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Original Paper

Conversion of Orange and Pineapple Fruit Peel Waste into Single Cell Protein Using *Saccharomyces Cerevisiae*

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Abstract*— The biological treatment of fruit wastes into single-cell protein has the potential to address the global protein shortage problem by producing a cost-effective product for food and feed. Pollution may be reduced if the substrate was made from the leftovers from the food processing industry. Herein, the submerged fermentation method was to make single-cell protein from pineapple and orange peels. Results indicated that fruit wastes analysed were highly hydrolysable. Proximate analysis of fruit wastes protein content of orange peel (10.73±0.02%) was significantly higher (P<0.05) than the protein content of pineapple peels (7.50±0.016%). When Saccharomyces cerevisiae was cultivated on fruit hydrolysate medium (FHM) without supplementation, the proportion of protein in single-cell protein was substantially lower, which produced (41.50±0.05% and 37.97±0.006%) total carbohydrate with (29.73±0.031% and 34.50±0.4%) crude protein, in both pineapple and orange medium respectively. The introduction of glucose to the supplemented fruit hydrolysate medium (GSFHM), increased the level of protein (45.50±0.031 and 37.73±0.011%) within the yeast cell, the comparison of treatment showed P<0.05, thus we can conclude that there is significant difference in the overall comparison of treatments in most of the parameters. The ITS2 region sequence analysis of Saccharomyces cerevisiae MA851180, revealed 99% identity with Saccharomyces cerevisiae. Thus, Saccharomyces cerevisiae produces higher amount of Single Cell Protein by submerged fermentation of pineapple peel hydrolysate as compared to orange peel hydrolysate. Hence, the single cell protein production by yeast depends on the growth substrates or media composition. SCP from yeast using fruit waste can serve as cheaper alternative to the conventional protein gotten from plant and animal and by selecting the most suitable prospective microbe and cost-effective technique.

Keywords*—Bioconversion, Single Cell Protein, Fruit Peel Waste, Fungus

I. INTRODUCTION

The ever-increasing population expansion in developing countries necessitates a rise in food availability. As the world needs for foods rich in protein grows, researchers are looking for other sources of protein to supplement established ones. An important step toward this goal is the synthesis of single-cell proteins (SCP). It represents a fresh and different strategy to resolving the global food crisis [22]. SCP stands for dead, dried microbial cells or full proteins that are extracted from microbial culture. In addition to a significant amount of protein, the SCP also has a high concentration of minerals, lipids, nucleic acids, vitamins, and carbohydrate with 60-82% of dry cell weight [14]. SCP also contains a lot of vital amino acids like lysine and methionine, two nutrients that are typically lacking in meals from plants and animals. Instead of more costly sources like fish and soybeans, this protein can be used to supplement the diet [11].

Several microbes have the ability to create SCP, including bacteria, fungi, mold, algae, and yeasts [5, 28]. Fungi like *Fusarium venenatum*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Pleurotus floria* are some of the best fungal sources because of their high protein content [24].When fungi are farmed primarily for the manufacture of SCP, they can contain up to 63%protein. The FAO's recommendations for the intake of amino acids and proteins by humans are also met by their amino acid profiles [23]. Fungi proteins are low in methionine and cysteine but high in threonine and lysine, which are amino acids that contain sulphur [23, 29]. Additionally, foods rich in vitamin B-complex vitamins such streptogramin, choline, niacin, pantothenic acid, thiamine, biotin, folic acid, pyridoxine, glutathione, riboflavin and amino benzoic acid may be found in SCP derived from fungi [26]. When compared to algae (which have a nucleic acid level of up to 6%), the nucleic acid content of fungus is greater, up to 10% [23].

Fruit wastes that are high in glucose and other essential nutrients may be able to support microbial development [2].As a result, fruit processing wastes can be used as a source of proteins produced by microorganisms. Reducing pollution and resolving the issue of waste disposal will be made possible by using wastes from fruits in the production of SCP to some extent, as well as meet the world's protein-rich food shortfall [9]. an investigation of the development of *Saccharomyces cerevisiae*, *Kefir* sp. and *Kluyveromyces marxianus*, by submerged fermentation utilizing substrates made up of several forms of typical wastes from food industry (orange, molasses, brewer's solid waste, whey, and potato residues), and found that *Saccharomyces cerevisiae* has a protein content of 39%, while

