Waste and Biomass Valorization
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ORIGINAL PAPER

Augmenting Protein-Rich Biomass from Desert Vegetable Waste: Effects of Various Fermentation Parameters

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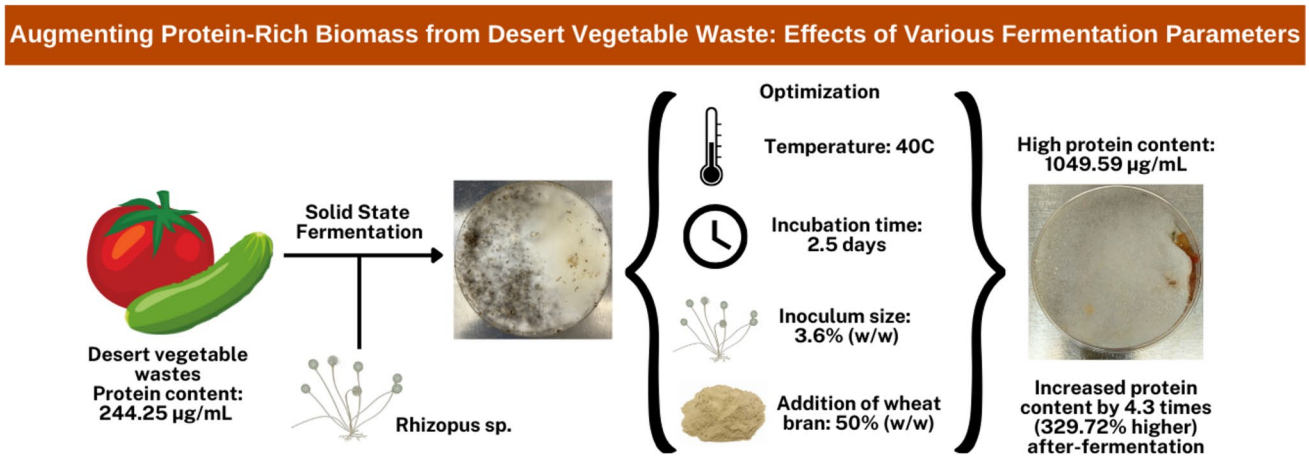
Abstract

The accumulation of vegetable waste from agriculture, especially in desert regions, poses significant environmental challenges and represents untapped potential for resource utilization. To address this, we investigated a sustainable solution for converting desert vegetable waste into valuable protein through solid-state fermentation (SSF). This study used *Rhizopus oligosporus* to transform vegetable waste into protein-rich biomass, optimizing critical parameters like incubation temperature, time, inoculum size, and wheat bran addition through a single-variable-at-a-time (SVAT) approach. The results revealed a maximum protein yield of 1049.59 µg/mL under optimal conditions: 40 °C for 2.5 days, 3.6% (w/w) inoculum size, and 50% (w/w) wheat bran. This represents a 4.3-fold increase in protein yield, or a 329.72% improvement compared to unfermented waste. Our findings underscore the potential of SSF as an effective method for valorizing desert agricultural waste, contributing to enhanced food security and reduced environmental impact in arid regions.

Graphical Abstract



Keywords Solid-state fermentation · Vegetable waste · *Rhizopus oligosporus* · Protein-rich biomass · Waste valorisation

Nomenclatures

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BSA	Bovine Serum Albumin
g/L	Grammes per Litre
GRAS	Generally Regarded as Safe
µg/mL	Microgrammes per millilitre

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NADH	Nicotinamide Adenine Dinucleotide (Reduced form)
SVAT	Single-variable-at-a-time
R. oligosporus	*Rhizopus oligosporus*
rpm	Revolutions per Minute
SSF	Solid-State Fermentation
TCA Cycle	Tricarboxylic Acid Cycle (also known as the Krebs Cycle or Citric Acid Cycle)
w/w	Weight/Weight Percentage
%	Percentage

Introduction

The world's population is expected to grow to 9.7 billion by 2050, resulting in a substantial rise in the demand for sustainable food sources, particularly those rich in protein [1]. This surge in population intensifies the pressure on existing food systems, which makes it crucial to explore alternative protein sources that can meet both nutritional needs and environmental sustainability. Conventional protein production methods, especially those relying on livestock farming, contribute significantly to environmental challenges. Livestock production contributes around 14.5% of global greenhouse gas emissions, playing a significant role in driving climate change [2]. Furthermore, livestock farming uses about 70% of agricultural land and consumes one-third of freshwater resources, further intensifying the competition for these scarce resources [3]. The increasing pressures of climate change and resource scarcity require urgent action to develop sustainable alternatives to conventional protein sources that can alleviate the strain on environmental resources and food systems. Numerous methods have been employed to increase the protein content in biomass, such as solid-state fermentation (SSF), Submerged Fermentation (SmF), enzyme hydrolysis, chemical treatment, and Ultrafiltration and fractionation. Each method has its advantages and limitations, as tabulated in Table 1.

Amid the growing challenges of food security and the environmental effects of conventional protein production, solid-state fermentation (SSF) has gained recognition as an effective method for converting agricultural waste and food industry waste into high-value products, such as proteins. SSF involves cultivating microorganisms on solid substrates in the absence or near-absence of free-flowing water, setting it apart from traditional submerged fermentation methods [4]. SSF mimics the natural environment for the growth of microorganisms, resulting in higher product yields, improved product characteristics, and reduced energy and water requirements compared to submerged fermentation [5, 6]. Table 2 tabulates examples of a few studies that have experimentally explored SSF with various microorganisms and substrates. Research shows that SSF not only increases the nutritional value of plant-based materials, but also improves their overall digestibility, making them favourable for human and animal consumption. SSF using *Rhizopus oligosporus* on maize cobs resulted in an improved nutritional value with 12.9 ± 0.3 mgg-1 soluble protein [7]. SSF improved digestibility by breaking down complex proteins into more accessible forms, generating bioactive peptides, and eliminating antinutritional factors [8]. Furthermore, SSF is characterised by lower energy and water requirements compared to submerged fermentation, making it a more sustainable choice in resource-constrained environments [9]. The use of solid substrates also allows better aeration and control over fermentation conditions, which can lead to higher metabolite production. This fermentation method is capable of efficiently using a wide variety of substrates, such as organic waste, food processing by-products, and agriculture residues, offering a versatile method for producing biobased products. SSF presents a promising path not only for addressing protein scarcity but also for fostering sustainable agricultural practices and contributing to the circular economy.

Despite significant advances in SSF and its application to a wide variety of agricultural by-products, limited attention has been paid to the valorisation of desert vegetable waste. In arid regions, agricultural production produces waste that is often underutilized. Current research focuses predominantly on common agricultural waste streams like fruit peels and cereal residues, with little exploration into the potential of desert-grown vegetables such as tomatoes and cucumbers. These crops, often produced in harsh, resource-scarce environments, present an untapped resource for sustainable protein production. The novelty of this study lies in its focus on bioconverting desert vegetable waste into protein-rich biomass using *Rhizopus oligosporus*, a process that addresses both food security and waste management challenges specific to arid regions. As tabulated in Table 1, diverse types of waste have been utilised in SSF to produce valuable products. However, vegetable waste, which is produced in millions of tons worldwide, remains underutilised. Consisting of peels, stems, leaves, and other by-products, it is often discarded in landfills or incinerated, contributing to environmental pollution [10]. These wastes are rich in nutrients, carbohydrates, proteins, and fibres, making them suitable substrates for fermentation [11]. Recent studies have highlighted the potential of vegetable waste as a substrate for the production of protein and bioactive compounds through microbial fermentation [12]. In addition, valorisation of these wastes not only addresses waste management issues but also promotes sustainable practices in the food production and processing industries. However, unlike earlier studies that focused on conventional agricultural waste, this research explores the underutilised potential of vegetable waste from desert agriculture, highlighting the unique

Table 1 Comparison of different methods for protein rich biomass production

References	Method	Description	Advantages	Limitations
[17]	SSF	SSF improved the nutritional and physicochemical properties of wheat bran and whole wheat bread	Enhanced nutritional content, physicochemical properties, and bioactive compounds content while reducing harmful mycotoxins in agricultural by-products	Requires specific environmental conditions with close monitoring of fermentation parameters and potential contamination risks
[18]	SSF	SSF released rich enzyme system, converting proteins in soybean into small peptides and amino acids	Enhanced nutritional content and bioactive compounds, with improved digestibility of substrates	Requires precise control of environmental conditions, potential contamination risks, and requires specific microbial strains
[19]	Submerged Fermentation	Single-cell protein production by *Pleurotus ostreatus* LGAM 1123 in submerged cultures and valorise fiber sludge	Higher biomass yield, better control over environmental conditions, and reduced contamination risks	Require expensive equipment and complex nutrient media
[20]	Submerged Fermentation	Protease production by submerged fermentation of *Bacillus* sp	Higher enzyme yields, better control over environmental conditions, and reduced contamination risks	Metabolic by-products may inhibit growth and enzyme activity, thus, requires optimization of parameters for maximized protein yield
[21]	Enzymatic Hydrolysis	Recovery and transformation of protein from biomass into protein hydrolysates by enzymatic catalysis	Less environmental impact, improved product quality by formation of low molecular weight peptides, and enhanced biological activities	High cost of enzymes, requires specific conditions for optimal enzyme activity, and challenges in enzyme stability and recyclability
[22]	Enzymatic Hydrolysis	Enzymatic protein hydrolysis of monkfish heads using Alcalase proteolytic enzyme	Enhanced protein recovery, improved functional properties, and the potential for bioactive compound extraction	Requires specific enzyme conditions causing high operational costs and requires optimization to achieve desired outcomes
[23]	Chemical Treatment	Chemical modifications of biochar for environmental remediation	Enhance nutrient availability and digestibility	Potential toxic byproducts and requires careful management of treatment conditions
[24]	Chemical Treatment	Optimization of nitrogen and carbon source addition on biomass and protein production by *Rhodopseudomonas*	Improved nutrient availability, increased biomass yield, and the potential for protein recovery	Potential for chemical toxicity to microbial populations, requires careful management of treatment and costs associated with chemical inputs
[25]	Algae cultivation	Integrated biorefinery approach that repurposes food waste hydrolysate for the cultivation of microalgae	High biomass productivity and significant protein accumulation	Variability in degradation rates of food waste, which can affect nutrient availability and overall biomass yield
[26]	Algae cultivation	Semi-continuous cultivation strategy to enhance protein production from indigenous freshwater microalgae	Efficient nutrient removal and high protein content in biomass	Requires high nutrient media and optimization to maintain consistent biomass production
[27]	Ultrafiltration and fractionation	Protein concentrates recovery from wheatgrass using ultrafiltration	Enhanced nitrogen recovery rates and the ability to produce high-quality protein concentrates	Potential losses of nitrogen in by-product streams and the process require specific conditions and equipment
[28]	Ultrafiltration and fractionation	Ultrafiltration of an aqueous rapeseed protein concentrate	Enhanced protein concentration, improved purity, and the ability to separate valuable components efficiently	Potential membrane fouling, which can decrease efficiency and increase operational costs, and the need for pre-treatment processes

