Scientific Research Publishing

Food and Nutrition Sciences, 2025, 16(5), 517-535
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Optimization of Process Parameters for Single Cell Protein Production from *Trichoderma atroviride* Strain Using Cassava Peel Substrates

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How to cite this paper: Sitati, L.C., Matofari, J.W. and Nduko, J.M. (2025) Optimization of Process Parameters for Single Cell Protein Production from *Trichoderma atroviride* Strain Using Cassava Peel Substrates. *Food and Nutrition Sciences*, **16**, 517-535.
https://doi.org/10.4236/fns.2025.165029

Received: March 31, 2025
Accepted: May 23, 2025
Published: May 26, 2025

Abstract

This study aimed to optimize process variables for the enhanced production of single-cell protein (SCP) using *Trichoderma atroviride* EGER23 from cassava peels through Response Surface Methodology (RSM). A Box-Behnken design was applied to investigate the influence of three key factors viz. moisture content (60.0% - 75.0%), pH (6.5 - 8.5), and incubation time (4 - 8 days) on protein content. A second-order polynomial model effectively explained the variation in experimental data and showed a strong correlation between the independent variables and the response. The optimized conditions (moisture content of 66.52%, pH of 6.50, and incubation time of 7.39 days) resulted in a maximum protein yield of 9.5% with a desirability of 91.66% after optimization. High moisture content lowered the protein yield by restricting oxygen transfer, whereas variations from the optimal pH inhibited the activity of enzymes involved in fungal metabolism. In addition, prolonging the fermentation period beyond the optimal period led to protein degradation rather than further accumulation. The optimization model showed that the optimization of process parameters maximizes Single Cell Protein production. The findings highlighted the potential of cassava peels as a sustainable substrate for microbial protein production, aligning with the circular economy strategies to enhance resource efficiency in food and feed production.

Keywords

Trichoderma atroviride, Single-Cell Protein, Cassava Peel, Optimization, Response Surface Methodology, Circular Economy

1. Introduction

The global food system faces more challenges as it strives to meet the nutritional

demands of an ever-growing population under the constraints of environmental sustainability [1]. Therefore, rapid increases in the world population and the rising pressures on food security call for efficient and sustainable protein production systems [2]. Traditional animal-based proteins, while nutritionally rich, are environmentally costly due to higher greenhouse gas emissions, extensive land use, and significant freshwater consumption [3]. Microbial protein, or so-called single-cell protein (SCP), therefore, is an ideal alternative to meet counter protein deficiency, especially in developing nations. SCP refers to protein extracted from pure or mixed cultures of microorganisms such as microalgae, yeast, fungi, or bacteria, and it can be used as a substitute for the conventional protein sources destined for human and animal consumption [4]. SCP production is more environmentally friendly, consumes less water, requires smaller land areas, and its effect on climate change is much less pronounced than in the case of agriculturally derived proteins [5]. Aside from being rich in protein, it positively contributes to waste management and a circular bio-economy approach [6].

Cassava (*Manihot esculenta* Crantz) is a drought-resistant staple food in tropical and subtropical regions, containing starch in higher amounts [7]. Cassava is grown primarily for its starchy root as it is a major source of carbohydrates for millions of people, especially in regions where other staple foods are not readily available. However, its by-products, such as leaves and tuber peels, are usually disposed of as waste, which ultimately pollutes the environment [8] by releasing greenhouse gases, particularly methane, during decomposition, contributing to global warming. Cassava peels, being the main residues from processing, account for 20 - 35 percent of the tuber's weight, especially when peeling by hand [9]. Cassava peels are lignocellulose material that can be treated biologically, chemically or physically, to remove components such as lignin and starch in order to produce hemicellulose-based compounds [10]. The peels are a good source of fibre and can be used as animal feed, in paper production, and as biodegradable packaging. Despite the great potential of cassava peels, their use is limited due to their high levels of anti-nutritional components, such as cyanogenic glycosides (linamarin and lotaustralin), which can be harmful to human and animal health if not properly detoxified [11]. Studies have shown that fermentation can effectively decrease cyanogenic contents while enhancing protein levels in a way suitable for SCP production [12]. However, effective bioconversion processes need an efficient fermentation strategy with the objective of maximizing the growth of the microorganisms and protein yield.

Among all the microorganisms, filamentous fungi have emerged as a preferred option due to their facile cultivation, harvesting as pellets and their capability to degrade lignocellulosic material and store quality protein [13]. *Trichoderma* spp is widely recognized for secreting hydrolytic enzymes such as cellulases, hemicellulases, and proteases that make it possible for it to hydrolyse complex carbohydrates into fermentable sugars [14]. In particular, *Trichoderma atroviride* has been identified as a potential producer of SCP because it has a rapid growth rate, mul-

tiple fermentation resistance environments, and has the potential to produce high levels of biomass protein. In addition, the species has antagonist characteristics against plant pathogens and can be employed in integrated cropping systems [15].