Kluyveromy marxianus and *Kefir* sp. has 34% and 23% respectively [3]. On the other hand, [19] researched how to minimize pollution from the potato starch industry's waste; SCP might be created by combining wastewater with potato pulp, and then used as animal feed. Potato wastewater and dextrose medium were used to grow *Candida utilis* for 48 hours at 28°C yielded a protein content of 46% and 49% [17]. Hashem, M.; Hesham, L.; Alamri, SA. and Alrumman, A. [12] employed *Zygosaccharomyces rouxii* and *Hanseniaspora uvarum* yeast strains from spoilt dates using *in vitro* and bioreactor production for single-cell protein production. After 60 hours, both strains produced at their peak levels (48.9 g/L).

Food and feed industries have been unable to meet the world's dietary protein needs because of an ever-increasing population, which has increased the demand for protein-rich food. Single-cell proteins have shown to be a viable strategy since agricultural leftovers like fruit wastes may be used in production. This can serve as an alternative to meet the global protein inadequacy and high cost of protein and minimize wastage/pollution. This study's aim is to convert fruit wastes into single-cell protein using *Saccharomyces cerevisiae.* The objectives of this study are to (1) isolate and identify *Saccharomyces cerevisiae* from palm wine; (2) characterize isolated *Saccharomyces cerevisiae* by molecular method; (3) degrade different fruit wastes (Orange and pineapple) by isolated *Saccharomyces cerevisiae*, and (4) determine the proximate composition of substrate and biomass yield.

II. Material And Methods

A. Study area

This research was conducted at Gwagwalada Area Council. The town of Gwagwalada is located in Federal Capital Territory FCT, north-central Nigeria. Its entire land area is 1043 km2, its yearly humidity is 20 to 30 percent, and its average temperature ranges from 27 to 35 degrees. It has a total land area of 1043km2, an annual humidity of 20–30%, with a temperature range of 27 to 35 °C. It is situated between latitudes 80–90°N and 70–80°E. The regional council's population has skyrocketed, and as of the 2006 census, it was estimated to be 157,770.The Koro, Bassa, Hausa Fulani, Gede, and Gwari, who were the initial settlers, as well as other Nigerians and foreigners, make up this population. The area council grows a number of important crops, including sorghum, millet, melon, yam, soya beans, cassava, and rice. Animals can live there as well because there is plenty of grazing land [13].

B. Sample Collection and Preparation of Substrates

Fruit peel leftovers from several fruit vendors in Gwagwalada market Abuja, Nigeria, was collected aseptically into sterile containers, and it was then brought to the microbiology laboratory at the University of Abuja. They were separated, thoroughly cleaned many times with sterile water, oven-dried to a constant weight (at 40–50°C), ground in a Scanfrost electric blender (UK), and then sieved through a mesh screen. The samples that were acquired in this way were put in see-through Zip-lock bags and stored at room temperature for further study [18].

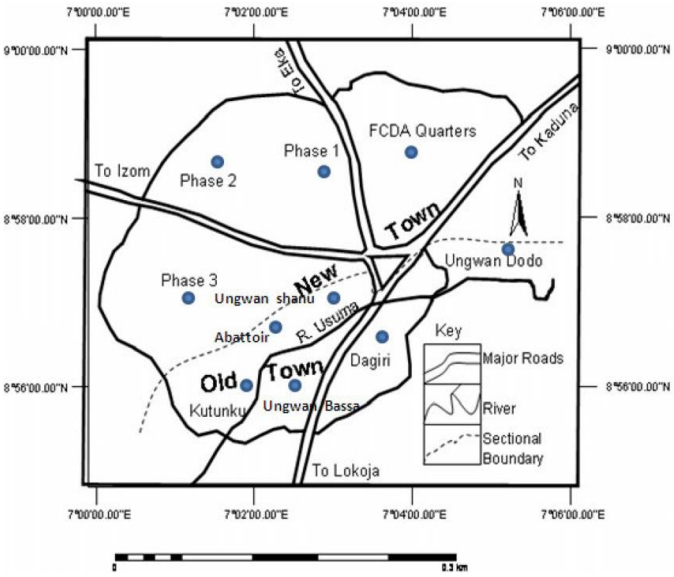

Fig. 1. Map of Gwagwalada town showing points of sample collection [13].

