that cereal-heavy diets tend to lack[2].

Oyster mushrooms of the genus Pleurotus are among the most efficient white-rot fungi for turning plant residues into food[3]. They release cellulases, hemicellulases, laccases and manganese and lignin peroxidases that loosen the cellulose, hemicellulose and lignin of plant cell walls and make the sugars inside available for growth[4,5]. Their dry-weight protein usually falls between 20% and 35%, and it moves with the substrate and the growing conditions[2,6].

Tropical agriculture leaves behind large amounts of material that fits this biology. Banana pseudostem is cut and left after every harvest, and it is rich in cellulose, hemicellulose, soluble sugars and minerals such as potassium and calcium that help drive extracellular enzymes[7]. Jackfruit seeds, a common by-product of fruit processing, are starch-rich, carry a moderate amount of protein, and are mostly thrown away[8,9]. When such residues are dumped or burned, they add to pollution and waste nutrients that could be recycled[7].

The physical matrix matters as much as the nutrients. Coconut-coir (coco-bed) material holds water well, stays open enough for oxygen to diffuse, and breaks down more slowly than cereal straw, which keeps moisture and aeration steadier during long incubations[10]. Earlier work has tested one residue at a time on straw or sawdust[11]. Pairing a slowly degraded lignocellulosic carbon source with a quickly metabolized starch source on coir has received little attention, even though balancing the two should support early hyphal growth and later amino-acid synthesis[12].

This study therefore had three aims: (i) to prepare an enriched cultivation medium from banana pseudostem extract and jackfruit seed powder on coco-bed for *P. pulmonarius;* (ii) to optimize the ratio of the two residues together with temperature,

humidity, pH and moisture by statistical design; and (iii) to assess protein accumulation in the harvested mycelial biomass with the Lowry, Biuret and Kjeldahl methods.

MATERIALS AND METHODS

Materials

Fresh banana pseudostem came from local farm fields after fruit harvest. Jackfruit seed waste was collected from local market sources and carried to the laboratory in clean containers. Coco-bed (coconut coir) was obtained from a coir processing unit. A pure culture of *Pleurotus pulmonarius* maintained on potato dextrose agar (PDA) supplied the inoculum. Analytical-grade chemicals and distilled water were used throughout.

Preparation of Banana Pseudostem Extract

The pseudostem was washed with distilled water to remove soil and surface debris, then cut into small pieces with sterile tools to widen the surface available for extraction. The pieces were crushed in a laboratory blender to release the plant sap, and the slurry was passed through Whatman filter paper and muslin cloth. The clarified filtrate, which holds soluble sugars, organic acids and minerals, was kept as the carbon-rich extract[7].

Preparation of Jackfruit Seed Powder

Seeds were washed free of pulp, sun-dried briefly and then oven-dried at moderate temperature to constant weight. The dry seeds were ground in a laboratory grinder, sieved through a mesh to obtain an even particle size, and stored in sterile airtight containers under dry conditions until use[8],[9].

Conditioning of Coco-Bed Substrate

Coir was rinsed with distilled water to remove dust, soluble salts and other impurities, soaked for 24 h for uniform hydration, and sterilized in an autoclave at 121 °C for 15 min[10].

Formulation of the Enriched Medium

Measured volumes of pseudostem extract and jackfruit seed powder were blended into the conditioned coir under aseptic conditions until evenly distributed. Four substrate configurations were prepared: coir alone (control), coir with pseudostem extract, coir with jackfruit seed powder, and coir with both supplements (combined treatment). The pseudostem-to-jackfruit-seed ratios examined in the optimization study were 100:0, 90:10, 70:30, 50:50 and 30:70 (w/w basis).

Inoculum Preparation and Inoculation

Discs were cut from the actively growing margin of PDA colonies with a sterile cork borer, because the margin carries the most metabolically active hyphae. All handling was carried out in a laminar airflow cabinet, and tools were disinfected with alcohol and flame. Substrate was filled to a uniform depth in sterile bags, discs were placed at several points through the substrate to speed even colonization, and the bags were closed with breathable coverings.

Incubation Conditions

Bags were held in an incubation chamber at 25–30 °C and 70–85% relative humidity. Moisture was checked regularly and topped up with sterile distilled water when needed, while adequate aeration was maintained so that oxygen-dependent lignin-degrading enzymes could stay active[10],[11]. Colonization was followed by recording the first visible mycelium, the rate of hyphal extension and the evenness of substrate coverage.