Although SCP production via *Trichoderma atroviride* is promising, different processing parameters such as pH, substrate level, temperature, incubation time, incubation period and carbon to nitrogen ratio have significant effects on fungal metabolism, secretion of enzymes, and protein yield [16]. The absence of optimal fermentation conditions calls for sequential optimization to achieve optimal protein content and process efficiency. Recent advances in bioprocess engineering have shown that statistical optimization methods, including Response Surface Methodology (RSM), can effectively optimize process parameters to maximize microbial growth and SCP production [16] [17].

The study aimed to maximize the fermentation conditions of SCP production from *Trichoderma atroviride* using cassava peels. The Box-Behnken design, a statistical optimization tool, was used to determine the optimal conditions and evaluate how process variables affected the correlation between controlled experiments and selected variable outcomes. The outcome gave important information on microbial valorisation of cassava peel to facilitate global initiatives towards circular bio-economy approaches and alternative sources of proteins for food and animal feed.

2. Materials and Methods

2.1. Substrate Preparation

The cassava peels (KEMI variety) were sourced from Kenya Agricultural and Livestock Research Organization (KALRO), Njoro, Nakuru County. The peels were first washed with distilled water to remove dirt and other foreign materials. The peels were then cut into small pieces and oven-dried at 60˚C for 48 hours to stop the destructive activity of microorganisms and any biological reactions. The dried cassava peels were grounded into powder using an electric miller (NIMA NM-8300) and screened to a particle size of less than 4 mm. The ground peels were then stored in airtight containers for further analysis. (**Figure 1**)

(a) (b)

Figure 1. Cassava peels. (a) Dried cassava peels; (b) grounded cassava peels.

2.2. Microorganism Cultivation

The filamentous fungus *Trichoderma atroviride* Eger23, isolated from decaying soft wood, was collected from the Food Microbiology lab, Egerton University, cultured on agar slants and incubated at 28˚C for 5 days. The freshly inoculated slants were stored at 4˚C for preservation.

2.3. Inoculum Preparation

The spore suspension of *Trichoderma atroviride* EGER23 was prepared by adding 10 ml of sterile distilled water to the slants. The surface of the fungal mycelia was gently scraped with a sterilized loop to homogenize the spore suspension. The sample was filtered, and the filtrate was counted as inoculum after measuring its concentration (spores/mL). The filter paper and distilled water were all sterilized in an autoclave.

2.4. Experimental Design

The optimization of Single Cell Protein production conditions was carried out using the Response Surface Methodology employing the Box-Behnken design (BBD) approach [18]. The variable parameters, moisture content (60.0%, 67.5%, and 75.0%), time intervals (4, 6 and 8 days), and pH levels (6.5, 7.5, and 8.5) were selected as independent variables while the single cell protein was the response. The purpose of variation was to identify optimal conditions that would maximize the yield of single-cell protein. A total of 45 experiments were carried out within the BBD framework, with each experiment comprising 15 runs and 3 replicates. A second-order polynomial equation model was used to fit the experimental data and establish the correlation between independent variables and the response. According to [19], the formula of the second-order polynomial model is as follows

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

where:

Y: Response of output variable (Single Cell Protein);

B_0: model intercept that represents the constant baseline value of the response variable;

X_1, X_2 and X_3: are the coded values of the independent factors under study: moisture content, time and pH);

β_1, β_2, and β_3: linear coefficient terms, which measure the effect of each factor (x_1, x_2 and x_3) on the response variable;

β_{11}, β_{22}, β_{33}: quadratic coefficient terms, which capture the curvature or non-linearity of the response variable for each factor;

β_{12}, β_{13} and β_{23}: interaction coefficient terms, which account for the combined effect of two factors on the response variable.

2.5. Fermentation Process

To prepare the substrate for Single Cell Protein production, a total of 30 grams of

dried cassava peels were used. Distilled water was mixed with the cassava peels to adjust the sample into different moisture contents of 60.00%, 67.50%, and 75.00%. Nitrogen supplementation via peptone at a rate of 10 g/kg of the sample was incorporated into the moistened mash. The mixture was aseptically transferred to 250 mL Erlenmeyer flasks and sterilized at 121˚C for 15 minutes, and then cooled to room temperature (25˚C). To create different experimental conditions, the initial substrate pH was adjusted to 6.5, 7.5, and 8.5 using a 0.1 M NaOH solution. Aseptic inoculation involved the addition of 3 mL of spore suspension to each flask, ensuring uniform mixing. The flasks were incubated at a constant temperature of 28˚C ± 2˚C and monitored periodically on days 4, 6, and 8. After the incubation period, the samples were oven-dried at 60˚C for 48 hours, and the dried samples were used for subsequent analysis of the Single Cell Protein. (**Figure 2**)