C. Sample Preparation

Ground wastes were chemically broken down to release more easily accessible sugars from the cellulose component. Each sample (40gm) in a conical flask was added 50 (ml) of 10% (w/v) HCl, respectively. The combination or solution spent an hour in the water bath at 100°C. It was filtered using number 1 Whatman filter paper after cooling for a while. The filtrates were treated with sterile distilled water at varied concentrations before being placed in the autoclave for 15 minutes at 121°C. The sterile solution produced in this way served as a source of carbon and nitrogen for the development of biomass [18].

D. Sterilization and Media Preparation

Glucose, Sabouraud dextrose agar, and yeast potato dextrose agar (all from Hi-Medium Laboratory, pot Ltd., India) were the media used. However, all of the chemicals were of the analytical quality and came from the American Difco Detroit, Michigan Laboratory. The autoclave was used to sterilize every piece of equipment for 15 minutes at 121°C. The workstation was swabbed with around 70% ethanol, and other equipment was sterilized. In accordance with IP (2014), Sabouraud Dextrose Agar with Chloramphenicol Medium 4 (Hi-Media Laboratory, Pot Ltd, India) was one of the media utilized in this study. Medias were all made in accordance to the directives of the manufacturers, all media were prepared.

E. Isolation of Microorganisms

Using a tenfold serial dilution to extract the yeast (Saccharomyces cerevisiae) from Palm wine, there was a 1 ml addition of the stock solution to a test tube with 9 ml of sterile water, as specified by Avishai and Charles [7]. The content was then homogenized by mixing. 48 hours of incubation at 28°C followed the inoculation of the plates. The isolates were identified in the Department of Biotechnology/Microbiology Laboratory, National Institute for Pharmaceutical Research and Development (NIPRID), Abuja. The yeast was sub cultured

severally on Sabouraud dextrose agar before being preserved in agar slants and incubated at 28°C.

F. Identification of Microorganism

Using standard yeast identification techniques, the isolates were identified based on their appearance, sporulation, and fermentation characteristics [21].

i. Colony Morphology

The Sabouraud dextrose agar was used to establish a pure culture of Saccharomyces cerevisiae. Then the organism was observed and confirmed [27].

ii. Yeast Viability Test

A tiny amount of the culture was used on the slide for the yeast viability test, and the slide was then inundated by adding methylene blue and left to dry. Next, the slide was examined using a microscope to confirm the results following the procedure of Acharya [1].

iii. Lacto phenol Cotton Blue Staining

Using this process, it was possible to identify yeast by examining the microscopic structure. On a slide, a little amount of culture was placed and spread out to create a thin film.

A drop of LCB was used to stain this film, which was then covered with a cover slip. According to the procedure outlined by Yadav and Tiwar [27] the slide was then examined with a 40X objective and confirmed.

iv. Test for Hydrolysis of Urea

This experiment was done on Christensen's urease agar slants to see if the organism could make the urease enzyme and cause urea to be hydrolyzed. The recovered organism was then streaked on Christensen's urease agar slants, incubated at 25°C for 48 hours, and validated using the procedure outlined by Yadav and Tiwar [27].

v. Hydrogen Sulphide Production

An organism's capacity to convert sulfur-containing substances to sulphite during metabolism was examined in this experiment using Bismuth sulphite agar. In accordance with the procedure outlined by Tong and Herbert [25] following a 48-hour inoculation on the medium, the organism was verified by looking for a color change.

G. Molecular-based identification of the Isolate

i. DNA extraction

The technique outlined by Ausebel, M., Brent, R., Kingston, E. and Moore, D. [6] for DNA extraction was used. On Sabouraud dextrose agar, Hi-Media Laboratory, pot India, yeast was sub cultured and then incubated at 28 °C for 24 to 48 hours. Colonies of these species were immersed in saline and turbidity of McFarland standards 0.5 at 530 nm was measured. A milliliter of the cell suspension was centrifuged in a mini centrifuge at 5,000 g for three minutes. According to the manufacturer's instructions, use the Tissue and Blood Genomic DNA Extraction Mini prep apparatus to extract the genomic DNA (Vio-gene, Taiwan).For later usage, the extracted DNA was kept in a freezer at 20°C.