Statistical Optimization

A Plackett–Burman screening layout[13] extended to a quadratic response model was used to evaluate five factors: carbon-to-nitrogen (pseudostem : jackfruit seed) ratio, temperature, humidity, pH and substrate moisture. Twenty runs were analysed by ANOVA, and numerical optimization was run with the C:N ratio and humidity set to maximize, temperature, pH and moisture kept within range, and protein content and biological efficiency (BE) as responses to maximize. A separate seven-cycle run sheet recorded measured protein and BE at selected factor combinations.

Harvest and Sample Preparation

Colonized substrate containing well-developed mycelial networks was collected with sterile forceps, separated from residual particles, rinsed gently with distilled water and dried under controlled laboratory conditions before extraction.

Protein Estimation

Lowry method. Standards of 20, 40, 60, 80 and 100 µg/mL were prepared from the working standard (0.1, 0.2, 0.4, 0.6 and 0.8 mL, made up with distilled water). Each tube received 2.1 mL of alkaline copper reagent and stood at room temperature for 20 min, after which 0.2 mL of Folin–Ciocalteu reagent was added. Absorbance was read at 660 nm against a reagent blank, and 0.4 mL of sample extract was treated identically and read at 30-s intervals over 150 s[14].

Biuret method. Extracts from each treatment were mixed with Biuret reagent (copper ions in alkaline medium) and the violet colour was compared visually as a qualitative index of protein level[15].

Kjeldahl method. One gram of sample was digested with concentrated sulfuric acid, potassium sulfate and copper sulfate until a clear greenish digest formed. The digest was cooled, made alkaline with 40% NaOH, and the liberated ammonia was distilled into 4% boric acid containing a mixed indicator (methyl red and bromocresol green). The distillate was titrated with standard hydrochloric acid to a

persistent light-pink endpoint, with a reagent blank for correction[16]. Nitrogen percentage was calculated as:

N (%) = (V1 - V2) x N x 14 x 100 / (W x 1000)

where V1 − V2 is the net titre (mL), N the normality of the acid, 14 the atomic weight of nitrogen, and W the sample weight (g). Crude protein was obtained by multiplying nitrogen by a nitrogen-to-protein conversion factor.

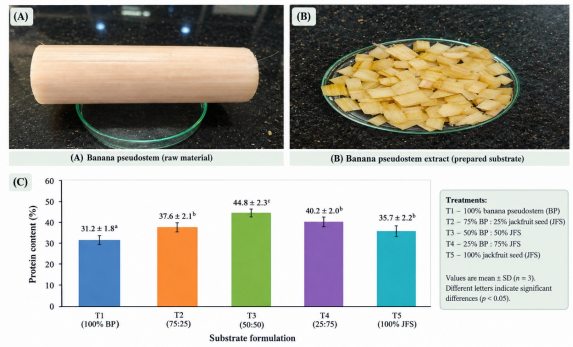


Figure 1. (A) Banana pseudostem (raw material); (B) Banana pseudostem extract (prepared substrate); (C) Protein content of oyster mushroom biomass produced from different substrate formulations.

RESULTS AND DISCUSSION

Statistical Optimization of the Medium

The quadratic model fitted to the twenty runs was highly significant ($F = 28.34$, $p < 0.0001$), and each of the five factors contributed significantly (Table 1). Using the sums of squares, the model accounts for about 96% of the total variation (1482.45 of 1540.57). The carbon-to-nitrogen ratio carried the largest share (sum of squares 520.12; $F = 89.47$), followed by temperature, pH, humidity and moisture, so the balance between the pseudostem and the seed powder was the main lever on protein formation. A pH of 6.32 together with 67.5% moisture was the combination that favoured breakdown of the fibrous banana tissue, in line with the known dependence of fungal cellulolytic and ligninolytic enzymes on water availability and pH[4,5,10].

Table 1. ANOVA for the quadratic model

Source	Sum of squares	df	Mean square	F-value	p-value
Model	1482.45	9	164.72	28.34	< 0.0001
A: C:N ratio	520.12	1	520.12	89.47	< 0.0001
B: Temperature	192.34	1	192.34	33.09	0.0002
C: Humidity	88.50	1	88.50	15.22	0.0030
D: pH	105.67	1	105.67	18.18	0.0017
E: Moisture	75.40	1	75.40	12.97	0.0048
Residual	58.12	10	5.81		
Cor total	1540.57	19			

Numerical optimization settled on a 50:50 pseudostem-to-jackfruit-seed mixture, 26.8 °C, 88.5% relative humidity, pH 6.32 and 67.5% moisture (Table 2). Under these settings the model predicted 44.80% protein and 89.40% biological efficiency.