Table 2 Various recent studies involving SSF

Microbes Used	Waste Valorised	Result/Product	Maximum product	References
Aspergillus ibericus and *Rhizopus oryzae*	Fruit peels	Nutritional value enrichment	200% increase in protein content	[29]
Corynebacterium glutamicum	Agricultural by-products (palm kernel cake, soybean cake, groundnut cake, and rice bran)	L-lysine	3.27 ± 0.02 (mg/gds)	[16]
Recombinant *Aspergillus sojae AsT3* and *Aspergillus niger A42*	Coffee waste, ground coffee, and coffee extract	beta (β)-mannanase, inulinases and oligosaccharides	71.17 and 564.07 U/mg of protein respectively	[30]
Bacillus cereus	Sardine fish waste	Crude protein	42.09%	[31]
Aspergillus protuberus and *Aspergillus unguis*	Castor husk, rice husk, groundnut fodder, sugarcane bagasse and saw dust	Amylase	1.614 U/g of substrate	[32]
Aspergillus niger	Cocoa pod husk	Crude protein	7.93%	[15]
Saccharomyces cerevisiae and *Candida tropicalis*	Cottonseed cake	Enhanced crude protein	Increased from 4 to 12%	[33]
Pleurotus ostreatus	Rapeseed meal	Crude protein	Increased protein content by 19.2%	[34]
Pleurotus ostreatus	Quinoa flour	Increased protein content	2.11%	[35]
Aspergillus niger	Soy husk and soy bran	Proteases and soluble proteins	677 U g^{-1} proteases and 4154 mg mL^{-1} soluble proteins	[36]
Rhizopus oligosporus	Maize cob	Enhanced nutritional values	12.9 ± 0.3 mgg^{-1} soluble protein	[7]

challenges and opportunities presented by arid environments where resource scarcity requires innovative waste utilisation strategies.

Rhizopus sp., a group of filamentous fungi, has been widely used in various fermentation processes due to its strong ability to produce valuable metabolites at high yields. *Rhizopus oligosporus* is recognised as a GRAS (Generally Regarded as Safe) organism and is commonly used in food and feed production because they do not produce any toxic compounds and lack pathogenic ability [13]. This fungus has been extensively used in the fermentation of soybean to produce tempeh, a traditional Indonesian fermented food, highlighting its efficiency in protein enrichment and substrate [14]. Despite its widespread application in traditional food fermentation, its potential for valorising vegetable waste through SSF for protein production remains underexplored. Additionally, *Rhizopus oligosporus* has shown potential in bioconversion processes, where it can enhance the nutritional profile of various agricultural by-products, making them more digestible and beneficial for both human and animal consumption. Furthermore, its ability to produce enzymes such as amylases and proteases plays a crucial role in breaking down complex carbohydrates and proteins, further improving the bioavailability of nutrients in these substrates. Compared to *Aspergillus niger* has been used to ferment substrates like cocoa pod husk, achieving a 7.93% increase in the crude protein content [15]. Similarly, *Corynebacterium glutamicum* was used to produce L-lysine from agricultural by-products, yielding up to 3.27 ± 0.02 mg/g [16]. However, *Rhizopus oligosporus* has shown greater efficiency in protein enrichment, especially when used on substrates with high carbohydrate content, making it an ideal choice for the SSF of vegetable waste.

In fermentation processes, optimizing critical parameters such as temperature, incubation time, inoculum size, and nutrient supplementation is essential to maximize product yield and efficiency. For SSF using *Rhizopus oligosporus*, temperature control significantly impacts fungal metabolism and protein synthesis. Studies have shown that an incubation temperature of 35 °C–40 °C facilitates optimal growth, enhancing protein production [37]. Similarly, the incubation time is a crucial parameter; while shorter periods may result in incomplete substrate colonization, extended incubation durations can lead to reduced yields due to nutrient depletion . Moreover, the inoculum size plays a vital role in ensuring adequate fungal biomass and nutrient supplementation such as wheat bran, is often required to enhance the carbohydrate content of substrates, providing a more robust medium for microbial activity and protein production. The optimization of these parameters is thus pivotal to achieving maximum efficiency and sustainability in protein-rich biomass production [5, 6, 38].

This research aims to optimise the conditions for solid-state fermentation of desert vegetable waste using R.

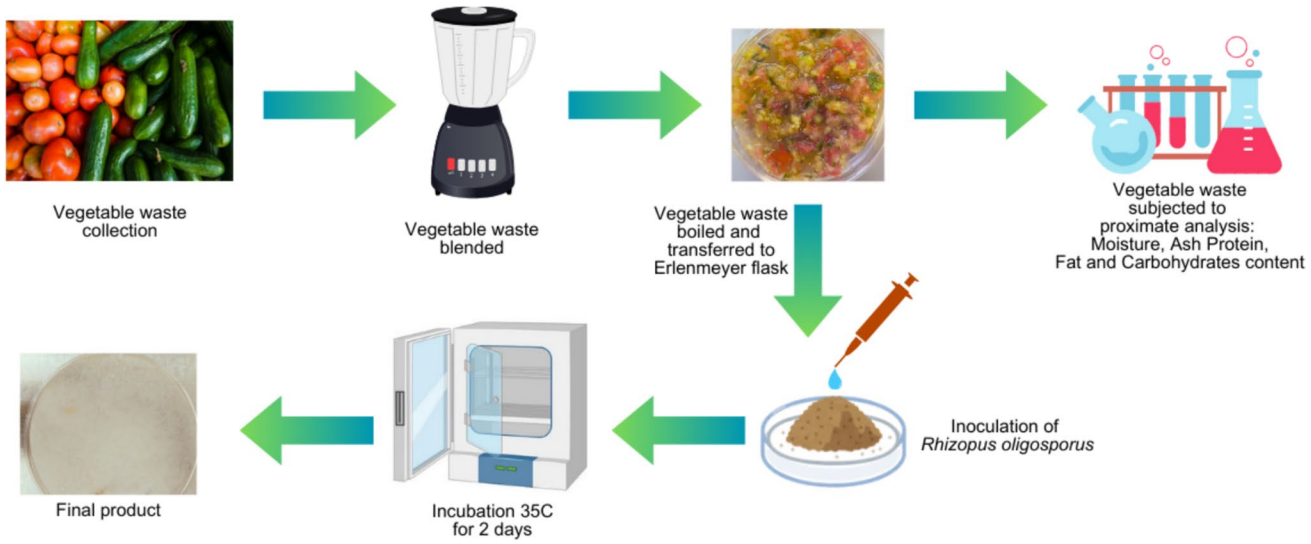

Fig. 1 SSF Process of vegetable waste for protein production

oligosporus to maximise protein production. Using a Single-variable-at-a-time (SVAT) approach, we systematically investigated the effects of key parameters, including incubation temperature, time, inoculum size, and addition of wheat bran, on protein yield. The findings of this study will not only contribute to sustainable waste management practices, but also enhance the nutritional profile of food products derived from these fermented substrates. Additionally, the results can pave the way for innovative applications in the food industry, promoting the use of underutilised resources while addressing food security. Furthermore, the insights gained could inspire further research on the scalability of this fermentation process, potentially leading to commercial viability and broader adoption in various regions facing similar waste management issues. In addition to optimising solid-state fermentation conditions, it is essential to consider the broader implications of using vegetable waste for protein production in desert regions. Integration of such innovative practices not only addresses food security but also contributes to environmental sustainability by reducing the dependency on conventional agricultural inputs that are scarce in arid environments. This study pioneers the valorization of desert vegetable waste, an underutilized resource in arid regions, through solid-state fermentation (SSF) with Rhizopus oligosporus to produce protein-rich biomass. Optimizing fermentation parameters via a single-variable-at-a-time approach, we achieved a 329.72% protein yield increase (1049.59 µg/mL), surpassing prior SSF studies on conventional agricultural waste. This sustainable process transforms waste into a valuable protein source, reducing environmental impact and promoting a circular economy in resource-scarce desert environments.

Methodology

Figure 1 illustrates the entire process, from the initial collection of vegetable waste through to the final production of fermented biomass, highlighting each critical step involved in the experiment. The experiment begins with vegetable waste collection (such as tomatoes and cucumbers). The collected waste is then blended to increase its surface area, facilitating efficient microbial fermentation. After blending, the waste is boiled and transferred to petri dish to ensure sterility, preventing contamination before fermentation. The boiled vegetable waste is subjected to proximate analysis, where the moisture, ash, protein, fat, and carbohydrate content are measured to determine the nutritional composition of the substrate. After analysis, the waste is inoculated with *Rhizopus oligosporus*, the fungal strain responsible for fermentation. The inoculated waste is then incubated at 35 °C for 2 days to allow fungal growth and protein production. Finally, the experiment yields the fermented product, a protein-rich biomass.