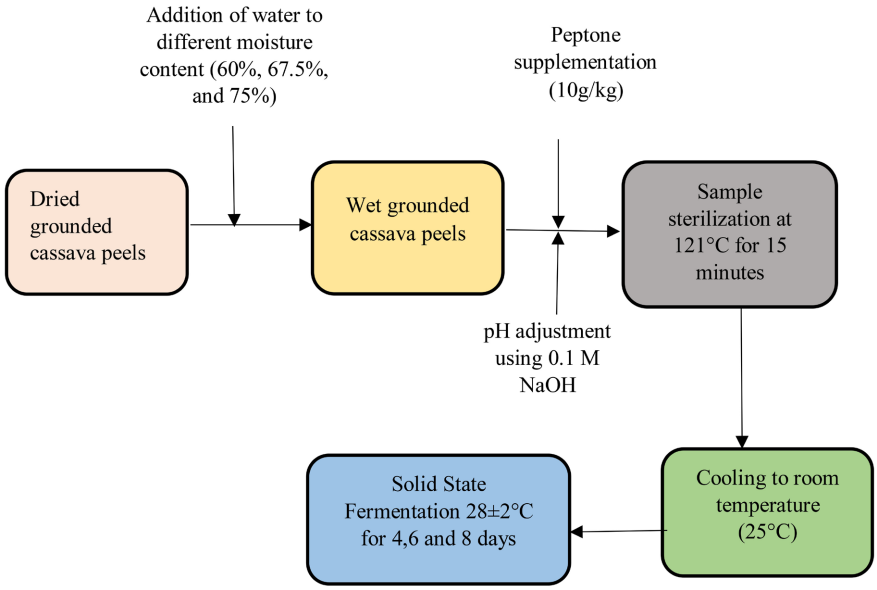

Figure 2. Flowchart for fermentation process.

2.6. Protein Determination

The crude protein content in the cassava peels was determined using the Kjeldahl method as per AOAC (2012), method number 920.87 [20]. About 1 g of the sample was precisely weighed into a test tube and digested with 10 mL of concentrated sulphuric acid (H_2SO_4), along with a selenium catalyst, which was added into the test tube. The mixture was then heated until a noticeable colour change occurred, typically turning blue. After digestion, steam distillation was conducted using 100 mL of 40% NaOH solution. During this process, as the digestion products were heated, ammonia was released and carried over into a solution containing boric acid, where it was captured. The resultant distillate containing the trapped ammonia was collected.

To determine the protein content, approximately 60 mL of the distillate was titrated against a 0.1 M hydrochloric acid (HCl) solution, with the aid of a meth-

ylene blue indicator. The titration was carried out until a noticeable colour change to orange occurred. The volume of HCl solution required to bring about this colour change was recorded. To calculate the protein content, a conversion factor of 6.25 was used. This calculation was based on the percentage of nitrogen content, which was determined from the volume of HCl solution utilized during the titration.

$$(\%)\mathrm{N} = N\mathrm{HCL} \times \frac{Corrected\ acid\ volume}{Weight\ of\ sample} \times \frac{14\ gM}{Mol} \times 100$$

$$\mathrm{Protein}(\%) = \mathrm{Nitrogen}(\%) \times 6.25$$

where Corrected acid volume = (volume of acid sample – volume of acid blank);

NHCl = normality of HCl in mol/1000 ml;

Corrected acid volume = (ml std. acid for sample) − (ml std. acid for blank);

14 g = atomic weight of Nitrogen;

6.25 = conversion factor equivalent to 0.16 g nitrogen per gram of protein.

2.7. Data Analysis

Minitab 16 was used to analyze the optimized fermentation conditions. To test the study hypothesis, a one-way analysis of variance (ANOVA) was conducted with a significance level of $p < 0.05$, corresponding to a confidence level of 95%.

3. Results and Discussion

3.1. Model Fitting and Statistical Analysis

A second order polynomial equation that includes linear, quadratic, and interactive terms defines the correlation between the independent variables (Moisture content, pH) and time and the response (SCP). The model equation is

$$\mathrm{Protein} = 8.7378 - 0.3208A - 0.0721B + 1.0530C - 0.6122A^2 + 0.2969B^2 - 0.9197C^2 - 0.1225A*B + 0.0392A*C - 0.2400B*C$$

where A, B and C are moisture content, pH and fermentation time respectively.