Table 2. Numerical optimization (point prediction)

Parameter	Goal	Optimized value
C:N ratio (pseudostem : jackfruit seed)	Maximize	50:50
Temperature	In range	26.8 °C
Humidity	Maximize	88.5%
pH	In range	6.32
Moisture	In range	67.5%
Protein content	Maximize	44.8
Biological efficiency	Maximize	89.4

Run-Sheet Verification

The seven-cycle run sheet supports the model (Table 3). Cycle 3, run at the optimized settings, produced 44.8% protein and 89.4% BE, the highest of all cycles. Pseudostem-heavy mixes performed worst: the 90:10 and 100:0 cycles gave only 16.3% and 14.2% protein, which suggests that a lignocellulosic carbon source on its own is released too slowly to drive early growth. Adding the starch-rich seed powder lifted protein in the 70:30 and 30:70 cycles to 32.1% and 28.4%, and the balanced 50:50 mix did best. Cycle 7 used the same 50:50 ratio but a

cooler 22 °C and 85% humidity, and protein fell to 36.5%, showing that the ratio alone does not guarantee the result and that temperature and humidity must be kept close to the optimum. Because several factors changed from cycle to cycle, these comparisons are indicative rather than strictly one-factor-at-a-time.

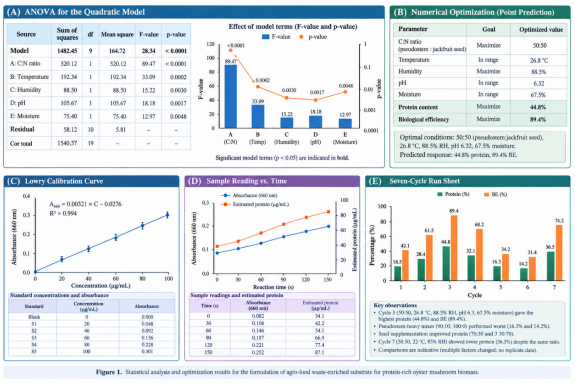


Figure 1. Statistical analysis and optimization results for the formulation of agro-food waste-enriched substrate for protein-rich oyster mushroom biomass.

Table 3. Seven-cycle run sheet

Cycle	C:N ratio	Temp (°C)	Humidity (%)	pH	Moisture (%)	Protein (%)	BE (%)
1	0.479 (as recorded)	25	80	6.0	65	18.5	42.1
2	30:70	25	80	6.0	65	28.4	61.5
3	50:50	26.8	88.5	6.3	67.5	44.8	89.4
4	70:30	28	90	6.5	70	32.1	68.2
5	90:10	28	90	6.5	70	16.3	34.2
6	100:0	25	85	6.2	68	14.2	31.4
7	50:50	22	85	6.3	67.5	36.5	74.2

Protein Estimation by the Lowry Method

Absorbance at 660 nm rose steadily with standard concentration, from 0.048 at 20 µg/mL to 0.301 at 100 µg/mL (Table 4), giving a near-linear calibration suited to quantification. Sample readings climbed from 0.082 at the start to 0.252 at 150 s, all inside the calibrated range, which shows that the extract contained measurable soluble protein and that colour development was still under way during the early part of the reaction.

Table 4a. Lowry calibration and sample readings (660 nm)

Standard	Concentration (µg/mL)	Absorbance
Blank	0	0.000
S1	20	0.048
S2	40	0.092
S3	60	0.156
S4	80	0.228
S5	100	0.301

Sample reading time (s)	0	30	60	90	120	150
Absorbance	0.082	0.108	0.146	0.187	0.221	0.252

Fitting the five standards by least squares gave A660 = 0.00321 × C − 0.0276 (C in µg/mL; R^2 = 0.994). Applying this line to the sample readings gives the estimated concentrations in Table 4b. The values rise with reaction time, so the reading time should be fixed in future runs; converting these figures to a percentage of biomass dry weight is not possible here because the extract volume and the dry weight extracted were not recorded.