Sample Collection

Vegetable waste, specifically rejected tomatoes and cucumbers, was sourced from Agrico Farm in Al-Khor, Qatar. These vegetables were selected due to their high availability and favourable biochemical composition, particularly their rich carbohydrate and protein content, which are essential for effective fungal fermentation. The vegetable waste was then subjected to a proximate analysis.

Proximate Analysis

Proximate analysis is a method used to determine the basic composition of a sample such as moisture, ash, lipids, carbohydrates and protein content. Moisture content was determined by measuring the initial weight of the sample. Then, the sample dried in a drying oven (Heratherm OMS60, Thermo Scientific, Germany) until a constant weight was achieved. The difference in the initial weight and dried weight of the sample was calculated to determine its moisture content [39]. To determine the ash content, the sample was combusted in a muffle furnace (FD1545M, Thermo Scientific, Germany) at 550 ◦C for four hours. Then, the sample was allowed to cool in a desiccator and weighed [39]. To determine the protein content, the sample was homogenised and filtered. The protein concentration of the filtrate was quantified using the Pierce™ Modified Lowry Protein Assay Kit (Thermo Scientific, IL, USA) and measured using a spectrophotometer at 750 nm [40]. For fat content analysis, extraction was done by placing 4 g of dried vegetable waste in a container and mixed with 20 mL of methanol and 10 mL of chloroform. Then, 10 mL of chloroform was added and mixed thoroughly. The mixture was then filtered using filter paper and the filtrate were transferred into a 50 mL graduated cylinder for phase separation. Once the mixture was separated into layers, the volume of the chloroform phase was recorded, and the methanol phase was discarded. 5 mL of the chloroform extract was transferred into a pre-weighed aluminium dish and placed in an oven at 105 °C for 15 min to evaporate the solvent. Once dried, the dish was removed from the oven and allowed to cool in a desiccator to room temperature. Lastly, the dish was weighed to determine the fat content extracted from the vegetable waste sample [39]. The carbohydrate content was determined by calculating the weight difference [41].

Substrate Preparation

Upon collection, the vegetable wastes were thoroughly washed with distilled water to remove contaminants and residual pesticides. The cleaned vegetables were then blended using a kitchen blender to increase the surface area, facilitating efficient fungal colonization. To minimize the risk of contamination, the blended substrate was subjected to boiling at 100 °C for 10 min, effectively inactivating unwanted microbial activity and preventing contamination. The substrate was cooled to room temperature before inoculation.

Inoculum Preparation

The fungal inoculum, *Rhizopus oligosporus*, was obtained from Raprima, a reputable supplier specialising in tempeh (fermented soybean) cultures. This specific strain is recognized for its Generally Regarded as Safe (GRAS) status, ensuring safety in food applications. The required inoculum size was calculated based on the total weight of the substrate (vegetable waste), targeting 1%–5% (w/w) inoculum. The measured quantity of Raprima tempeh starter was suspended in 10 mL of sterile distilled water per gram of starter to create a uniform inoculum suspension. The suspension was thoroughly stirred to ensure even dispersal of fungal spores. The inoculum suspension was then added to the sterilized vegetable waste substrate and mixed thoroughly to ensure homogeneity.

Solid State Fermentation

The solid-state fermentation (SSF) process was illustrated in Fig. 1, it was conducted in sterile petri dish. Each petri dish was filled with 10 g of the prepared substrate and inoculated with 1% (w/w) of the tempeh inoculum. The choice of inoculum size was based on preliminary studies indicating that this concentration would optimize fungal growth while minimizing competition for nutrients. The petri dish was then incubated at a controlled temperature of 35 °C for 48 h, chosen for its compatibility with the optimal growth range for *R. oligosporus*. Fermentation conditions were varied in terms of incubation temperature, incubation time, inoculum size, and wheat bran supplementation (Table 3):

- Incubation Temperature: The temperature was varied between 30 °C and 40 °C, as previous studies by [42] indicated this range as optimal for the growth and metabolic activity of *R. oligosporus*.
- Inoculum Size: Different inoculum sizes (1%–5% w/w) were used to evaluate the impact of fungal inoculum concentration on protein production.
- Addition of Wheat Bran: Wheat bran was added to the substrate at varying levels (0%–50% w/w) to determine the optimal nutrient balance for enhanced fermentation, as wheat bran is known to improve protein synthesis by supplying necessary carbohydrates and preventing excess moisture that could lead to anaerobic conditions [17].
- Incubation Time: Fermentation times were varied between 1 and 3 days to identify the optimal incubation duration for maximum protein production [43].

Each petri dish was periodically agitated to promote aeration and ensure even distribution of fungal spores throughout

the substrate, thus enhancing fungal colonization and nutrient uptake.

Protein Extraction

Following fermentation, the biomass was harvested for protein extraction. The fermented substrate was mixed with Tris–HCl buffer (0.1 M, pH 8.0) to solubilize proteins. The mixture underwent homogenization using a laboratory blender to disrupt cellular structures and release proteins effectively. The homogenate was filtered through Whatman No. 1 filter paper, and the resultant filtrate was centrifuged at 8000 rpm for 15 min to separate the supernatant, which contained the crude protein [44].

Estimation of Protein Content

The protein concentration was quantified using the Pierce™ Modified Lowry Protein Assay Kit (Thermo Scientific, IL, USA), a widely accepted method for total protein estimation due to its reliability and accuracy. A calibration curve was generated using bovine serum albumin (BSA) as a reference standard, allowing for precise calculation of protein concentration in the samples. The absorbance of the reaction mixtures was measured at 750 nm using a spectrophotometer, ensuring consistent and accurate readings.

The data were analyzed using Design Expert software (Stat-Ease Inc., USA) to identify the optimal fermentation conditions for maximizing protein production. The predicted values for protein yield under varying experimental conditions were generated using statistical models built through the software, specifically through regression analysis. These predicted values were based on the experimental data obtained from the various fermentation parameters such as temperature, incubation time, inoculum size, and wheat bran addition. By comparing these predicted values with the actual experimental results, we were able to assess the accuracy and robustness of the models. The high correlation between the predicted and actual protein yields further supports the reliability of the modeling approach used to optimize the fermentation conditions.

Statistical Analysis

Statistical evaluations were conducted using ANOVA (Analysis of Variance) to assess the significance of various factors influencing protein production. The significance level was set at $p < 0.05$ to determine the relevance of the models. The ANOVA results provided insights into the individual contributions of each factor, while the lack of fit test was employed to assess the adequacy of the models in explaining the variability observed in the data.

The ANOVA output included metrics such as sum of squares, degrees of freedom, mean square, F value, and p-value, allowing for a comprehensive assessment of the experimental results. The p-values determined the statistical significance of each factor, while the F values indicated the ratio of the variance explained by the model to the variance unexplained, providing confidence in the reliability of the findings.

Result and Discussion

In this study, solid-state fermentation for protein production was optimized by focusing on key factors, including temperature, incubation time, inoculum size, and nutrient supplementation (wheat bran). These factors were selected based on their significant influence on fungal metabolism and protein yield. SVAT strategy was applied in the optimisation of temperature, incubation time, addition of wheat bran and inoculum size for protein production. This approach was chosen because it allows for a systematic evaluation of each variable's influence on the fermentation process while maintaining other conditions constant. By examining single variable at a time, the specific impact of each parameter on protein yield was examined, providing a clearer understanding of its contribution to the overall process. The protein content of the vegetable waste measured as 244.25 µg/mL and serves as a control to compare with the protein content post-fermentation.

Proximate Values of Vegetable Waste

The proximate analysis of the vegetable waste, which included rejected tomatoes and cucumbers, revealed its fundamental nutritional composition. The results, summarized in Table 4, show the moisture, ash, protein, fat, and carbohydrate content of the waste material.

The moisture content was measured as $93.23 \pm 0.14\%$, indicating that the vegetable waste was predominantly composed of water. High moisture levels are typical for fresh vegetable waste and significantly affect microbial activity and the efficiency of the fermentation process. High moisture content is beneficial for solid-state fermentation as it supports fungal growth, but excess water can also pose challenges for substrate aeration and fermentation control.

The ash content, representing the total mineral composition, was $11.09 \pm 0.04\%$, which is crucial for understanding the inorganic nutrient content of the waste. A moderate ash content suggests that the vegetable waste contains essential minerals that could potentially contribute to microbial metabolic activities during fermentation.

The protein content was relatively low at $0.024 \pm 0.002\%$, emphasizing the need for fermentation to enhance its

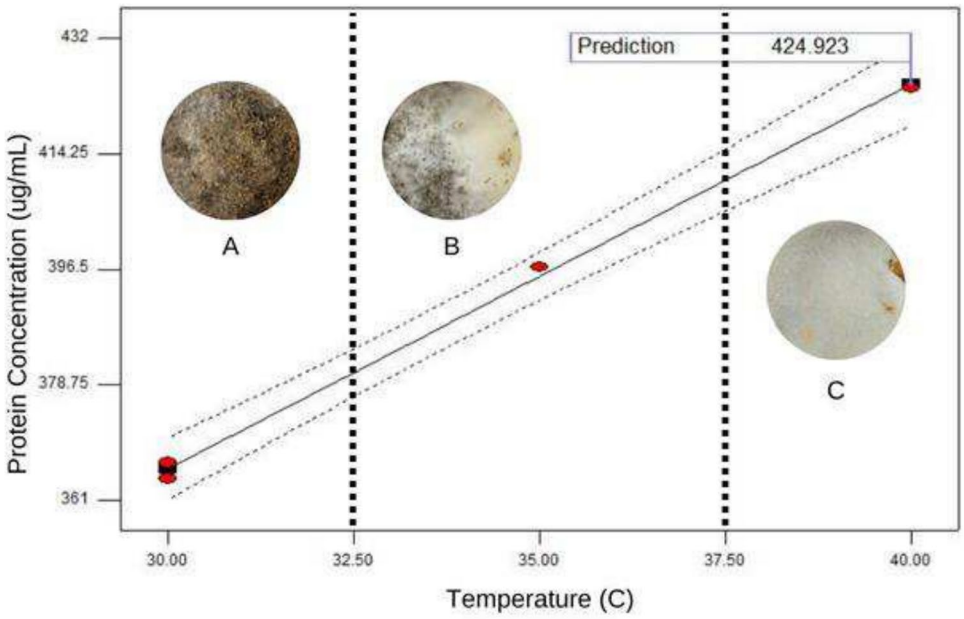

Fig. 2 Effect of temperature on protein production

Table 3 The range of variables for experiment key parameters

Key parameters	Ranges		
	− 1	0	1
Temperature	30 °C	35 °C	40 °C
Incubation Time	1 day	2 days	3 days
Inoculum Size	1% (w/w)	3% (w/w)	5% (w/w)
Addition of wheat bran	0%	25%	50%

Table 4 Proximate analysis of vegetable waste

Proximate Analysis	Value (%)
Moisture	93.23 ± 0.14
Ash	11.09 ± 0.04
Protein	0.024 ± 0.002
Fat	4.66 ± 0.06
Carbohydrate	84.2 ± 0.13

nutritional profile. This low baseline value provides a clear reference point for evaluating the protein enrichment achieved through the fermentation process.

