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From Scraps to Protein Powerhouse: Transforming Potato Peels into Single Cell Protein

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The utilization of low-cost industrial and agricultural wastes through emerging scientific approaches can contribute to the economic expansion of developing countries. In this research, the aim was to produce single-cell protein from a fungus by utilizing submerged fermentation and the agro-industrial waste of potato peels as a substrate. Four types of broths (control, glucose broth, potato peel broth, and mix broth) were prepared and studied. The mix broth yielded the highest dry cell biomass (0.523 g/100 ml) and was chosen for further investigation. All broths were supplemented with potassium dihydrogen phosphate, magnesium sulfate, sodium chloride, and yeast extract to enhance growth. A comparison was made between the growth of fungal biomass in a stirred tank fermenter and a bubble column fermenter, and the optimum yield (5.45 g/100 ml) was obtained in the bubble column fermenter. This bioconversion process not only provides a source of protein-rich food but also contributes to environmental pollution control.

Keywords: Single cell protein, Potato peels, Fermentation

INTRODUCTION

In developing countries, economic expansion can be achieved by employing emerging scientific approaches to convert low-cost industrial and agricultural wastes into more valuable products. One such waste material is potato peel, which can be transformed into various value-added compounds including enzymes, biogas, bio-sorbents, biohydrogen, and more (Javed et al. 2019). The generation of potato peel waste has increased due to the growing consumption of manufactured edible potato products. Biodegradable wastes have the potential to be utilized for the production of highly nutritious and high-quality products without causing harm. Various approaches have been developed to convert these waste materials into valuable products, aiming to reduce environmental pollution and address protein deficiency in the global population (Paraskevopoulou et al. 2002; Sharif et al. 2021).

Aquaculture farming practices, which require a significant amount of protein in animal feed, can benefit from the conversion of low-cost agricultural waste into single-cell protein (SCP) using bacteria or yeast (Øverland et al. 2013; Mahan et al. 2018). Food industries generate large amounts of waste and by-products that not only contribute to environmental pollution but also pose risks to living organisms. Additionally, malnourishment affects a significant number of people worldwide. Converting different types of waste into single-cell protein offers a solution to both environmental pollution and protein deficiency (Khan et al. 2010; Bacha et al. 2011).Potato peels, a substantial byproduct in the potato-processing industry, are often considered waste despite containing

valuable components such as lignin, protein, lipids, cellulose, ash, starch, and non-starch carbohydrates (pectin, cellulose, and hemicellulose) (Liang and McDonald, 2014; Dos Santos et al. 2016; Calcio Gaudino et al. 2020). While fermentable sugars are present in relatively low quantities, potato peels are associated with exceptional properties like anti-inflammatory, antioxidant, apoptotic, antibacterial, and chemo preventive effects (Liang and McDonald, 2014; Wu, 2016; Dos Santos et al. 2016; Calcio Gaudino et al. 2020).

The composition of potato peels may vary slightly depending on factors such as the breed, variety, and geographical region. Typically, raw potato peels consist of water (83.3-85.1%), protein (1.2-2.3%), carbohydrates (8.7-12.4%), lipids (0.1-0.4%), starch (7.8%), phenolic compounds (1.02-2.92%), dietary fiber (2.5%), flavonoids (0.51-0.96%), and ash (0.9-1.6%) (Javed et al. 2019; Calcio Gaudino et al. 2020). The peeling method employed can also impact the composition, with abrasion peeling resulting in higher starch content and lower lignin content compared to steam peeling (Javed et al. 2019).Given the increasing demand for protein and the pressure on conventional protein sources, researchers are exploring alternative protein sources to meet this demand. The objective of this experimental work is to produce single-cell protein from microorganisms using a cheap and readily available low-cost substrate that is otherwise considered waste and contributes to environmental pollution (Haddish, 2015).

Substrate and Microorganisms

Potato peels were obtained from a local market in Lahore and used as the substrate. They were washed with tap water to remove dust particles, dried at room temperature, and stored in a zipper bag at 4 °C. The fungus Rhizopus oligosporus, obtained from PCSIR testing laboratories complex in Lahore, was used for the production of single-cell protein. The fungus was grown and maintained on potato dextrose agar (PDA) plates, and slants were stored at 4 °C.

Pretreatment / Potato Peel Extract Preparation

The potato peels were chopped into small pieces. Then, 20 g of potato peels were placed in a 250 ml Erlenmeyer flask, and 100 ml of distilled water was added. The pH of the mixture was adjusted to 3.5 using concentrated HCl, and it was autoclaved at 121 °C for 15 minutes. After cooling, the mixture was filtered with muslin cloth to separate the potato chunks. The resulting filtrate was designated as potato peel extract (PPE).