Analysis of variance (ANOVA) was used to check the statistical significance of the model developed. The coefficients of the second order polynomial equation regression (Coefficient) and p values are presented in **Table 1**. They indicate that the equations best describe the response correlation with their significant variables. The high coefficient of determination (R^2) value (98.91%) clearly verified that the developed model is accurate, which indicates an adequate correlation between the response and independent variables. The value of adjusted-R^2 (98.91%) for the Single Cell Protein was also high, showing a good relationship between the experimental and predicted values. In this study, the predicted R^2 (98.07%) value for the Single Cell Protein was in line with the adjusted R^2 (98.63%).

3.2. Influence of Process Variables on the Response

The main and interactive effects of independent variable, e.g., moisture content,

Table 1. Analysis of variance (ANOVA) for response surface quadratic model for the production of Single Cell Protein.

Source	Degree of freedom	Coefficient	Sum of Squares	F Value	p Value
Model	9	8.7378	44.5782	354.18	<0.001
MC	1	−0.3208	2.4698	176.60	<0.001
pH	1	−0.0721	0.1247	8.92	0.005
Time	1	1.0530	26.6093	1902.72	<0.001
MC*MC	1	−0.6122	4.1512	296.84	<0.001
pH*pH	1	0.2969	0.9764	69.82	<0.001
Time*Time	1	−0.9197	9.3690	669.94	<0.001
MC*pH	1	−0.1225	0.1801	12.88	<0.001
MC*Time	1	0.0392	0.0185	1.32	0.258
pH*Time	1	−0.2400	0.6912	49.42	<0.001
R^2			98.91%		
Adjusted R^2			98.63%		
Predicted R^2			98.07%		

pH and fermentation period were noted on the response variable (protein) as follows. In Table 1, the linear terms, moisture content and pH had significant negative coefficients ($r = -0.3208$, $p < 0.05$) and ($r = -0.0721$, $p < 0.05$), respectively, meaning that higher values of these factors led to decreased protein content, while incubation time was positively correlated ($r = 1.0$, $p < 0.05$) with protein content. This suggests that optimal levels lie within the tested range, beyond which further increase/decrease could have diminishing or even negative returns. Moisture content is a crucial factor in fermentation processes that influences the physical state of the substrate, availability of nutrients, oxygen-carbon dioxide exchange, and microbial activity. This relationship is largely governed by water activity, which determines the availability of water for cellular functions. Water activity (*aw*) is responsible to a large extent because it controls the amount of water available for cellular functions. Microorganisms have specific characteristic *aw* requirements, on which the majority of bacteria develop at values above 0.91, while fungi and yeasts are able to tolerate lower levels, generally between 0.7 and 0.9 [21]. High moisture levels during fermentation could have led to reduced protein content due to the decreased porosity of cassava peel particles, increased substrate stickiness, and agglomeration. These factors reduced gas volume and gaseous diffusion, which in turn impaired oxygen transfer. Limited oxygen transfer inhibited microbial growth and metabolism [22]. *Trichoderma atroviride* EGER23 growth and reproduction were inhibited below the optimal moisture level, which led to decreased protein production. This reduced the proteolytic breakdown of SCP proteins into peptides and amino acids, resulting in a lower overall protein content in the cassava peels. The high moisture supported microbial proliferation but also

had a tendency to dilute intracellular constituents, impairing metabolic efficiency and protein yield. Low moisture content caused higher water tension, altered microorganism cellular structure, reduced the solubility of nutrients, limited microorganisms' metabolism and growth, and lowered the degree of swelling [23]. The low moisture caused the microbial cells to experience dehydration, leading to structural instability, enzyme inactivation and metabolic inhibition.

Biochemically, water acts as a vehicle of enzymatic reactions, nutrient transport, and cellular hydration [24]. Hydrolytic enzymes like proteases, lipases, and amylases depend on adequate moisture levels to maintain their three-dimensional structure and catalytic activity. Similarly, glycolysis, oxidative phosphorylation, and the electron transport chain are highly water dependent [25]. A decrease in moisture interrupts these processes, resulting in lower ATP yield, substrate conversion inhibition, and oxidative stress. When there is not enough water, the effectiveness of metabolic enzymes is reduced, restricting microbial growth and reducing protein biosynthesis. Microorganisms have several mechanisms for coping with moisture fluctuations. Among them is the biosynthesis of compatible solutes, including trehalose, proline, and glycine betaine, which stabilize cell membranes and proteins during osmotic stress conditions [26]. Microbial cells also adjust the membrane lipid composition so that they become fluid-resistant to desiccation. Under conditions of excessive moisture, however, the activation of stress response proteins, including heat shock proteins and proteases, becomes essential to prevent protein unfolding and degradation. Such processes highlight the delicate balance that microbes have to sustain in order to survive and remain metabolically active in varying states of water.