The fat content was 4.66 ± 0.06%, which plays a minor role in the overall composition of the waste. Although fat is not the primary focus of this study, its presence may contribute to the energy available for microbial metabolism during fermentation.

Lastly, the carbohydrate content was 84.2 ± 0.13%, making it the dominant macronutrient in the vegetable waste. Carbohydrates serve as an essential energy source for *Rhizopus oligosporus*, the microorganism used in the fermentation process, facilitating its growth and metabolic activity.

These proximate values provide essential insights into the composition of the vegetable waste, serving as a baseline for assessing the fermentation process's effectiveness in increasing protein content. The high carbohydrate content and relatively low protein levels make this substrate ideal for solid-state fermentation, where fungal metabolism can be harnessed to convert available carbohydrates into protein-rich biomass.

Standard Curve

A standard curve was generated using Bovine Serum Albumin (BSA) as a reference protein to determine the protein concentration in the samples, following the Lowry method [40]. The equation derived from the standard curve, $y = 0.0004x + 0.1573$, where y is the absorbance and x is the protein concentration, allowed for precise interpolation of protein concentrations in the experimental samples. This calibration equation was used to quantify the protein content of the samples from the fermented vegetable waste.

Effect of Temperature on SSF and Protein Yield

In this section, we explore the effect of temperature on protein production by *Rhizopus oligosporus* during solid-state fermentation (SSF) using vegetable wastes as substrates. Fungal growth and protein formation can be influenced by temperature, with an optimal range for metabolic activity

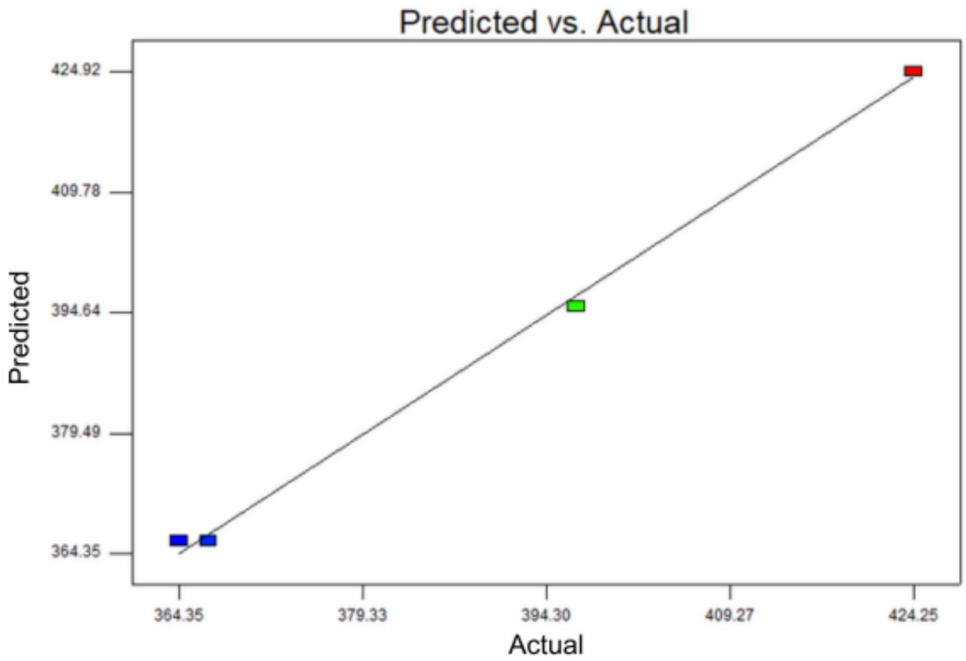

Fig. 3 Plot of actual against predicted values for the effect of temperature

and protein synthesis [45]. The temperature range investigated in this study was from 30 °C to 40 °C, as *Rhizopus oligosporus* typically thrives in temperatures between 28 °C and 37 °C for optimal growth and metabolic activity [46]. The data revealed a clear upward trend in protein yield with increasing temperature, with the maximum yield of 424.92 µg/mL observed at 40 °C, suggesting that higher temperatures enhance fungal metabolism by boosting enzymatic activity and nutrient absorption.

Further statistical analysis using ANOVA confirmed the significant effect of temperature on protein yield ($p < 0.05$), with no significant lack of fit ($p > 0.05$), indicating that the model sufficiently explains the observed data variability. The relationship between predicted and actual protein yields at various temperatures showed minimal deviation, confirming the robustness of the model and its accuracy in predicting protein production.

Figure 2 shows the results of protein concentration as a result of the change in temperatures. The red points represent experimental data, while the black line is the fitted linear model with 95% confidence intervals (dashed lines). Images A, B, and C illustrate the culture conditions at specific temperatures (30 °C, 35 °C, and 40 °C, respectively). The data revealed a clear upward trend in protein yield with increasing temperature, with the maximum yield of 424.92 µg/mL observed at 40 °C. This suggests that higher temperatures enhance fungal metabolism, likely by boosting enzymatic activity and nutrient absorption in *R. oligosporus*. These findings align with the results reported by A. K. Pandey & Negi [47], where *Rhizopus sp.* displayed increased cellulase and protease production within a similar temperature range during SSF. Notably, the increase in protein yield from 365.89 µg/mL at 30 °C to 424.92 µg/mL at 40 °C underlines the critical role of temperature optimization in maximizing fermentation efficiency. At 40 °C, the higher protein production likely stems from enhanced protease activity, facilitating efficient breakdown and uptake of substrate proteins into fungal biomass. In Image C, most of the waste was covered with white mycelium indicating that the fungus fully colonised the substrate. This finding also in line with previous work on protease production by *Bacillus cereus,* maximum enzyme activity observed at 40 °C [31]. The similarity in temperature ranges suggests that both microorganisms exhibit enhanced enzyme activity under moderate thermal conditions, making this temperature range ideal for protein synthesis in SSF processes.

ANOVA Analysis of Temperature's Effect on Protein Yield

The ANOVA performed using Design Expert revealed that the model assessing the effect of temperature on protein production is significant ($p < 0.05$), as indicated in Table 5. This implies that temperature variation has significant effects on the protein production by R. *oligosporus*. These results are consistent with the established understanding that temperature can modulate microbial growth and metabolic activity, thereby affecting protein production rates [48]. Furthermore, the lack of fit is non-significant ($p > 0.05$), indicating that the model sufficiently demonstrates the data variability. This suggests that the chosen model effectively illustrates the correlation between temperature and protein production. The increase in protein production up to 40 °C can be attributed

Table 5 ANOVA for effect of temperature of SSF

Parameters	Sum of squares	Degree of freedom	Mean square	F value	p-value	Significant
Model	2396.14	1	2396.14	892.57	0.0011	Yes
A-Temperature	2396.14	1	2396.14	892.57	0.0011	
Residual	5.37	2	2.68			
Lack of Fit	2.49	1	2.49	0.86	0.5232	No
Pure Error	2.88	1	2.88			
Cor Total	2401.51	3				

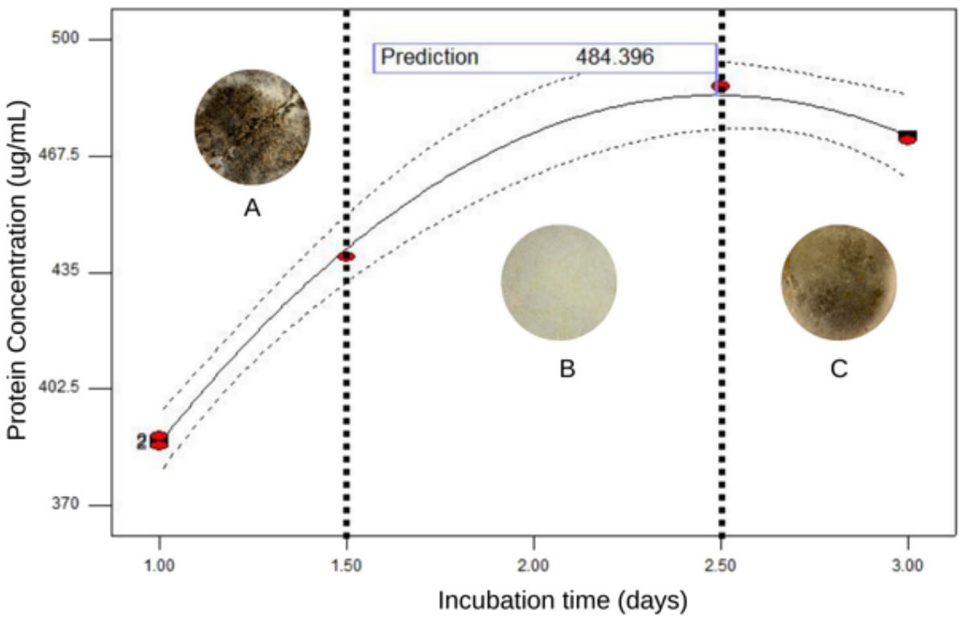

Fig. 4 The effect of incubation time on protein production

to improved enzymatic hydrolysis of the substrate and optimal fungal growth conditions. However, temperatures exceeding 40 °C may lead to reduced fungal activity, potentially due to protein denaturation or heat stress, as observed most fungal systems [48]. The "Predicted vs Actual" chart (Figure 3) demonstrates the relationship between predicted protein yields and actual yields at various temperature conditions. The data points in the chart, representing different temperature points, align closely with the 1:1 diagonal line, indicating that the predicted values are highly accurate when compared to the actual protein yields. The blue points at lower temperatures show minimal deviation from the line, suggesting that the model accurately captures the protein yield at these temperatures. As the temperature increases, represented by the green and red points, the predicted values continue to match the actual values closely, reinforcing the model's accuracy across a wide range of temperatures.