Inoculum Preparation

For fermentation, an inoculum was prepared using a subculture of R. oligosporus grown on PDA slants. The plants were flooded with 20 ml of sterilized distilled water to dislodge spores from the fungal hyphae. The inoculum size was adjusted to 106-7 spores/ml using a hemocytometer for inoculation in all experiments. Fresh inoculum was prepared for each investigation of the parameter.

Selection of Broth

Four types of broths were prepared: a control broth, a potato peel extract broth, a glucose broth, and a mixed broth.

MATERIAL AND METHOD

Control broth:

The control broth comprising of yeast extract, peptone, glucose, sodium chloride and magnesium sulphate prepared. The composition of control is mentioned in table 1.

Table 1: Chemical composition of control medium

Sr. No.	Chemicals	Quantity (g/100 ml)
1.	Yeast extract	0.32
2.	Peptone	0.5
3.	Glucose	1.6
4.	NaCl	0.21
5.	$MgSO_4$	0.1

The pH of the potato peel extract solution was adjusted to 6.5 using HCl or NaOH. This control solution was then transferred to a fermentor and autoclaved along with the fermentor at 121 ℃ for 15 minutes.

The control solution was inoculated with 2% (v/v) of Rhizopus oligosporus. After 3 days of fermentation, the fermented material was harvested, and the wet weight of the biomass was measured. The biomass was then dried by placing it in an oven for 24 hours at a temperature below 80 ℃ until a constant mass was achieved. The dried biomass was recorded.

Preparation of Potato Peel Extract Broth:

The Potato Peel Extract Broth consists of 100 ml of the pretreated potato peel extract described earlier.

Preparation of Glucose Broth:

Glucose broth (100ml) was prepared by adding 3 g glucose in 100ml distilled water.

Preparation of Mix broth:

Mix broth comprising of 100 ml potato peels extract broth and 1.2 g glucose.

The potato peels extract, glucose and mix broth were supplemented with chemicals mentioned in Table 2.

Table 2: Common chemicals used in all media

Sr. No.	Chemicals/ suplements	Quantity (%)
1.	Yeast extract	0.50
2.	KH_2PO_4	0.05
3.	$MgSO_4$	0.025
4.	NaCl	0.025

The pH of all the broths was adjusted to 5.5 using diluted HCl or NaOH. Subsequently, the broths were autoclaved at 121 °C for 15 minutes. After cooling, the broths were inoculated with 2% (v/v) of a freshly prepared inoculum of R. oligosporus.

The cultures were incubated in an incubator at 35 °C for a duration of three days. After the incubation period, the biomass was filtered using Whatman filter paper, and the wet biomass was weighed using a digital balance. The biomass was then placed in an oven set at 80 °C for 24 hours until a constant weight was achieved. All experiments were conducted in triplicates.

Impact of Fermenter Type on Biomass Yield

Fermenters play a crucial role in providing optimal conditions for microbial growth and are utilized for the production of various products. Stirred tank fermenters are recognized for their agitation capabilities through impellers, while airlift fermenters rely on air for mixing instead of impellers (Gaikwad et al. 2018).

In both types of fermenters, the temperature was maintained at 35 ℃ with an aeration speed of 1.0 vvm. The mixed media was fermented with Rhizopus oligosporus for a duration of 3-4 days in each fermenter. Following fermentation, the cell biomass was separated from the filtrate using filtration of the fermented media. The wet cell biomass was subsequently dried in an oven at a temperature of 70-75 °C until a constant weight was achieved. All experiments were conducted in triplicates.

Analytical Procedures

The reducing sugars were determined using the Benedict's quantitative test (Hernández-López et al. 2020). The crude protein content of the single-cell protein was determined using the Kjeldahl procedure (Sáez-Plaza et al. 2013). The total protein in the growth media was estimated using the Lowry method (Lowry et al. 1951).

RESULTS

The objective of utilizing waste potato peels was to alleviate the strain on conventional protein sources. To achieve this, a series of experiments were conducted to produce single-cell protein from Rhizopus oligosporos through submerged fermentation using waste potato peels. These peels were chosen due to their abundant availability and cost-effectiveness, making them an ideal resource for developmental purposes.

Media Screening for Fermentation

Three different broths were formulated: glucose broth, potato peels broth, and a mixed broth containing both potato peels and glucose. While there were slight variations in their compositions, the same supplements were added to all of them. The results presented in Table 3 indicate that the highest single-cell protein (SCP) production (0.523 g/100 ml) was achieved with the mixed media, followed by the potato peels media. The crude protein content of the dried biomass ranged from 45% to 55%. The control medium, included for comparison purposes, resulted in a dry cell biomass of 0.045 g/100 ml. The comparison revealed that potato peels served as a better substrate, providing essential elements for the fungus.