ii. Amplification of the ITS1-5.8S-ITS2 Region of Isolates

The isolates were identified based on the length of the rDNA region covering the 5.8S rRNA gene and bordering the internal transcribed spacers 1 and 2 using the primers ITS1 (5′ -TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′ -TCCTCCGCTTATTGATATGC-3′). This method was reported by Alvarez-Martins, P.; Florez A.; Lopez-Diaz, T. and Mayo, B [4], after that, the cell suspension received 40 µl of PCR reaction mixture. The ITS1 and ITS4 primers (5′ -TCCGTAGGTGAACCTGCGG-3′) from Metabion in Germany and 1 mMdNTPs from Thermo Scientific in the United States were used in the PCR reaction mix, along with 0.8 UTaq polymerase and 1xPCR buffer (Thermo Scientific, USA).As a result, the tip of sterile forceps was used to transfer biomass from a new colony into a PCR tube that was filled with 10µl of sterile de-ionized water. The PCR 2720 Thermal Cycler (Bio Systems, Germany) was used to perform the amplification, and the following program was used: Initial denaturation at 95 °C for 10 min, then 35 cycles of denaturing at 94 ° for 30 sec, annealing at 55 ° for 30 sec, extension at 72° for 1 min, and final extension at 72 ° for 7 min.

iii. Agarose Gel Electrophoresis

PCR products were observed on a 2% agarose gel stained with Safe View (NBS Biological, UK) at 100V for 50 minutes using the VWR Mini Electrophoresis System (VWR, Germany) and Mini Bis Pro (DNR Bio-Imaging Systems, Israel) for gel visualization. The molecular marker was GeneRuler1kb plus from Thermo Scientific in the United States. The image-editing program Image J64 was used to calculate the size of the fragments (freeware). After being digested at 37°C for 4 hours with Hae III, Cfo I, and Hinf I, three restriction enzymes (Thermo Scientific, USA), the PCR products were then submitted to RFLP analysis. 2% agarose gel that was stained with Safe View at 100V for 55 minutes allowed restriction fragments to be seen.

iv. DNA sequencing and analysis

The DNA sequencing-based identification of the fungus was confirmed using the RLFP profiles that were acquired (Macro Gen Europe, Inc.).The BLAST program was used to match the nucleotide sequences to the Gen Bank database [4].

H. Inoculums Preparation

The inoculums were made from a *Saccharomyces cerevisiae* culture that had grown for 24 hours were spent incubating at 28°C on Sabouraud dextrose agar slants. Ten milliliters of sterile, distilled water were used for the dilution of the developing colony. For future usage, the suspension inoculums were stored at 4°C in a refrigerator.

I. Single cell protein fermentation and harvesting

Three experimental medium were used for submerged fermentations in Erlenmeyer flasks. Supplemented fruit hydrolysate (SFH), which is one of them, included the following, KH_2PO_4 (1.0gm), $MgSO_4.7H2O$ (0.5gm), $(NH_4)_2SO_4$ (2.0gm), NaCl (0.1gm), $CaCl_2$ (0.1gm) made up to 1 liter of fruit hydrolysate (FH).The second medium is called glucose supplemented fruit hydrolysate (GSFH), and it contains glucose (2 gm/l) as well as all the other components of SFH. Only the fruit hydrolysate medium was present in the third media (FHM). Due to the acidic pH of the medium, the test was performed in triplicate in each of the media and the initial pH was corrected to 5.8 for each media using 1N NaOH. Each media (98 ml) was put into an Erlenmeyer flask with a capacity of 250 ml, disinfected for 15 minutes at 121°C, and then used.2 ml of the *Saccharomyces cerevisiae* suspension were aseptically introduced as inoculums into each medium, with 100mg/l chloramphenicol added to the stock solution to prevent contamination [8].

J. Bioconversion of Fruit Waste

Following fermentation, in order to extract the biomass from the culture broth, sterile water was first used to vacuum-filter the biomass. Before being weighed, the extracted biomass was placed in an aluminium disc. To achieve the necessary temperature and weight, it was oven-dried for one hour at 105°C before being chilled in desiccators.

K. Proximate Analysis of SCP

The chemical elements of dried biomass that have been separated, such as the amount of total carbohydrate and crude protein, were then examined using the Association of official analytical chemist AOAC technique after an analysis of the composition of fruit peels, including crude protein, carbohydrate, fiber, lipids, and ash content. By heating 5g of the sample to a constant mass in a crucible kept at 105°C in an oven, the moisture content was ascertained. The determination of the other variables used the dry matter. 5g of the sample were used to calculate crude protein using the Kjeldahl technique (% total nitrogen x 6.25); 5g of crude fat were produced by exhaustively extracting the sample in a soxhlet device using petroleum ether (boiling point range 40-60%) as the extractant. The amount of ash was calculated by the five-hour, 550°C muffle furnace incineration of 6g of material. 4g of the sample were digested with H2SO4 and NaOH to produce the crude fiber, which was then produced by burning the leftover material for five hours at 550°C in a muffle furnace. Difference was used to determine the total amount of carbohydrates.