The relationship between incubation temperature and protein production was modelled using a linear regression equation. Equation 1 quantifies the relationship between temperature and protein yield. In the equation:

$$\text{Protein} = 188.78 + 5.9\text{T} \quad (1)$$

where T is the incubation temperature (°C).

The intercept value of 188.78 indicates the baseline protein concentration at 0 °C. The coefficient of 5.9 indicates that for every 1 °C increase in temperature, protein yield increases by 5.9 µg/mL. This positive correlation demonstrates the effect of R. *oligosporus* growth to temperature, with higher temperatures promoting increased metabolic activity and protein synthesis. The model can be used for varying temperature conditions to predict the protein production, providing a tool for optimizing the SSF process.

The rate of change of protein production with increasing temperature is given by:

$$\frac{\text{d(Protein)}}{\text{dT}} = 5.9 \quad (2)$$

Equation 2 indicates that for each 1°C increase in temperature, protein production rises by 5.9 µg/mL. This suggests that higher temperatures promote faster fungal growth

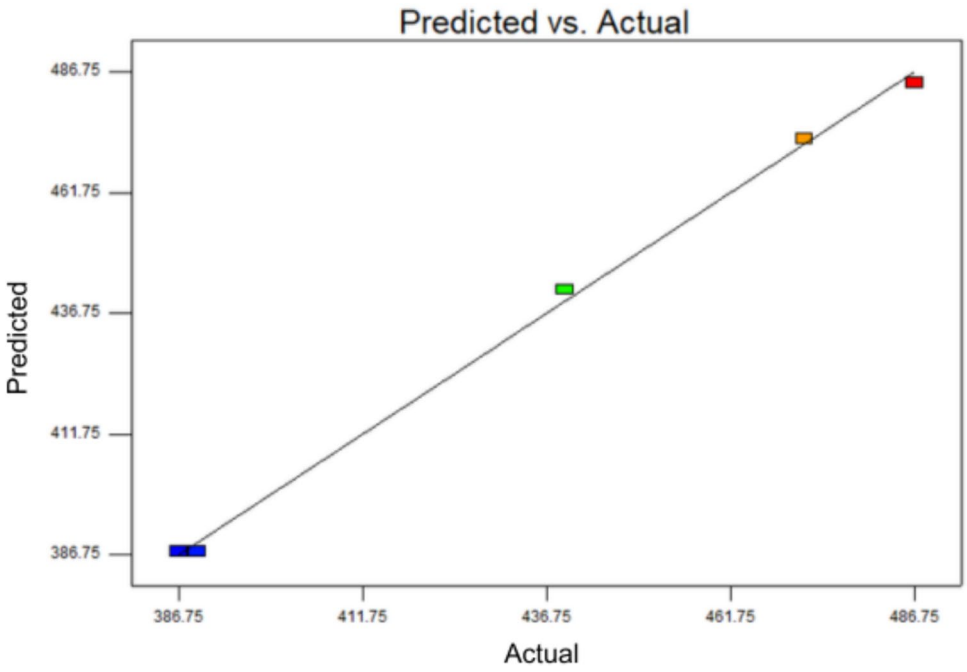

Fig. 5 Plot of actual against predicted values for the effect of incubation time

and metabolic activity, leading to enhanced protein production. However, temperatures beyond 40 °C could potentially inhibit fungal activity, due to enzyme denaturation or resource depletion, thus limiting the positive effect of temperature. Having established that 40 °C is the optimal temperature for protein production, the next critical factor analysed was the effect of incubation time on protein yield.

Effect of Incubation Time on SSF and Protein Yield

Incubation time plays a critical role in SSF, affecting fungal growth and protein production. The optimal duration for fermentation was found to be 2.5 days, where protein production peaked at 484.39 µg/mL. Shorter incubation times (1–1.5 days) resulted in partial substrate colonization, leading to lower protein yields, while longer incubation periods (beyond 2.5 days) caused a slight decline in protein concentration, likely due to nutrient depletion or the onset of sporulation.

ANOVA analysis revealed that the incubation time had a significant effect on protein production ($p < 0.05$), and the model provided a good fit to the data. The "Predicted vs Actual" chart showed a strong alignment between predicted and observed protein yields, further validating the model's predictive accuracy.

The range of incubation time investigated is from 1 to 3 days as generally, *Rhizopus oligosporus* optimum incubation time are 1 to 2 days [49]. Figure 4 shows the protein concentration at different incubation times (1, 1.5, 2, 2.5 and 3 days). The graph shows a quadratic relationship between incubation time (days) and protein concentration (µg/mL), indicating a complex kinetic behaviour. Images A, B, and C show the visual state of the culture at 1.5, 2.5, and 3.0 days, respectively.

The highest protein yield of 484.39 µg/mL was reached at 2.5 days of incubation. Protein concentration increased from 387.41 µg/mL at day 1.0 to 439.25 µg/mL by day 1.5, corresponding to partial colonization of the substrate by *R. oligosporus* (Image A). At 2.5 days, the protein concentration peaked at 484.396 µg/mL, with the substrate fully colonized by the fungus (Image B), marking this as the optimal incubation period for protein production. During this phase, the fermentation process is in an active metabolic state, converting substrates into protein effectively. Subsequently, the protein concentration slightly declines to 472.93µg/mL on 3.0 days could be linked to sporulation, as indicated by the blackening of the culture (Image C). This decline could be attributed to several factors, such as nutrient depletion and accumulation of inhibitory by-products, leading to decreased protein production. This observation is consistent with the typical growth curve of fungi in SSF systems, where rapid protein production is followed by a decline due to nutrient depletion and the accumulation of metabolic by-products [49].

ANOVA Analysis of Incubation Time's Effect on Protein Yield

Table 6 shows the ANOVA analysis for the Response Surface Quadratic Model performed using Design Expert 7.0. It showed that the model is significant ($p < 0.05$), meaning that the model explains a significant portion of the variability in the data. This implies that variation in incubation time

Table 6 ANOVA for an incubation time of SSF

Parameters	Sum of squares	Degree of freedom	Mean square	F value	p-value	Significant
Model	8451.05	2	4225.53	513.82	0.0019	Yes
A-Incubation time	5790.84	1	5790.84	704.17	0.0014	
A^2	1241.03	1	1241.03	150.91	0.0066	
Residual	16.45	2	8.22			
Lack of Fit	13.32	1	13.31	4.26	0.2871	No
Pure Error	3.13	1	3.13			
Cor Total	8467.50	4				

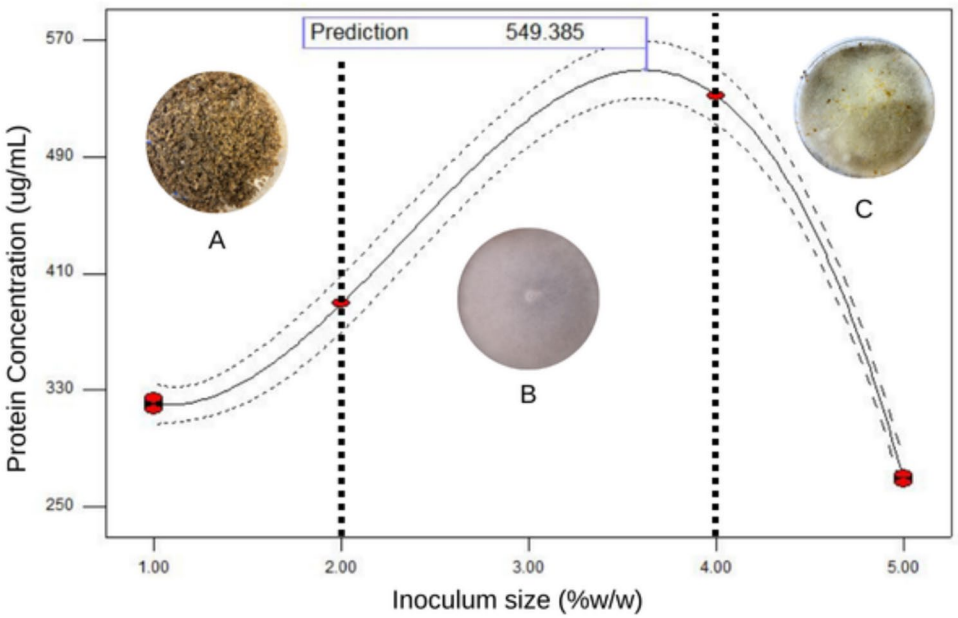

Fig. 6 The effect of inoculum size on protein production

has a significant effect on protein production. Meanwhile, the lack of fit is not significant ($p > 0.05$), suggesting that any remaining unexplained variability in the data is not statistically significant, implying that the model fits the data well. The "Predicted vs Actual" chart (Figure 5) illustrates the relationship between predicted protein yields and actual yields at varying incubation times. The close fit of predicted to actual values suggests that incubation time is a significant factor affecting protein yield in this study. Specifically, the blue data point, representing a shorter incubation period, is nearly identical to the predicted value, indicating high accuracy at this incubation time. As incubation times increase, represented by green, yellow, and red points, the predicted values continue to align closely with the actual measurements, showing that the model remains accurate across the full range of incubation times studied. Therefore, this model serves as a valuable tool for optimizing incubation conditions to maximize protein production.