Statistical analysis demonstrated that the dry biomass yield obtained from all three media was significantly different ($p < 0.001$). The mixed medium and potato peels medium yielded more biomass compared to the glucose medium, with the mixed medium being selected as the most favorable yield-producing medium. Figure 1 presents a clustered bar graph illustrating the total dry biomass, biomass yield, and sugar consumption for each medium.

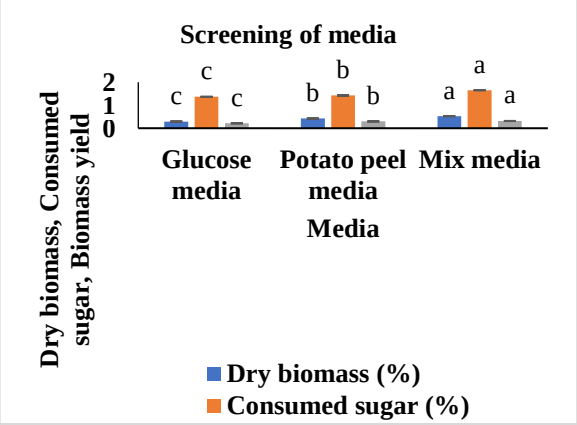

Figure 1: Screening of medium with different carbon sources for biomass production.

Table 3: Screening of best yield giving media based on total biomass quantity

Sr. No.	Media	Dry biomass (%) Mean ± SD	Consumed sugar (%) Mean ± SD	Biomass yield (g/g) Mean ± SD
1.	**Glucose media**	$0.21^{c} \pm 0.003$	$1.37^{c} \pm 0.009$	$0.21^{c} \pm 0.001$
2.	**Potato peel media**	$0.42^{b} \pm 0.001$	$1.12^{b} \pm 0.006$	$0.30^{b} \pm 0.015$
3.	**Mix media**	$0.12^{a} \pm 0.003$	$1.65^{a} \pm 0.004$	$0.32^{a} \pm 0.404$
Significance level (95%)	$P < 0.001$			

Means that do not share a letter are significantly different in a column.
Initial sugar:
Glucose=3%, Potato peel media=1.8%, Mix media=3%

Impact of Fermenter Type on Biomass Yield

The influence of fermenter type on the production of single-cell protein (SCP) was investigated by fermenting one liter of mixed media in both a Stirred Tank Reactor (STR) and a Bubble Column Fermenter, as depicted in Figure 3 and 4, respectively. After three days of fermentation, the biomass from both fermenters was harvested. The results, provided in Table 4, revealed that the Airlift Fermenter yielded the highest dry cell biomass (5.452 g/L).

Statistical analysis indicated a significant difference in the total quantity of dry cell biomass between the STR and bubble column fermenter ($p < 0.001$). The variation in total dry biomass in relation to the fermenter type is illustrated in Figure 2.

Table 4: Effect of fermenter type on the yield of biomass

Biomass	Stirred-Tank Bioreactor	Bubble Column Fermenter
Dry mass (g/L) ± SD	2.52^{b} ± 0.019	5.30^{a} ± 0.019
Significance level (95%)	P < 0.001	

