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Potato peel as substrate for Single Cell Protein in animal feed by submerged fermentation

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ORIGINAL ARTICLE

Abstract

Single-cell protein (SCP) has gained attention in scientific research as a vital source of protein in animal feed and human food. Taking into account the importance of SCP, an attempt was made in the current research to grow *Rhizopus oligosporus* and *Saccharomyces cerevisiae* biomass by selecting potato peel as a substrate. The potato peel was treated with sulfuric acid to form potato peel hydrolysate following acid hydrolysis. In 1.0-L potato peel hydrolysate, 2.5-g/L KH_2PO_4, 0.5-g/L $MgSO_4$, 0.5-g/L NaCl, and 10 g/L yeast extract were taken to prepare growth media. The moisture content of potato peel was examined by following standard procedure of the Association of Official Agricultural Chemists (AOAC). The Kjeldahl method was used to determine the protein content, and glucose was examined by using the dinitrosalicylic acid (DNS) method. Optimization of SCP yield was accomplished by following the 'one factor at a time' method. The effect of each parameter, such as incubation period, temperature and pH on SCP production was analyzed. Bioreactor scale-up production of SCP was performed in a jar fermenter. To enhance SCP, yield co-culture of *Saccharomyces cerevisiae* and *Rhizopus oligosporus* was used in a bioreactor that yielded 19 g/L of SCP. Thus, as a nutritional supplement for living organisms, SCP can effectively fulfill protein scarcity globally.

Keywords: bioreactor; optimization of SCP production; *Rhizopus oligosporus*; single cell protein, submerged fermentation

Introduction

According to a report of the Food and Agriculture Organization of the United Nations (FAO), the world population is predicted to grow to 9.7 billion by 2050. Food scarcity is a serious problem due to the drastically increasing global population (Abdullahi *et al.*, 2021). More than 1 billion global populations are suffering from malnutrition because of widening gap between food consumption and accessibility, inflation, decline in forest cultivation, global warming, and other factors. Proteins rich in nitrogen are required by humans and animals to produce new structural and functional proteins, which include enzymes and hormones essential for developmental processes and regeneration of cells (Aziz *et al.,* 2024; Benkirin *et al.,* 2024; A. Bacha *et al.,* 2024; Kurek *et al.*, 2022). In the context of the current global protein deficiency, single-cell protein (SCP) derived from microbes is receiving considerable recognition as a significantly secure and cost-effective source of proteins.

Microorganisms have long been utilized for food fermentation and the production of foodstuffs, such as bread, cheese, yogurt, soy sauce beverages, and beverages (Chaudhary *et al.,* 2024; Ejaz *et al.,* 2024; Afsar *et al*., 2024; Khatoon *et al.,* 2023; Ullah *et al*., 2023a). However, the utilization of microbes as a substitute source of nutritional components is not an innovative approach (Riesute *et al*., 2021).

Single-cell protein is described as dead desiccated cells of microorganisms or proteins from single-celled algae and cyanobacteria that are nurtured on different growth media and utilized as an alternative source of proteins. SCP is a rich source of protein constituting 60–80% of total dry weight (Allah and Saleem, 2021). SCP also contains a significant amount of amino acids, such as methionine and lysine, that are deficient in most plant and animal sources. Moreover, other nutritional components include fatty acids, carbohydrates, vitamins, and minerals. Due to its healthy nutrient composition, SCP has very appealing qualities as a food source (Suman *et al*., 2015). According to Spalvins *et al.* (2018), the problem of the unavailability of protein sources for animal feed was easily resolved by using SCP in the specific regions of Indonesia where natural sources of proteins, such as fish and soybeans, were insufficient. SCP has gained consideration as an excellent alternative source of proteins because it could be easily produced from inexpensive carbon sources by using organic waste materials from agricultural leftovers and the food manufacturing and processing industry (Bakratsas *et al*., 2023).

Important factors considered while selecting substrate media for fermentation include accessibility, cost, and expenses of subsequent processing of substrate (Li *et al.,* 2023; Arooj *et al.,* 2023; Shah *et al.,* 2023; Brastosin *et al*., 2021). Potato peel in bulk is discarded as a waste material of the potato processing industry, although it is an excellent source of protein, lipids, cellulose, starch, ash, glucose, lignin, and non-starch carbohydrates (Chaudhary *et al.,* 2024; Ullah *et al*., 2023b; This waste potato peel is used as a carbon source for the production of industrial food products (Arifeen *et al.,*2024; Shehzadi *et al.,* 2024; Aziz *et al.,* 2023; Calcio *et al*., 2020).