A mathematical model was developed to quantify the relationship between incubation time and protein yield, providing a predictive tool for optimizing the fermentation process. The relationship between incubation time on protein yield was modelled using a quadratic equation:

$$\text{Protein} = 213.33 + 217.85t - 43.77t^2 \quad (3)$$

where t represents the incubation time (in days).

In Eq. 3, the positive linear term (217.85) reflects the initial increase in protein yield with time, while the negative quadratic term (-43.77) suggests a decline in protein production beyond the optimal point.

The derivative of the Eq. 3 provides the rate of change of protein yield over time:

$$\frac{d(\text{Protein})}{dt} = 217.85 - 2(43.77t) \quad (4)$$

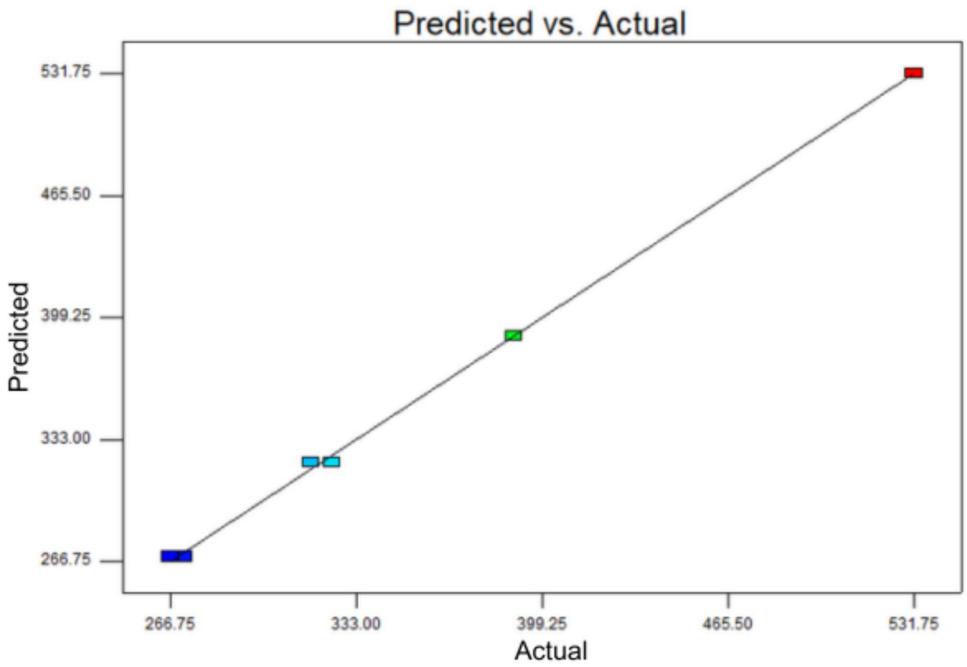

Fig. 7 Plot of actual against predicted values for the effect of inoculum size

Setting this derivative in Eq. 4 to zero allows us to find the optimal incubation time:

$$t = \frac{217.85}{2 \times 43.77} \approx 2.49 \text{ days} \quad (5)$$

The result calculated in Eq. 5 is consistent with experimental data, which showed maximum protein production at approximately 2.5 days. However, beyond this point, protein production declines due to nutrient depletion or sporulation. Thus, the model is valid for incubation times between 1 and 3 days but extending the process beyond this time may lead to a reduction in protein yield.

Effect of Inoculum Size on SSF and Protein Yield

Inoculum size is a crucial factor in SSF, influencing the extent of microbial colonization and protein production. In this study, inoculum sizes ranging from 1 to 5% (w/w) were tested. Protein yield increased with inoculum size, reaching a peak at 3.6% (w/w), where the maximum protein concentration of 549.39 µg/mL was achieved. Beyond this point, protein production declined, possibly due to substrate overloading or inhibition from excessive microbial biomass.

ANOVA results indicated that inoculum size significantly affected protein yield ($p < 0.05$), and the model demonstrated a high degree of accuracy in predicting protein production across the different inoculum sizes tested. The relationship between inoculum size and protein yield was modeled using a cubic polynomial regression, which confirmed that the optimal inoculum size for maximum protein production is 3.6% (w/w).

Figure 6 illustrates the optimisation of inoculum size for maximising protein concentration in solid-state fermentation. The graph shows a cubic relationship between inoculum size (%w/w) and protein concentration (µg/mL). Images A, B, and C show the visual state of the culture inoculated with 1%, 2% and 4% (w/w) of R. oligosporus, respectively. Initially, the protein concentration increases with inoculum size, reaching a peak at around 3.6% w/w before declining. When 2.0% w/w (image A) inoculum was used, the amount of fungus was not sufficient to fully utilise the substrate, resulting in lower protein production. As the inoculum size increases to 3.0% w/w (image B), the substrate utilisation improves, leading to higher protein concentration. The predicted maximum protein concentration of 549.385 µg/mL is achieved at 3.6% w/w, suggesting this is the optimum inoculum size for maximum protein production. Beyond this optimal point, at 4.0% w/w (image C), the protein produced starts to decline. This decrease may be attributed to substrate overloading or possible inhibition effects due to excessive microbial load, which can lead to competition for nutrients and the accumulation of inhibitory metabolites.

ANOVA Analysis of Inoculum Size's Effect on Protein Yield

Table 7 shows the ANOVA analysis for the Response Surface Quadratic Model performed using Design Expert 7.0. It showed that the model is significant ($p < 0.05$), meaning that the model explains a significant portion of the variability in the data. This implies that variation in inoculum size has a

Table 7 ANOVA for inoculum size of SSF

Parameters	Sum of squares	Degree of freedom	Mean square	F value	p-value	Significant
Model	49,355.21	3	16,451.74	809.93	0.0012	Yes
A-Inoculum size	11,000.59	1	11,000.59	541.57	0.0018	
A^2	36,575.52	1	36,575.52	1800.64	0.0006	
A^3	12,562.67	1	12,562.67	618.47	0.0016	
Pure Error	40.63	2	20.31			
Cor Total	49,395.83	5				

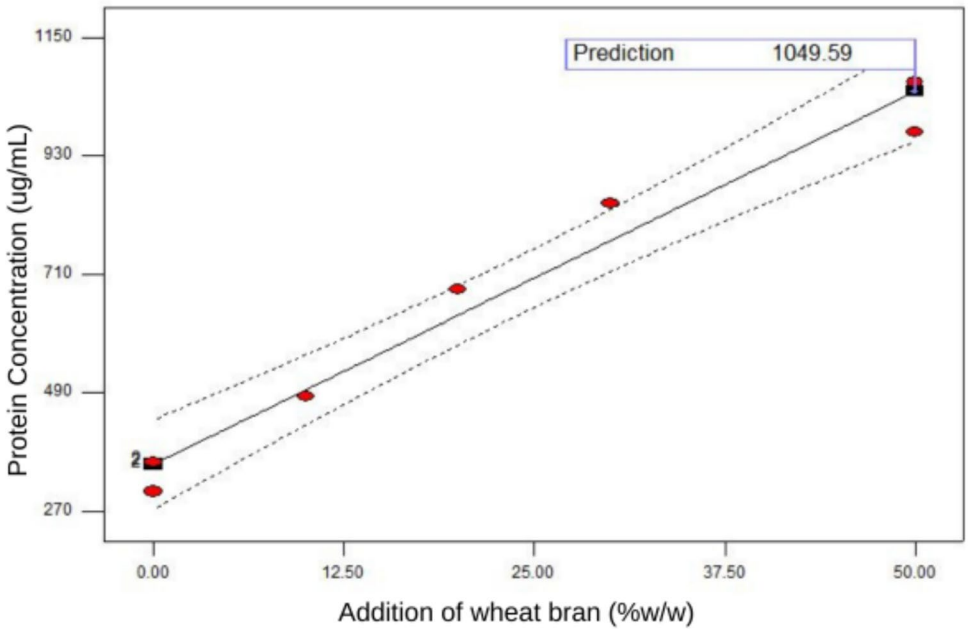

Fig. 8 Effect of wheat bran addition on protein concentration

significant effect on protein production. The "Predicted vs Actual" chart (Fig. 7) illustrates the relationship between predicted and actual protein yields under varying inoculum sizes. The data points are located along the 1:1 diagonal line, suggesting that actual protein yields across the range of inoculum sizes tested matched the predicted values accurately. The lowest inoculum size represented by the blue data point, the predicted yield aligns perfectly with the actual yield, indicating a high level of accuracy in the model's predictions. As the inoculum size increases, represented by the cyan, green, and red points, the predicted values continue to match the actual values closely. The red data point at the highest inoculum size also lies almost perfectly on the line, further demonstrating the model's ability to accurately predict protein yield at different inoculum sizes.

The relationship between inoculum size and protein concentration was modelled using a cubic polynomial regression, which provided a good fit for the experimental data and provided a tool to predict the optimal temperature for maximising protein production during SSF. Equation 6 represent the mathematical model:

$$\text{Protein} = 477.58 - 326.04A + 196.98A^2 - 28.02A^3 \quad (6)$$

where *A* represents the inoculum size (%w/w).

Equation 6 illustrates a non-linear relationship between inoculum concentration and protein production. The local maximum at A = 3.6% (w/w) inoculum size suggests an optimal balance between fungal colonization and nutrient availability.