L. Statistical Analysis

Each analysis was carried out in triplicate. A two-way ANOVA was used to compare group means, a P-value of 0.05 (5%) was used to calculate the significance level.

III. RESULTS

A. Yeast Isolation and Identification.

Saccharomyces cerevisiae was isolated from freshly produced palm wine. Table 1 showed the morphological and biochemical characteristics of isolates, the morphology of isolates showed isolates appeared to be cream-colored, smooth, glistering, flat, raised and moist with a raised edge. When viewed under the microscope, the isolates showed budding as a means of yeast reproduction when stained with lacto phenol cotton blue stain. The result for utilization of urea and hydrogen sulfide production showed negative. Meanwhile, in the yeast viability test, the live cells were colorless, while the dead cells absorbed the dye and took on a blue appearance.

TABLE I. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF ISOLATES

Sample	H_2S	LCB	Urease	Morphology
1	-	Budding	-	Smooth, glistering, flat, moist, cream coloured
2	-	Budding	-	Smooth, glistering, flat, moist, cream coloured
3	-			Smooth, glistering, flat, moist, cream coloured
4	-	Budding	-	Smooth, glistering, flat, moist, cream coloured
5	-			Smooth, glistering, flat, moist, cream coloured
6	-	Budding	-	Smooth, glistering, flat, moist, cream coloured
7	-			Smooth, glistering, flat, moist, cream coloured
8	-	Budding	-	Smooth, glistering, flat, moist, cream coloured
9	-			Smooth, glistering, flat, moist, cream coloured
10	-	Budding	-	Smooth, glistering, flat, moist, cream coloured

Probable Organism- Saccharomyces cerevisiae
Key-
LCB- Lacto phenol cotton blue staining
H2S-Hydrogen sulphide production
Urease - Test for Hydrolysis of Urease
_- Negative
Morphology - Colony morphology.

B. Molecular Identification of the Isolates

Successful amplification of the ITS1-5.8S-ITS2 region from a DNA strain (plate 1), with size ranging between 50 – 1500 bp. Table 2 showed that the ITS2 region sequence analysis of Saccharomyces cerevisiae MA851180 revealed 99% identity with Saccharomyces cerevisiae.

TABLE II. MOLECULAR IDENTITY OF SAMPLE

S/N	ID	Organism match	Primer used	Strain	Similarity (%)
1	**SC**	*Saccharomyces cerevisiae*	ITs1-F/ITs-4R	MA851180	99

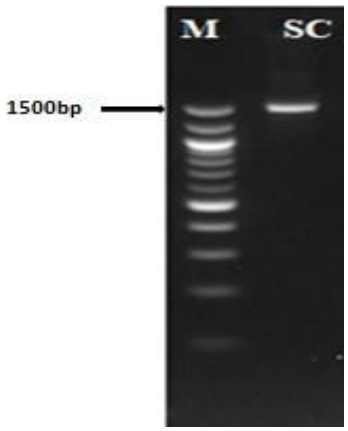

Fig. 2. Agarose gel electrophoresis showing PCR result with the bond point. Key- From left to right, Legend: M=1kb –Ladder, SC=isolate SC (Saccharomyces cerevisiae). Lane M represents protein molecular mass markers (50 to 1500bp), while Lane S represents the Saccharomyces cerevisiae. A target bond point is indicated by arrows.