Table 4b. Estimated protein concentration in the sample extract (from the calibration line)

Reading time (s)	0	30	60	90	120	150
Absorbance (660 nm)	0.082	0.108	0.146	0.187	0.221	0.252
Estimated protein (µg/mL)	34.1	42.2	54.1	66.9	77.4	87.1

Protein Estimation by the Biuret Test

All biomass extracts gave a positive Biuret reaction, and colour depth separated the treatments in a clear order (Table 5). The coir-only control produced a pale violet, the pseudostem-enriched substrate a moderate violet, the jackfruit-seed substrate a dark violet, and the combined treatment a deep violet-purple. The ranking agrees with the run-

sheet trend and indicates that the two residues act together: the seed starch gives quick energy for early amino-acid synthesis, and the pseudostem keeps carbon flowing later in incubation[8,9].

Table 5. Biuret test observations

Treatment	Colour observed	Relative protein level
Control (coco-bed only)	Light violet	Low
Banana pseudostem enriched	Moderate violet	Moderate
Jackfruit seed enriched	Dark violet	High
Combined (pseudostem + seed)	Deep violet / purple	Highest

Nitrogen Estimation by the Kjeldahl Method

For a 1 g sample, the net titre was 15.2 mL of 0.1 N acid, which gives

N (%) = (15.2 x 0.1 x 14 x 100) / (1 x 1000) = 2.128 %

So the sample held 2.128% nitrogen. With the conventional factor of 6.25 this corresponds to roughly 13.3% crude protein. Fungal biomass also contains non-protein nitrogen such as chitin, so the factor chosen affects the result, and the Kjeldahl figure refers to the single sample digested rather than to the optimized run. It is therefore not directly comparable with the model values in Tables 1–3; digesting biomass from each cycle would be the logical way to tie the two together.

Context, Significance and Limitations

Reported protein in Pleurotus fruiting bodies usually sits in the 20–35% dry-weight range[2,6], so the 44.8% obtained at the optimum is high; it is a model-supported value for mycelial biomass from a controlled laboratory system and should be confirmed by repeat measurements before it is treated as a general figure. The findings are consistent with earlier reports that coir offers favourable moisture and aeration[10], that banana residues are colonized readily by oyster mushrooms[7,11], and that starch-rich supplements shorten the lag phase and raise protein[12]. Using two locally available wastes at once also reduces the need for cereal straw, which competes with feed and soil uses, and keeps the system within a circular-bioeconomy approach[1,7].

Several limits should be kept in mind. Replicate values and standard deviations were not recorded, so no error estimates are given and the run-sheet figures come from single runs. The work was done at laboratory scale, so yield and economics at larger scale are untested. Environmental drift in temperature, humidity and moisture can shift growth. The Lowry assay is sensitive to interfering substances and to calibration quality, the Biuret test here is only qualitative, and the Kjeldahl method counts all nitrogen. Amino-acid composition, digestibility and food-safety analysis (including heavy metals and microbial load) were not examined.

CONCLUSION

Banana pseudostem extract and jackfruit seed powder, supported on a coconut-coir matrix, formed an effective low-cost medium for growing protein-enriched *Pleurotus pulmonarius* mycelial biomass. Statistical optimization identified a 50:50 pseudostem-to-seed mix at 26.8 °C, 88.5% humidity, pH 6.32 and 67.5% moisture as the best condition, with 44.8% protein and 89.4% biological efficiency recorded in the matching single run, which was not replicated. Biuret observations ranked the combined treatment highest, the Lowry calibration was dependable, and Kjeldahl analysis of one sample gave 2.128% nitrogen, about 13.3% crude protein. Turning two common wastes into edible fungal protein supports both food security and cleaner residue management. Before commercial use, the work needs repeat Kjeldahl and Lowry measurements on biomass from every run, amino-acid and nutritional profiling, food-safety testing and scale-up trials.

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AUTHOR CONTRIBUTIONS

Mr A Praveenkumar and Mr K Sharmesh carried out the experimental work and data collection. Ms P Subhakarini has contributed to the experimental design and statistical analysis. Dr R Balakrishnaraja conceived and supervised the study and revised the manuscript. All authors read and approved the final version.

ETHICAL STATEMENT

The study used only microbial culture, plant residues and laboratory assays; no human or animal subjects were involved and ethical approval was not required.

DATA AVAILABILITY

The data supporting the findings are contained in this article; further details are available from the corresponding author on reasonable request.

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