The aim and objective of the current research were to investigate the possibility of bioconversion of potato peel, easily accessible locally, into SCP through the process of submerged fermentation (SmF). Considering this, the present investigation attempted to figure out the potential of harvesting SCP from *Rhizopus oligosporus* by liquid state fermentation utilizing plenty of locally accessible potato peels as an affordable substitute of carbon sources (Perveen *et al.,* 2023). This study tried to determine an alternative best substrate for SCP production and to enhance the amount of protein generated in potential substrate and reduce cost of production. Furthermore, optimization of fermentation conditions, such as pH (3.5–7.5), temperature (30, 37 40, and 45°C), and duration of fermentation (5, 7, and 9 days), in contrast to the control medium (glucose), was also performed. Multiple methods were employed to conduct a proximate analysis of potato peel. Moreover, mixed cultures of *Rhizopus oligosporus* and *Saccharomyces cerevisiae* were used for raising the production of SCP.

Methodology

Source of microorganisms

Saccharomyces cerevisiae and *Rhizopus oligosporus* were collected from the PCSIR Laboratory in Lahore, Pakistan. Cultured potato dextrose agar medium slants were incubated at 30°C for 7 days. The inoculated slants were stored at 4°C and subcultured once every 2 weeks.

Collection and pretreatment of potato peel

Potato peel was collected in sterilized glass containers from a nearby market in Lahore, Pakistan. Potato peel was washed and cleaned in distilled water, and converted to fine powder in an electric grinder after a 3-day drying at 40°C in an oven. Some of the potato peel was used for analysis of nutrient composition.

Optimization of acid hydrolysis

Different concentrations of sulfuric acid (H_2SO_4) were used to analyze the optimum value which generated maximum glucose content in potato peel slurry. In each test tube, 1-g potato peel powder and 9-mL distilled water were used to make slurry. Six samples were prepared in test tubes with H_2SO_4 concentration of 1–5%; however, the control sample was not treated with H_2SO_4. Test tubes were heated at 90°C in a water bath for 1 h and centrifuged after cooling. The supernatant was used for glucose analysis using the dinitrosalicylic acid (DNS) method.

Glucose analysis

Six test tubes were labeled according to used H_2SO_4 concentrations, and 0.2-mL supernatant of the above-mentioned sample was added to each test tube. Then 0.8 mL of distilled water and 3 mL of DNS solution were added into each test tube. These test tubes were heated for 15 min in a beaker containing boiling water. Glucose absorbance was examined at 540 nm.

Hydrolysate preparation

Potato peel powder was further processed by treating 100-g of it with 1% H_2SO_4. This media was heated for 2 h on a hot plate to form potato peel hydrolysate, which was filtered using a muslin cloth. This supernatant was autoclaved and used as a substrate for submerged fermentation.

Proximate nutritive composition of potato peel

The nutritive composition of potato peel, including moisture, ash, and protein, was examined. For ash determination, five potato peels were taken in a crucible flask and burnt on flame. This burnt potato peel was converted into ash in a furnace at a temperature of 5000°C, and its mass was measured.

The moisture content of potato peel was examined following the standard procedure of the Association of Official Agricultural Chemists (AOAC, 2016). The Kjeldahl method (Bremner,1960) was used to determine the protein content of potato peel.

Production and harvesting of SCP

Inoculum preparation
In a 100-mL conical flask, 50-mL distilled water was taken along with two loops of *Rhizopus oligosporus* spores. This inoculum was used in the fermentation process for SCP production.

Fermentation at flask level

Screening of nitrogen source
Nitrogen source has a significant effect on SCP yield. Different nitrogen sources were used to analyze their effect on SCP yield. These include inorganic sources of sodium nitrate and organic sources of urea, peptone, and yeast extract.

Optimization of parameters

Optimization of certain parameters was performed by following the "one factor at a time" procedure. These factors include the incubation period (5, 7, and 9 days), temperature (30, 37, 40, and 45°C), and pH (3.5, 4.5, 5.5, 6.5, and 7.5).

Bioreactor scale-up production

Scale-up bioreactor production of SCP was performed by maintaining optimized conditions in a jar fermenter with an inoculum of *Rhizopus oligosporus*. The biomass was filtered, dried, and weighed. To enhance the yield of SCP, co-culture of *Saccharomyces cerevisiae* and *Rhizopus oligosporus* was used in a bioreactor.

Statistical analysis of data

The results were obtained as mean value and standard deviation (SD) of three trials. Statistical Package for Social Sciences (SPSS) was applied for statistical analysis.

Results

Analysis of the proximate nutritive composition of potato peel

The nutritive composition of potato peel, such as nitrogen, protein, moisture, and ash, was examined (Table 1).