Furthermore, the rate in change of protein concentration with respect to inoculum size was further deduced in Eq. 7 to understand the kinetics of the protein production process.

$$\frac{dA}{dP} = -\ 326.04 + 393.96A - 84.06A^2 \quad (7)$$

Setting the derivative of Eq. 7 to zero allowed us to identify critical points where the rate of change is zero, indicating potential maximum or minimum in protein concentration. Solving the equation:

$$0 = -\ 326.04 + 393.96A - 84.06A^2 \quad (8)$$

Table 8 ANOVA for wheat bran addition of SSF

Parameters	Sum of squares	Degree of freedom	Mean square	F value	p-value	Significant
Model	5.253×10^5	1	5.253×10^5	167.19	< 0.0001	Yes
A-Wheat Bran	5.253×10^5	1	5.253×10^5	167.19	< 0.0001	
Residual	15,708.85	5	3141.77			
Lack of fit	9918.23	3	3306.08		0.4893	No
Pure Error	5790.63	2	2895.31			
Cor Total	5.41×10^5	6				

we found the critical points at $A = 1.07$ and $A = 3.60$.

To determine the nature of these critical points, we evaluated the second derivative:

$$\left(\frac{d^2AP}{dA^2}\right) = 393.96 - 2 \cdot 84.06A \tag{9}$$

At $A = 1.07$

$$\left(\frac{d^2AP}{dA^2}\right)_{A\approx1.07} = 393.96 - 2 \cdot 84.06 \cdot 1.07 = 214.68 \tag{10}$$

Since the second derivative is a positive value, A = 1.07A is a local minimum.

At $A = 3.60$

$$\left(\frac{d^2AP}{dA^2}\right)_{A\approx3.60} = 393.96 - 2 \cdot 84.06 \cdot 3.60 = -210.87 \tag{11}$$

Since the second derivative is a negative value, $A = 3.60$ is a local maximum.

These results indicate that the protein concentration reaches its maximum at an inoculum size of approximately $A = 3.60$. This critical point is crucial for optimising the inoculum size to achieve the highest protein yield in our experimental setup. On the contrary, the local minimum at $A = 1.07$ suggests an inoculum size that should be avoided to prevent reduced protein production. This kinetic study underscores the importance of precise control over inoculum size in optimising solid-state fermentation. By selecting an inoculum size near the identified maximum, we can enhance protein production efficiency, potentially leading to cost savings and improved scalability of the process. Future work should consider further empirical validation of these findings and explore the underlying biological mechanisms that contribute to the observed kinetic behaviour.

Effect of Wheat Bran Addition

Wheat bran addition significantly impacted protein production during SSF. The highest protein yield of 1049.59 µg/mL was obtained when 50% (w/w) wheat bran was added to the substrate, as illustrated in Figure 8. Wheat bran acts as a nutrient-rich supplement, providing essential carbohydrates and improving the overall fermentation environment by regulating moisture content and preventing anaerobic conditions [50, 51].

The statistical analysis confirmed the significant impact of wheat bran addition on protein yield ($p < 0.05$), with a strong correlation between the predicted and actual protein yields at varying levels of wheat bran. The regression model showed a direct linear relationship between wheat bran concentration and protein yield, providing valuable insights for optimizing the fermentation process.

Wheat bran, as a nutrient-rich additive, provides essential carbohydrates and proteins that promote fungal growth and metabolic activity [51]. Additionally, wheat bran acts as an adsorbent, helping to reduce excess moisture in the substrate, which can otherwise inhibit fungal growth by creating anaerobic conditions. The linear increase in protein yield with wheat bran addition suggests that the substrate's nutritional content was the limiting factor for protein production in the absence of supplementation. Figueiredo et al. [50] demonstrated similar findings, where wheat bran enhanced fungal enzyme production and protein synthesis in SSF systems. The ability of wheat bran to maintain an optimal moisture level is crucial for SSF, where free-flowing water is absent, and solid substrates can become overly wet or dry, affecting fungal colonization [4]. The addition of wheat bran provided an abundant source of carbohydrates, may resulting in activation of glycolysis and the tricarboxylic acid (TCA) cycle in *Rhizopus sp*. The glucose present in wheat bran is broken down into pyruvate, which subsequently enters the tricarboxylic acid (TCA) cycle, leading to the production of ATP and reducing equivalents (NADH and $FADH_2$). This energy supply is crucial for cellular growth and protein synthesis, thus the significant increase in protein yield. The maximum protein yield observed in this study represents a 329.72% increase from the unfermented vegetable waste, exceeding the 200% protein increase achieved in SSF of fruit peels by *Aspergillus ibericus* and *Rhizopus oryzae* [29]. This indicates that *Rhizopus oligosporus* is highly effective in converting desert vegetable waste into protein-rich biomass.

ANOVA Analysis of Wheat Bran Addition's Effect on Protein Yield

The ANOVA performed using Design Expert revealed that the model assessing the effect of the addition of wheat bran on protein production is significant ($p < 0.05$), as indicated in Table 8. This implies that wheat bran content variation has significant effects on the protein production by R. *oligosporus*. Furthermore, the lack of fit is non-significant ($p > 0.05$), indicating that the model sufficiently demonstrates the data variability. This suggests that the chosen model effectively illustrates the correlation between wheat bran addition and protein production. The "Predicted vs Actual" chart (Fig. 9) illustrates the relationship between predicted and actual protein yields under varying levels of wheat bran addition. The data points in the chart lie closely along the 1:1 diagonal line, indicating a high level of accuracy between the predicted and actual protein yields at various wheat bran additions. The blue data point at the lowest wheat bran addition level aligns well with the line, suggesting that the model accurately captures the protein yield at this level. As the amount of wheat bran added increases, represented by cyan, green, yellow, and red points, the predicted values continue to align closely with the actual values. The red data point at the highest wheat bran addition also follows the 1:1 line, reinforcing the model's ability to predict protein yields accurately across different wheat bran concentrations.

The relationship between the addition of wheat bran and protein concentration was analysed through regression analysis, yielding a linear model that provides a clear understanding of the impact of wheat bran on protein production. The final equation derived from the regression analysis in terms of actual factors is:

$$\text{Protein} = 357.58 + 13.84W \tag{12}$$

where *W* represents wheat bran addition (%w/w).

Equation 12 indicates a direct linear relationship between wheat bran addition (%w/w) and protein concentration (µg/mL). Specifically, the intercept (357.58 µg/mL) represents the baseline protein concentration when no wheat bran is added. The slope (13.84 µg/mL/%w/w) suggests that for every 1% increase in wheat bran addition, the protein concentration increases by approximately 13.84 µg/mL.

Furthermore, the rate of change of protein concentration with respect to wheat bran addition was deduced from a linear model to further understand the kinetics of the protein production process.

The rate of change of protein content with respect to wheat bran addition can be expressed as:

$$\frac{d(\text{Protein})}{dW} = 13.84 \tag{13}$$

Equation 13 indicates that for every unit increase in wheat bran addition, the protein content increases by 13.84 units.

To understand the temporal dynamics of protein content, we can model the rate of change over time. Assuming a constant rate of wheat bran addition over time, the rate of protein increase R is given by:

$$R = 13.84 \times \frac{dW}{dT} \tag{14}$$

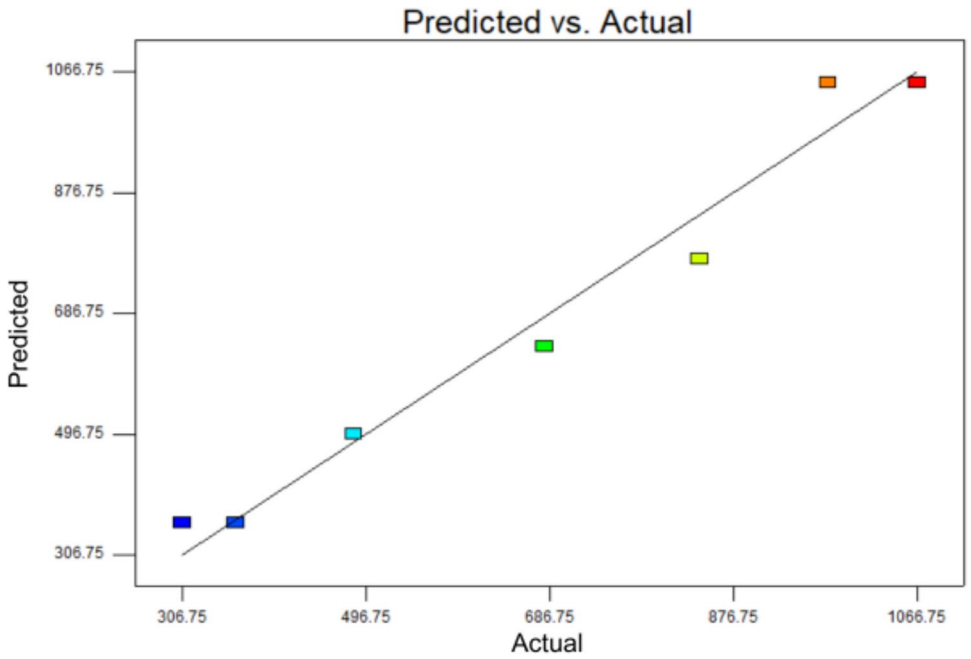

Fig. 9 Plot of actual against predicted values for the effect of wheat bran addition

Assuming a steady addition rate of wheat bran, we can simplify this to:

$$\frac{d(Protein)}{dT} = 13.84 \quad (15)$$

Equation 15 suggests that the protein content increases at a constant rate over time when the addition of wheat bran is constant.

The derived kinetic model is significant for practical applications where precise control of protein content is required. By adjusting the amount of wheat bran added, one can predictably influence the protein levels. This has potential applications in the formulation of dietary products and optimisation of nutritional content in food processing.

The models developed in this study provide a powerful tool for optimizing SSF conditions. For instance, the linear temperature model can be used in larger-scale fermentation processes to predict protein yield based on temperature control. The quadratic model for incubation time highlights the importance of timing in maximizing yield, which could help industrial processes achieve high efficiency by preventing over-incubation. These models could also be incorporated into process simulation software to predict outcomes in larger bioreactors, enabling better planning and cost control in industrial protein production.