The effect of water content on protein yield is most important in industrial microbial operations. Protein production is directly associated with the development of microbial biomass, which is highest at the best hydration conditions. Yet, as far as too high a water content is concerned, intracellular proteases could be too active, causing degradation of proteins and lowering the overall yield. In addition, water affects post-translational processes like glycosylation and phosphorylation, which are important for protein stability and function [27]. Humidity managed conditions are thus of greatest importance in biotechnology applications such as fermentation, pharmaceuticals, and food microbiology to maximize protein production with minimal proteolytic loss.

Changes in pH directly affect the concentration of hydrogen ions (protons) within the substrate. This can alter the structure of proteins [28], impacting their functionality. The negative correlation of pH on the protein content could be attributed to the higher pH values that altered the net charge of proteins, inducing repulsion among charged groups within the molecule. This caused the protein to unfold and lose its native structure [29], compromising its functionality and hindering protein production. The positive correlation of time to protein production revealed that protein production increased with the increase of the fermentation period. This is likely due to the high content of lignocellulosic compounds in the

cassava peel, which are converted to sugars for energy during fermentation [30], which helps the proper distribution of nutritional contents required for the growth of microorganisms at different fermentation times. This increased protein content reflects the enzymes themselves. As the fermentation progresses, the fungal biomass increases, contributing directly to the overall protein content of the product [31]. However, after reaching an optimal fermentation time, protein content starts to decrease. This is because beyond the optimal point, *Trichoderma atroviride* EGER23 may begin to break down its own proteins into peptides and amino acids for energy [30].

The quadratic terms: MC*MC, pH*pH, and Time*Time terms were also significant, highlighting the non-linear relationships between these factors and protein yield. This means that the protein content doesn't simply increase or decrease proportionally with changes in these factors. The interaction effects were also significant, indicating that the combined effects of these factors influence protein content more than their individual effects. This highlights the need to consider these combinations during optimization.

Figure 3 shows a 3D response surface plot from the developed mathematical model for protein content. The interaction of MC*time had a positive effect on SCP protein content. This means that an increase in the moisture content with an increase in fermentation time increases the SCP content. MC*pH and pH*Time interactions were significant and had a negative effect on SCP content, implying that an increase in pH with a decrease in moisture content and time reduced the SCP content.

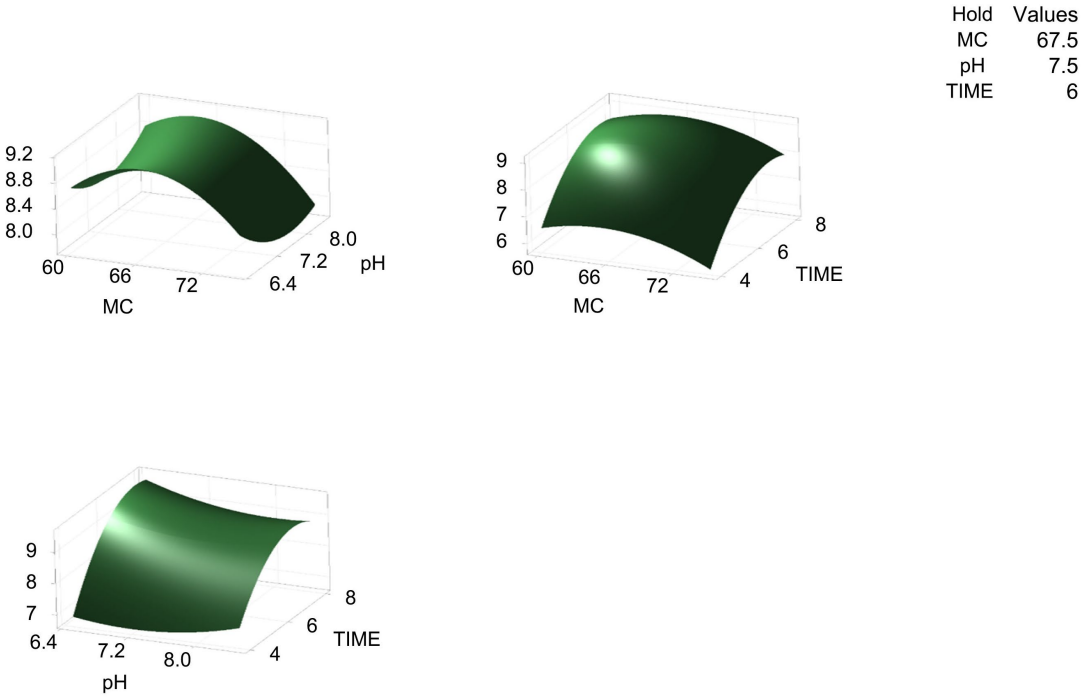

Figure 3. A 3D response surface plot showing the effect of process parameters (moisture content, pH and incubation time) on single cell protein.