Optimization of acid hydrolysis

For hydrolysate preparation, different concentrations of H_2SO_4 were used, with each concentration resulting in different glucose content. Maximum glucose content of 4% was observed by treating potato peel with 1% H_2SO_4. A small increase in the content was observed with other H_2SO_4 concentrations (Table 2). In further experiments, potato peel hydrolysate was prepared with 1% H_2SO_4.

Table 1. Proximate nutritive composition of potato peel.

Nutritive composition	Dry weight (%)
Protein	6.2 ± 0.53
Ash	5.3 ± 0.47
Moisture	83.2 ± 0.36

Results are shown as mean and standard deviation, n = 3.

Table 2. Effect of different concentrations of H_2SO_4 on hydrolysis of potato peel.

Concentration of H_2SO_4 (%)	Glucose (%)
1	4 ± 0.02^{c}
2	2 ± 0.05^{e}
3	$5 \pm 0.01^{a,b}$
4	$3 \pm 0.03^{c,d}$
5	6 ± 0.07^{a}

Note: Results are shown as mean and standard deviation, n = 3. Data in the same column with different superscript letters are statistically different ($p < 0.05$).

Nitrogen source screening

The effect of different nitrogen sources on SCP production was examined. Results revealed that the inorganic source of sodium nitrate and the organic nitrogen source of yeast extract yielded maximum SCP (12.6333 ± 0.50332 g/L and 12.6667 ± 0.08819 g/L, respectively, Table 3); these were used as an inexpensive and readily available optimum nitrogen source in further experiments.

Effect of temperature and pH on SCP yield

Optimization of temperature was performed by incubating culture media at different temperatures. Results presented in Table 4 revealed that 37°C was the optimum temperature for maximum growth of SCP (11.4667 ± 0.25166 g/L).

Optimization of pH was performed. Maximum SCP yield (11.6667 ± 0.25166) was obtained at an optimum pH of 4.5. Decline in yield was observed at basic pH of 6.5 and 7.5, as shown in Table 5.

Effect of incubation period on SCP

Results of SCP yield at different incubation periods are presented in Table 6. Biomass from each flask was gathered, dried, and weighed after respective periods as shown in table 6. The lowest yield was observed after 9 days of incubation. Maximum yield (11.7000 ± 0.20000 g/L) was obtained after 7 days of incubation, which was the optimum period for production of SCP.

Table 3. Nitrogen source screening.

Nitrogen source	SCP yield (g/L ± SD)
Control	4667 ± 0.30551[a]
Sodium nitrate	12.6333 ± 0.50332[c]
Yeast extract	12.6667 ± 0.08819[c]
Peptone	1.5333 ± 0.14530[b]
Urea	0.6667 ± 0.14530[a]

Note: Results are shown as mean and standard deviation, n = 3. Data in the same column with different superscript letters are statistically different ($p < 0.05$).

Table 4. Effect of temperature on SCP yield.

Temperature (°C)	SCP yield (g/L ± SD)
30	9.5333 ± 0.25166[c]
37	11.4667 ± 0.25166[d]
40	5.4000 ± 0.20000[b]
45	2.3333 ± 0.20817[a]

Note: Results are shown as mean and standard deviation, n = 3. Data in the same column with different superscript letters are statistically different ($p < 0.05$).

Table 5. Effect of pH on SCP production.

pH values	SCP yield (g/L ± SD)
3.5	1.4333 ± 0.15275[b]
4.5	11.6667 ± 0.25166[e]
5.5	9.6000 ± 0.20000[d]
6.5	4.3333 ± 0.25166[c]
7.5	0.3333 ± 0.15275[a]

Note: Results are shown as mean and standard deviation, n = 3. Data in the same column with different superscript letters are statistically different ($p < 0.05$).

Table 6. Effect of incubation period on SCP production.

Incubation period (days)	SCP yield (g/L ± SD)
5	7.9667 ± 0.30551[a]
7	11.7000 ± 0.20000[b]
9	3.8333 ± 0.40415[a]

Note: Results are shown as mean and standard deviation, n = 3. Data in the same column with different superscript letters are statistically different ($p < 0.05$).

Bioreactor Scale-up production of SCP

A jar fermenter was used for scale-up production of SCP by using *Rhizopus oligosporus*; 17-g/L SCP was obtained. To increase the yield, co-culture of *Rhizopus oligosporus* and *Saccharomyces cerevisiae* was used in a bioreactor, resulting in a yield of 19-g/L SCP.

Discussion

Rhizopus oligosporus was collected from PCSIR Laboratories in Lahore, Pakistan, and was preserved for the production of SCP using potato peel as a substrate by submerged fermentation.

Co-culture of *Saccharomyces cerevisiae* and *Rhizopus oligosporus* was also used for higher yield of SCP, compared to *Rhizopus oligosporus*. Higher production of SCP using potato peel as a substrate could be due to the composition of potato peel, microbial sources, or genetic composition of microbial strains. The composition of substrate has an important supportive role in the biomass production of microbes. Fungus growth is mainly affected by the nutritional composition of waste (Abdel-Rahman, 2016).