The findings of this study hold significant promise for industrial-scale protein production from agricultural waste, especially in regions where food security is a growing concern. The optimized fermentation conditions can be applied in large-scale setups to maximize protein outputs, contributing to both economic and environmental sustainability. Future studies could expand the tested range of conditions or use more advanced modelling techniques, such as response surface methodology (RSM), to explore interactions between multiple parameters simultaneously.

Evaluation of Findings in Light of Past Research

The outcomes of this study demonstrate the efficacy of *Rhizopus oligosporus* in significantly enhancing protein production through solid-state fermentation (SSF) of desert vegetable waste. The maximum protein yield achieved was 1049.59 µg/mL, representing a 329.72% increase in protein content compared to unfermented vegetable waste. This remarkable enhancement aligns with and surpasses findings from various studies, further positioning *R. oligosporus* as a key player in protein enrichment via SSF.

Araújo et al. [29] reported a 200% increase in protein content through SSF using *Aspergillus ibericus* and *Rhizopus oryzae* on fruit peels. The higher protein increase observed in this study may be attributed to the high carbohydrate content of the vegetable waste substrate, which provided an abundant energy source for *R. oligosporus*. Similar results were demonstrated by Zhang et al. [18], SSF using *R. oligosporus* enhanced the nutritional properties of soybean, improving its protein content and bioavailability. The increased protein content observed in both studies confirms the potential of *R. oligosporus* to effectively bioconvert various substrates into protein-rich biomass.

Moreover, Ndego et al. [7] utilized *R. oligosporus* for SSF of maize cob and observed a protein yield of 12.9 ± 0.3 mg/g, primarily driven by the fungus's ability to break down complex carbohydrates into simpler components. However, the present study demonstrated a notably higher protein yield, likely due to the carbohydrate-rich nature of vegetable waste and the optimization of fermentation parameters, such as temperature, inoculum size, and wheat bran addition. The addition of wheat bran in this study further facilitated the fermentation process, as it provided essential carbohydrates for fungal metabolism. This finding is consistent with study conducted by Figueiredo et al. [50], with result that wheat bran supplementation significantly enhances protein production during SSF.

In terms of temperature optimization, the present study aligns with the findings of Lima et al. [37], who reported that temperatures between 35 °C and 40 °C provide optimal conditions for *R. oligosporus* in SSF, leading to maximum protein production. Our study confirmed that 40 °C is the ideal temperature for protein synthesis by *R. oligosporus*, promoting increased metabolic activity and extracellular enzyme production. This is also in agreement with A. K. Pandey & Negi [47], who noted that *Rhizopus* species exhibit enhanced cellulase and protease production at similar temperature ranges during SSF.

When compared to submerged fermentation (SmF), solid-state fermentation offers several advantages. Bakratsas et al. [19] highlighted the limitations of SmF, such as the need for expensive nutrient media and highly controlled environments. Although SmF with *Pleurotus ostreatus* achieved high biomass yields, SSF, as demonstrated in our study, provided higher protein enrichment with simpler substrates and equipment. This confirms the viability of SSF as a more cost-effective and sustainable method for protein production, especially when processing agricultural by-products or food waste.

In a broader context, the results from this study further validate the potential of SSF to address global food security challenges by converting agricultural waste into valuable protein sources. Research by J. Wang et al. [9] similarly highlighted the ability of SSF to utilize agricultural by-products for protein production, noting the sustainability benefits and reduced environmental footprint of this approach. The current study contributes to this growing body of literature by focusing on desert vegetable waste, demonstrating the

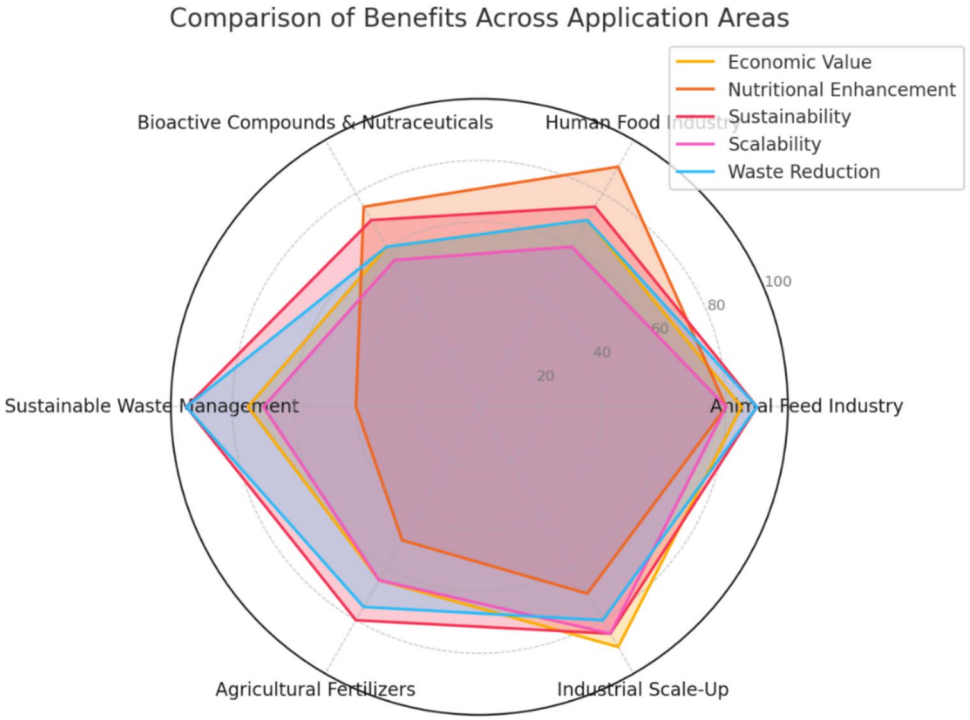

Fig. 10 The radar chart on comparison of the benefits across different application areas for the protein-rich biomass generated through SSF

feasibility of SSF under resource-scarce conditions typical of arid regions.

Overall, the findings of this study align well with previous research, while also providing new insights into the potential of *R. oligosporus* for protein enrichment of vegetable waste. The higher protein yields observed in this study, compared to earlier works, highlight the importance of optimizing key fermentation parameters, such as temperature, inoculum size, and nutrient supplementation. Future research should focus on scaling up this process and exploring its application to a wider range of waste materials, particularly in regions facing food security challenges.

Potential Application of Protein-Rich Biomass

The protein-rich biomass produced through solid-state fermentation (SSF) using *Rhizopus oligosporus* exhibits considerable potential across various application areas, each offering unique benefits and opportunities for commercial and sustainable use. Figure 10 visually captures the impact scores across these application areas, highlighting the high potential in the animal feed industry, industrial scale-up, and sustainable waste management sectors. This demonstrates the versatility and significant value that the protein-rich biomass from SSF offers across diverse industries, reinforcing the study's relevance to both commercial applications and sustainable development goals.

One of the most promising applications is in the animal feed industry, where the high protein yield and improved digestibility make the biomass a cost-effective and sustainable alternative to traditional feed ingredients like soybean meal. The ability to provide an affordable, high-quality protein source is particularly advantageous, especially in regions facing high feed costs or shortages, making it a suitable supplement for livestock, poultry, and aquaculture diets.

In the human food industry, the enhanced protein content and bioavailability of the SSF-derived biomass make it an excellent candidate for incorporation into plant-based protein products, such as protein bars, snacks, and meat alternatives. This aligns with the growing consumer demand for sustainable, plant-based protein sources. Additionally, the protein-rich biomass can serve as a functional ingredient to fortify cereals, baked goods, or pasta, thereby contributing to the development of healthier food options.

Another significant application is in the nutraceutical and bioactive compound sector, where the protein-rich biomass could be a source of bioactive peptides with potential health benefits, such as antioxidant or anti-inflammatory properties. Although the extraction and processing of these bioactive compounds may be more complex, their incorporation into dietary supplements or functional foods offers opportunities for product development in health and wellness markets.

The sustainable waste management potential of SSF is particularly noteworthy, as the process effectively converts vegetable waste into a valuable protein-rich product, aligning with circular economy principles. By transforming waste into a usable resource, the SSF process addresses environmental concerns associated with waste disposal while simultaneously providing an alternative protein source. This dual

benefit underscores the environmental sustainability of the process and its contribution to waste reduction.

In agriculture, the residual biomass left after protein extraction retains essential nutrients, making it suitable for use as an organic fertilizer or soil conditioner. This application not only enhances soil fertility but also supports sustainable agricultural practices by recycling nutrients back into the soil, particularly in arid regions where soil quality may be a concern.

Thus, the SSF process demonstrates substantial potential for industrial scale-up, offering an efficient method for producing protein-rich biomass from vegetable waste. The scalability of the process means it can be integrated into existing industrial operations, providing an economically viable and sustainable solution for large-scale protein production. This makes it attractive for industries looking to diversify their product portfolio while contributing to sustainable practices.

Conclusion

In conclusion, the optimisation of protein production through solid-state fermentation (SSF) of *Rhizopus oligosporus* using various vegetable wastes as substrates was investigated. Through the application of the single-variable-at-a-time (SVAT) approach, key parameters, including temperature, incubation time, inoculum size, and wheat bran addition, were systematically varied to determine their individual effects on protein production. Our findings revealed that all the factors studied significantly influence protein production. Furthermore, the SVAT approach allowed for the identification of optimal conditions for maximising protein production, which was found to be 40 °C, 2.5 days incubation time, 3.6% (w/w) inoculum size and 50% (w/w) addition of wheat bran. This study also demonstrated the feasibility of utilising vegetable wastes as cost-effective substrates for SSF, highlighting its potential as a sustainable solution for protein production. Overall, the findings of this research contribute to the understanding of SSF optimisation strategies and offer practical insights for enhancing protein production using *Rhizopus oligosporus* on vegetable waste. Future studies could explore further optimisation techniques, such as response surface methodology (RSM), to elucidate the potential synergistic effects of multiple factors on protein yield and to optimise the process for industrial-scale applications.

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Data Availability The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

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