C. Proximate Analysis of Fruit Peels and Biomass

The proximal findings of fruit peels are displayed in Table 3. It was observed that pineapple peel contains a higher percentage of carbohydrates (63.26±0.01%), which is significantly higher ($P<0.05$) than that of orange peel 58.52±0.011%. In comparison, orange peel has a higher percentage of protein (10.73±0.02%), which is also significantly higher ($P<0.05$) than 7.50±0.01% in pineapple peel. The fruit hydrolysate medium produced the lowest protein yield of 29.73±0.031% and 34.50±0.4% in orange and pineapple peel respectively (Table 3), while the supplemented fruit hydrolysate medium produced a higher protein and carbohydrate yield of 33.63±0.157% and 42.50±0.201% (Table 4).Table 5 showed the result of proximate analysis of biomass, where Glucose Supplemented Fruit Hydrolysate produced the highest protein yield of $45.50 \pm 0.031\%$ and the highest carbohydrate yield of $48.95 \pm 0.02\%$. The ANOVA result of the overall group mean showed that there is no significant difference between the group means with $P \geqslant 0.05$, similarly, the overall class is also observed to be not significant since its $P \geqslant 0.05$, thus we can conclude that there is no significant difference in the overall group mean of parameters for orange and pineapple peel. Meanwhile, analysis of variance result represented by the comparison of treatments (fruit peels, FHM, SFHM and GSFHM) over all the parameters (moisture%, ash%, lipids%, nitrogen%, protein%, crude fiber%, and carbohydrate %) showed ash%, nitrogen%, crude fiber%, Protein% and carbohydrate% all with $P \leqslant 0.05$, thus we can conclude that there is significant difference in the overall comparison of treatments over most of the parameters.

TABLE III. PROXIMATE ANALYSIS OF PINEAPPLE AND ORANGE PEEL

Parameters	Mean (Orange Peel) ± SD	Mean (Pineapple Peel) ± SD
Moisture%	6.01±0.021	5.02±0.016
Ash%	5.51±0.02	4.06±0.021
Lipids%	4.78±o.02	2.08±0.02
Nitrogen %	1.72±0.02	1.20±0.01
Protein%	10.73±0.02	7.50±0.016
Crude fiber%	14.45±0.021	18.08±0.02
Carbohydrate%	58.52±0.011	63.26±0.01

(g/100g dry peel) - Values are means ± standard deviation of three replicate measurements.

TABLE IV. PROXIMATE ANALYSIS OF BIOMASS FROM FRUIT HYDROLYSATE MEDIUM AND SACCHAROMYCES CEREVISIAE

Parameters	Mean (Orange peel) ±SD	Mean (Pineapple peel) ±SD
Moisture%	7.12±0.02	5.23±0.02
Ash%	5.16±0.01	4.16±0.01
Lipids%	3.30±0.096	2.08±0.598
Nitrogen %	4.75±0.06	5.52±0.03
Protein%	29.73±0.031	34.50±0.4
Crude fiber%	13.19±0.021	16.06±0.02
Carbohydrate%	41.50±0.05	37.97±0.006

Values are means ± standard deviation of three replicate measurements

TABLE V. PROXIMATE ANALYSIS OF BIOMASS USING SUPPLEMENTED FRUIT HYDROLYSATE MEDIUM AND *SACCHAROMYCES CEREVISIAE*.

Parameters	Mean (Orange Peel) ± SD	Mean (Pineapple Peel)±SD
Moisture%	7.19±0.051	4.32±0.03
Ash%	4.27±0.011	5.08±0.02
Lipids%	3.70±0.01	4.18±0.02
Nitrogen %	5.39±0.01	6.80±0.1
Protein%	33.73±0.157	42.50±0.201
Crude fibre%	6.19±0.006	7.28±0.02
Carbohydrate%	44.92±0.02	36.64±0.04

Values are means ± standard deviation of three replicate measurements.

TABLE VI. PROXIMATE ANALYSIS OF BIOMASS FROM GLUCOSE SUPPLEMENTED FRUIT HYDROLYSATE AND SACCHAROMYCES CEREVISIAE.

Parameters	Mean (Orange Peel) ± SD	Mean (Pineapple Peel)±SD
Moisture%	4.23±0.016	5.12±1.01
Ash%	2.27±0.02	3.08±0.01
Lipids%	2.63±2.327	3.04±0.02
Nitrogen %	6.03±0.03	7.28±0.011
Protein%	37.73±0.011	45.50±0.031
Crude fibre%	4.19±0.04	5.08±0.071
Carbohydrate%	48.95±0.02	38.18±0.01

Values are means ± standard deviation of three replicate measurements.