In this study, submerged fermentation was selected for SCP production because all the nutrients were thoroughly dissolved in a liquid medium and it was feasible to control parameters, such as temperature and pH. To

achieve maximum yield of SCP, we examined optimum fermentation conditions, such as the effect of the incubation period, temperature, pH, and nitrogen source.

Nitrogen acts as a protein-forming element in substrate media. Its structural properties have an important role for microbial growth in substrate media. Usage of peptone urea and sodium nitrate decreased SCP yield, while organic source of nitrogen as yeast extract enhanced it. Yeast extract included different metal ions, vitamins, proteins, and organic compounds, which may achieve the physiological requirements of *Rhizopus oligosporus* for production of SCP. Results were aligned with the study conducted by Ouedraogo *et al*. (2017), which obtained *C. utilis* biomass (3.25 g/L) with 0.75-g peptone. The authors observed that maximum biomass (4.56 g/L) was obtained by using 0.5% yeast extract.

Temperature is an important factor in microbial production of SCP. Biosynthesis of maximum SCP by *Rhizopus oligosporus* was obtained at 37°C. At higher temperatures, a decrease in growth was observed, which could be due to unfavorable conditions for microbial enzyme activity. Our results aligned with the study conducted by Milala *et al*. (2018), which reported 37°C as the optimum temperature for obtaining the highest yield of SCP with *S. cerevisiae*. The findings were comparable to those of Aslam *et al*. (2020), who presented the maximum production of pectinase from *Bacillus licheniformis KIBGE-IB3* at 37°C. pH has a significant role in the biosynthesis of SCP. *Rhizopus oligosporus* yielded a high production of SCP at pH of 4.5–5.5; maximum SCP yield was obtained at pH = 4.5. The results are in good agreement with a study conducted by Uçkun Kiran *et al*. (2015), who used *S. cerevisiae* and virgin grapes as substrate for maximum SCP yield at pH = 5.8. The results agreed with those of Chen *et al*. (2016), who studied the effect of different pH values (from 3.5 to 5.5) on SCP production from dry yeast, determining 5.0 as the optimum pH (Zhou *et al.,* 2023).

The experiment revealed that an incubation of 7 days was the optimum period. Decrease in SCP yield by increasing fermentation period of more than 7 days could be due to depletion of substrate concentration in growth media or accumulation of byproducts. The results aligned with the findings of Oshoma *et al*. (2019), who observed that incubation period of 6 days was optimum for SCP production with *A. niger* by using different fruit wastes as substrates, including pineapple, banana, orange, and watermelon wastes.

In a bioreactor, scale-up fermentation using *Rhizopus oligosporus* and co-culture of *Rhizopus oligosporus* and *Saccharomyces cerevisiae* resulted in 17 g/L and 19 g/L SCP, respectively. Our results were better than those reported by Sun *et al.* (2017), who obtained 13.5% SCP by using potato peel and wheat bran as a substrate and microbial strains of *Bacillus subtilize*, *Aspergillus niger*, and *Candida tropica*. The higher SCP yield could be due to the utilization of efficient microbial strains and optimized fermentation conditions.

Conclusion

Single-cell protein exhibits excellent qualities over plant- and animal-derived proteins and can be used to resolve universal problems related to protein scarcity, because it can be produced throughout the year and its production is not affected by season or climate changes. Another factor of importance is its healthy nutritional composition, such as phosphorus and potassium as well as lipids and carbohydrates, which establish SCP as a favorable dietary supplement. In this study, potato peel was selected as an inexpensive, readily available, and nutrient-rich substrate that resulted in maximum production of SCP and also reduced expenses of the experiment. This study also revealed an effective and useful solution to reduce environmental pollution caused by waste disposal of potato peel.

Recommendation

Production of SCP is the best solution to fulfill the needs of protein for increasing human and animal populations. SCP production could be accomplished at the industrial level by using potato peels as an inexpensive substrate.

Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

Conceptualization, Fakhar Un Nisa Yunus; methodology, Nimra Kiran; software, Muhammad Nadeem; validation, Farkhanda Manzoor; formal analysis, Robina Nelofar.; investigation, Abid Sarwar; resources, Tariq Aziz.; data curation, Najeeb Ullah.; writing—original draft preparation, Nimra Kiran; writing—review and editing, Majid Alhomrani; visualization, Walaa F Alsanie, Validation: Abdulhakeem S Alamri; supervision, Fakhar Un Nisa Yunus; project administration, Tariq Aziz, Funding Acquisition: Tariq Aziz

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