3.3. Optimization of the Process Parameters

Optimization was performed using the developed models for protein yield. The independent variables, moisture content, pH and incubation time were set in the range of 60% - 75%, 6.5 - 8.5, 4 - 8 days, respectively. The RSM of the Minitab program was run for the optimum conditions and the solutions. The optimization study revealed that the best conditions for single-cell protein production were 66.52% moisture content, a pH of 6.50, and 7.39 days of incubation, as shown in Figure 4. This combination of conditions resulted in an optimal desirability of 91.66%, indicating that the model was able to predict the response (protein) significantly and that it is the most favorable combination of factors for maximizing single-cell protein production.

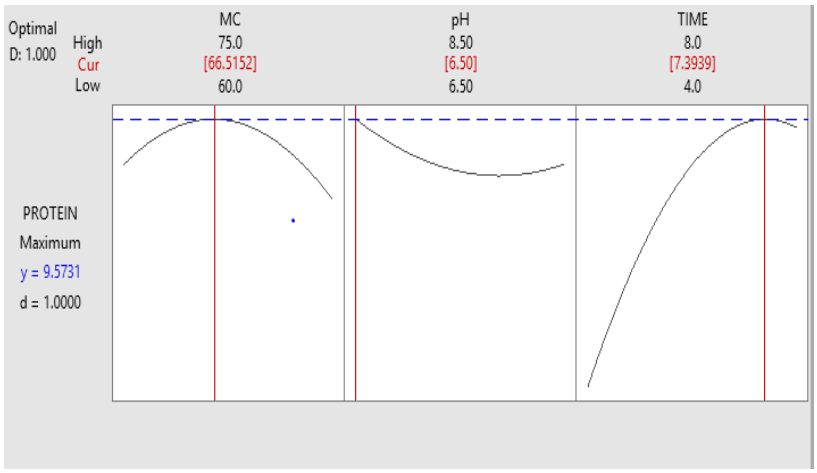

Figure 4. Optimum conditions for moisture content, pH and incubation time on Single Cell Protein.

While 7.39 days was identified as the optimal fermentation time under controlled laboratory conditions, during scale-up, several factors could cause deviations from this optimal duration. Temperature is essential for microbial growth, and deviations from the optimal temperature can significantly affect fermentation time. Elevated temperatures can accelerate fermentation but may also lead to increased production of by-products or even microbial death [32], while lower temperatures can slow down fermentation, potentially extending the entire fermentation process [33]. Inoculum concentration and size have an effect on microbial growth. If the inoculum concentration is too low, the microbial population could be insufficient for initiating the growth and production of the enzyme during the required fermentation time. This may result in inconsistent product quantity and quality due to an extended lag phase and slow fermentation. Conversely, a higher concentration would result in reduced enzyme activity due to a reduction in the nutrient availability caused by the competition for resources among the microorganisms [34]. In addition, high concentration can lead to generation of heat, hence limited oxygen within the available substrate. Substrates such as sugars act as the primary nutrient sources for microbial growth. Nutrients, such as vitamins or

minerals, provide essential building blocks for cell development. Insufficient substrates and nutrient concentrations may limit microbial growth and slow the fermentation process, while excess substrate levels may cause long lag times rate, osmotic stress or undesirable by-products and slow fermentation [35]. Therefore, it is necessary to monitor and control these concentrations to track substrate and nutrient consumption and analyse bioprocesses.

3.4. Detoxification Process of Cyanogenic Compounds in Cassava Peels

Detoxification of cassava peels for the production of single-cell protein (SCP) was carried out in multiple steps, *i.e.*, drying, milling and solid-state fermentation (SSF) with *Trichoderma atroviride* EGER23. All the steps were important for the cyanogenic compound reduction, with drying and milling helping in the initial breaking down of cyanides first, while fermentation with *Trichoderma atroviride* EGER23 ensured complete detoxification and protein enrichment. Drying and milling of the cassava peels before fermentation facilitated the liberation of the linamarase enzyme from the cell walls and the vacuole to interact with the cyanogenic glucosides (linamarin and lotaustralin) and convert them to hydrogen cyanide, and volatilize the remaining hydrogen cyanide [36]. The most significant detoxification step occurred during solid-state fermentation (SSF) with *T. atroviride* Linamarase enzyme produced during fermentation, which hydrolysed the linamarin and lotaustarlin into glucose and acetone cyanohydrins, to which were further decomposed by hydroxynitrile lyase enzyme into hydrogen cyanide [37] and acetone [38], as shown in **Figure 5**. The linamarase enzyme can be produced by microorganisms exogenously during fermentation and is capable of degrading the toxin compounds (linamarin and lotaustralin) in addition to the endogenous enzyme present in the cassava tissues [39].