Means that do not share a letter are significantly different

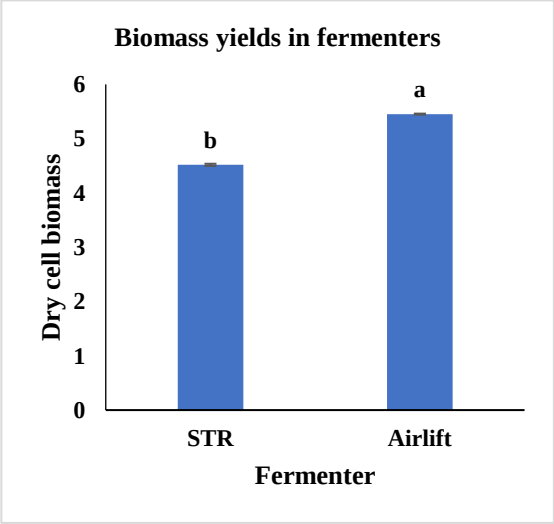

Figure 2: Dry cell biomass production in different bioreactors

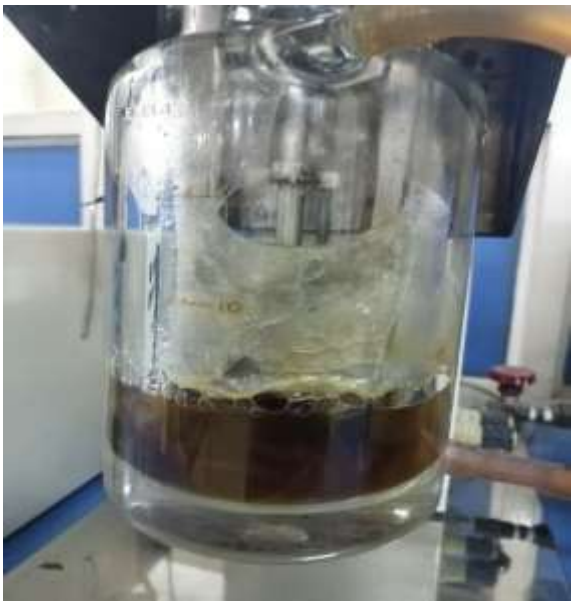

Figure 3: Stirred tank bioreactor

Figure 4: Bubble column Fermenter

Protein Estimation in Biomass

The total nitrogen and crude protein content in the Rhizopus oligosporous fungal biomass were determined using the micro-Kjeldahl method. The analysis revealed that the dry mass of the biomass contained 50% crude protein.

DISCUSSION

The process of fermentation is often followed by the steps of harvesting, filtration, centrifugation, washing, cell disruption, protein extraction, and purification (Paraskevopoulou et al. 2002).Different types of fermentation, including solid-state, semi-solid, and submerged fermentation, exist based on the moisture content of the substrate being fermented. Each type has its own advantages and disadvantages (Paraskevopoulou et al. 2002; Sharif et al. 2021).Substrates play a crucial role in providing essential nutrients for the microorganisms involved in the fermentation process. In submerged fermentation, all the required nutrients are dissolved in a liquid medium, allowing for easy control of factors such as pH and temperature (Al-Bakry et al. 2015).In our study, we utilized waste potato peels as the substrate in submerged fermentation to produce single-cell protein (SCP). The fermentation process was conducted at a temperature of 35 ℃ and a pH of 5.5 (i.e., pH 5.5). These conditions were selected based on previous studies and their suitability for SCP production (Reihani and Khusravi-Darani, 2019).

Furthermore, one study reported varying quantities of crude protein produced by different fungi using jackfruit peels as the substrate (A. niger-6.26%, R. stolonifera-7.25%, R. pusillus-6.63%, A. fumigatus-6.28%) (Reyes et al. 2018).Regarding oxygen flow rate, a review suggested that a rate of 1.0 vvm leads to better output. Additionally, the commonly used temperature range for fermentation is 25-38 ℃, while the pH range is 3.5-5.0 (Reihani and Khusravi-Darani, 2019). In our study, we used an oxygen flow rate of 1.0 vvm in both the STR and airlift fermenter, with a temperature of 35 ℃ and a pH of 5.5.

Our study results indicated that the maximum yield of dry biomass (0.523 g/100 ml) was obtained when the potato peel media was supplemented with glucose. Similar findings were reported by Mondal et al. (2012) in their study on biomass production from S. cerevisiae using glucose as a carbon source. The total crude protein content obtained from orange peels (30%) was lower than that obtained from cucumber peels (53.4%). In our study, we achieved a crude protein content of approximately 50% with Rhizopus oligosporus.Our findings align with those of Khan et al. (2009), who obtained crude protein from various waste materials using Rhizopus oligosporus as the fermenting microorganism.In a study conducted by Yousufi (2012a), SCP production using Rhizopus oligosporus and Aspergillus oryzae was explored with substrates such as okara and wheat grit at different pH levels. The maximum SCP production was achieved at pH 5.

Another study by Oshoma et al. (2017) investigated SCP production using banana peels and different media compositions. The highest biomass yield of 3.05 g/L was obtained in the supplemented banana peel medium. However, the protein content in their study (0.68 g/L) was lower compared to our research. These variations in results can be attributed to differences in substrates, microorganisms, and other factors influencing dry cell biomass production (Reyes et al. 2018).

CONCLUSION

Based on the information provided, it can be concluded that potato peels are a promising substrate for the production of single-cell protein (SCP). They contain sugars and other nutrients that are essential for supporting the growth of microorganisms. By supplementing the basic media with a nitrogen source, the yield of dry cell biomass can be further enhanced.

Furthermore, utilizing inexpensive agro-industrial sources, such as potato peels, for SCP production offers several advantages. It helps minimize environmental pollution by repurposing waste resources. Additionally, it contributes to reducing the cost of protein-rich animal feed. Single-cell protein serves as a viable alternative to protein sources derived from the agricultural sector.By exploring and utilizing such cost-effective and sustainable methods for SCP production, we can address environmental concerns, promote resource efficiency, and provide an affordable protein-rich feed option for animal husbandry.

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