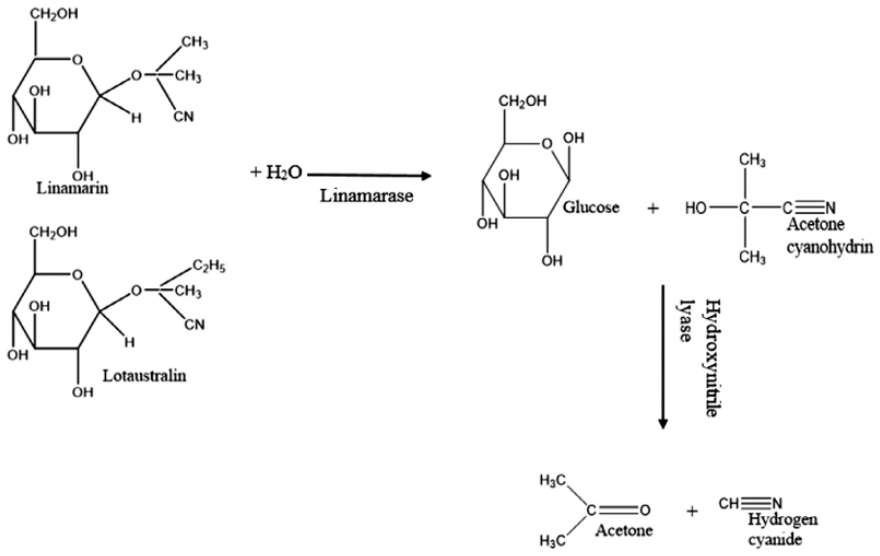

Figure 5. Linamarin or lotaustralin degradation through linamarase and hydroxynitrile lyase [39].

3.5. Safety of the Produced Single Cell Protein

To determine the safety of the final product, hydrogen cyanide (HCN) was determined using the Alkaline Titration method according to [40], with slight modifications. The cyanide content of the unfermented cassava peels was 46.20 ± 0.20 mg/kg, while that of the fermented ones was 7.6 ± 0.03 mg/kg. The cyanide levels were reduced by fermentation preparations, such as autoclaving, to below 10 mg HCN equivalent/kg dry weight. There was a significant ($p < 0.05$) decrease in the level of cyanide to below the acceptable safe limits of 10 mg HCN equivalent/kg dry weight after the fermentation process. The very low hydrogen cyanide is due to the initial drying of the cassava peels and the sterilization process of autoclaving before fermentation, as these processes are well known to reduce the available toxins [41]. This confirms that the fermentation processing of cassava peels greatly helps to reduce the cyanide level in the final product, thereby increasing palatability.

3.6. The Potential Economic Feasibility of Scaling up This Process for Commercial Use

Scaling up single-cell protein (SCP) production from *Trichoderma atroviride* using cassava peels as raw material involves techno-economic assessment based on the raw material cost, bioprocess yield, market demand, and sustainability. Cassava peels are a cheap, abundant agro-industrial waste material, but for commercial SCP production, investment in pre-processing, fermentation units, and downstream processing is needed. SSF is economical relative to submerged fermentation as it has reduced energy and water requirements, but substrate aeration, moisture control, and even nutrient uniformity problems have to be addressed [42]. A comparative cost-benefit analysis with traditional protein sources such as soybean meal and fishmeal is important to assess the economic viability of Single-Cell Protein (SCP). SCP's beneficial applications include the use as a supplement in animal feed and human nutrition [43], especially in areas with protein deficiencies, with regulatory approvals enabling wider uses. From a sustainability point of view, SCP production offers significant advantages by bio-converting agricultural residues, such as cassava peels, into high-value nutritional products. This process contributes to waste reduction, reducing air pollution from the emission of Greenhouse gases. However, challenges remain in scaling production, detoxifying cyanogenic glycosides, and meeting regulatory requirements. The economic viability of SCP production relies on the optimization of downstream processing, regulatory approval, and competitive market position. Techno-economic modelling, life cycle assessment (LCA), and pilot-scale testing are the future research requirements for Single Cell Protein development from laboratory-scale to industrially scaled production [44].

3.7. Key Challenges in Scaling up the Optimized Fermentation Conditions for Industrial Applications

It's quite challenging to achieve consistent moisture distribution across large vol-

umes for fermentation process. In large-scale processes, non-uniform mixing of raw materials leads to localized areas of higher or lower moisture content. These inconsistencies can negatively impact microbial growth and product quality, as uneven moisture affects both nutrient availability and microbial metabolism [45]. At high moisture content, solid substrate particles tend to agglomerate and thus reduce the surface-to-volume ratio of the solid material, leading to oxygen transfer limitations. As a result, a great decrease is observed in the production of microbial metabolites. On a larger scale, the heat and mass transfer conditions are quite different from small-scale laboratory experiments. Over-drying or the development of residual moisture can be caused by large-scale fermentation due to variations in rates of evaporation and moisture holding. Microbial activity during fermentation generates heat, which can accumulate within the solid substrate, leading to temperature increase that negatively affects microbial growth, product formation, and even denatures heat-sensitive products [46]. Ineffective mass transfer may lead to nutrient starvation, oxygen shortage, and accumulation of toxic end products, all of which may retard microbial growth and product formation [47] Fermentation operations, when up scaled from lab to industrial scale present nonlinear difficulties, especially to incubation time, because of differences in surface-area-to-volume and heat dissipation, which can cause asymmetric processing and longer incubation [48]. In laboratory-scale fermenters, the surface-area-to-volume ratio (SA/V) is relatively high, and heat removal and mixing are efficient. However, as the size increases, the surface area to volume ratio reduces, and it becomes increasingly difficult to dissipate the heat produced during fermentation. In big industrial reactors, the gradual loss of heat may lead to temperature gradients throughout the reactor and might influence microbial metabolism and growth [49].

3.8. Comparative Environmental Impacts of Single Cell Protein Production to Convectional Protein Sources

The life cycle assessment (LCA) of Single Cell Protein shows significant reductions in greenhouse gas (GHG) emissions compared to traditional protein sources. This is according to [50], who obtained as low as 0.81 - 1.0 kg CO_2-equivalent per kg of protein, after utilizing bacterial strains like *Cupriavidus necator.* In contrast, conventional protein sources show substantially higher emissions ranging from 10 - 30, 20 - 55, 45 - 640 CO_2 eq·kg^{-1}, 2.0 - 6.0 kg CO_2 eq·kg^{-1} for poultry, pork, beef and soy protein. [51] reported the lowest greenhouse gas emissions for yeast SCP production from crude pea starch, estimated at 0.61 kg CO_2 eq·kg^{-1} protein. Similarly, [52] obtained 1.6 kg CO_2 eq·kg^{-1} protein after considering a production system with hydrogen-oxidizing bacteria in which hydropower energy is used. These data suggest that SCP production from cassava peels can lead to over 90% reductions in GHG emissions compared to beef and up to 85% compared to soy protein.

In addition to their GHG savings potential, SCP manufacture has a very high potential for water saving. Data from the FAO and United Nations indicate that it takes 15,000 L of water to produce 1 kg of beef, which is seven times the amount

needed to produce 1 kg of rice or soybeans [53]. In addition, soy protein and fishmeal account for 1500 - 2500 and 5000 - 8000 litre per kilogram of protein, respectively [54]. Water consumption in SCP manufacture occurs primarily in terms of microbial culture media preparation and cooling plant facilities, while traditional protein sources consume large amounts of water in irrigation, drinking by animals, and the production of animal feed. Livestock production, specifically, causes enormous pressure on freshwater supplies through the concentrated water footprint of feed crop cultivation, animal upkeep, and meat processing. Additionally, SCP production reduces the potential for eutrophication by reducing the use of nitrogenous fertilizers, which stimulate algal blooms and aquatic ecosystem destruction. With freshwater supply continuing to decline everywhere in the world, SCP's low demand for water is a green solution for protein production.

Single-cell protein (SCP) production is very efficient and eco-friendly as an alternative to conventional protein sources, using only 0.1 - 0.3 m^2 of land per kilogram of protein, which is 90% - 99% less than conventional sources. In producing the same quantity of protein using beef, 326.21 m^2 would be needed, and with poultry at approximately 22.21 m^2 [55]. Conventional livestock farming occupies vast hectares of land for grazing animals and cultivating animal feeds that usually result in deforestation and destruction of vegetation and wildlife. SCP, however, is made in bioreactors under controlled conditions, entirely avoiding the necessity of further land cultivation. SCP utilizes agricultural waste such as cassava peels, reducing not only the waste but also holding precious agricultural land for the production of food crops. This renders it a suitable option for feeding an increasing population without further stressing natural ecosystems.

4. Conclusion

The study maximized process conditions for the production of single-cell protein by *Trichoderma atroviride* from cassava peels to obtain a maximum yield of 9.57% protein at 66.52% moisture content, pH 6.50, and 7.39 days of incubation. These results show the potential of cassava peels as a renewable substrate for protein production, allowing waste valorisation, minimizing environmental footprint, and upholding circular economy principles for improved food and feed security and providing a cost-effective alternative for protein supplementation in human and animal food.

Ethical Approval

The research work was approved by the Egerton University Research Ethics Committee and the National Commission for Science, Technology & Innovation (NACOSTI) of Kenya under Research License Number: NACOSTI/P/23/28821. There was no experimentation on human subjects in this study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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