IV. Discussion

Saccharomyces cerevisiae isolated from palm wine, inoculated on freshly prepared SDA agar, incubated for 24 hours at 28°C, were identified by their morphological and biochemical characteristics and confirmed by their molecular markers using ITS1-5.8S-ITS2 gene sequence. As shown in Table 4.1, the isolates showed Smooth, glistering, flat, moist, cream colored with the entire edge. The organism was elliptical-shaped, and a similar result was reported by Yadav and Tiwar, [27]. Some cells were observed to show budding when stained with Lacto phenol cotton blue stain. Consequently, *Saccharomyces cerevisiae* was identified as the organism, and Acharya [1] reported a similar result. The isolates were negative for hydrolysis of urea and hydrogen sulphide production as no color change occurred. This is similar to the findings of Yadav and Tiwar, [27].The yeast viability test shows that, the living cells were colorless, while the dead cells absorbed the dye and looked to be blue; the same outcome was noted by Tong and Herbert [25].The species was identified to be *Saccharomyces cerevisiae* using the BLASTn sequence analysis.

The findings of the proximate analysis, as shown in Table 1, recorded that orange peel contained 10.73±0.02% crude protein, which is significantly higher ($P \leq 0.05$) when compared to pineapple peel, which had 7.50±0.01% crude protein. Meanwhile, the carbohydrate equivalent to nitrogen-free extracts composition of the pineapple peel was also significantly higher ($P<0.05$), 63.26±0.01%, than the orange peel sample (58.52±0.011%). When compared to the values obtained for orange peels (14.45±0.021%), crude fiber was also significantly higher ($P<0.05$) in pineapple peels, with a value of 18.08±0.01%. Both orange and pineapple peel wastes included varying amounts of total carbohydrates and fiber that, when hydrolyzed, act as a source of carbon for microbial development during fermentation. As a result, the two peels employed in this investigation as substrates were suitable for microbial fermentation.

Microbial growth and metabolism require various media components, such as inducers, suppliers of carbon and nitrogen, and trace elements. On yeast growth medium used to produce SCP, the effects of adding nitrogen and glucose were examined, after seven days of fermentation, Table 2 showed the fruit hydrolysate media (FHM) without supplementation produced 41.50±0.05% and 37.97±0.006% total carbohydrate with 29.73±0.031% and 34.50±0.4% crude protein, Similarly, in Table 3, total carbohydrate (44.92±0.02% and 36.64±0.04%) and crude protein (33.73±0.157% and 42.50±0.201%) were higher in the supplemented fruit hydrolysate media (SFHM) thus, there is significant difference ($P \leq 0.05$) between treatments on all the parameters. Fruit hydrolysate medium with added glucose and nitrogen (GSFHM), shown in Table 4 on the other hand, produced the highest concentration of crude protein (45.50±0.031% and 37.73±0.011%) and carbohydrate (38.18±0.01% and 48.95±0.02%). The findings of this study showed that Pineapple fruit hydrolysate has the highest carbohydrate and protein content. Similarly, glucose, carbon and nitrogen have a favorable effect on *Saccharomyces cerevisiae* SCP production. Similar findings by DeAzeredo L.; Gomes, P.; Geraldo, S. and Castilho, L. [10], demonstrated that glucose at various concentrations increased growth and the generation of hydrolytic enzymes in *Penicillium restrictum* utilizing submerged fermentation, and that catabolite suppression was not seen.

As evidenced by the low biomass output in the absence of glucose supplementation, glucose may play a critical role in SCP synthesis by *Saccharomyces cerevisiae*. When the nitrogen (GFSHM) source was removed, poor biomass was also seen, though at a lower degree. This shows the synergistic impact of ammonium sulphate and glucose (as source of inorganic nitrogen and carbon, respectively), on SCP formation, as their existence significantly impacts *Saccharomyces cerevisiae* biomass growth. From the result of the submerged fermentation carried out in this study, pineapple peel hydrolysate when inoculated with *Saccharomyces cerevisiae* produces a more significant amount of SCP when compared to orange peel hydrolysate. This could be due to pineapple peels' high fiber content (18.08±0.01%), which releases considerable amount of carbon during cellulolytic digestion. The observed results agreed with the conclusions of Khalil, I.; Hoque, M.; Basunia, M. and Khan, A. [15]. The SCP synthesis of all fruit mixtures, however, was greatly increased when nutritional supplements (sugar and ammonium sulphate) were added; this is consistent with the results from Mondal, A.K., Samadrita, S, Jayati, B. and Bhattacharya, D.K. [20].The addition of glucose led to an improved production of biomass and SCP by providing a more readily usable source of carbon, which is critical during early stages of microbial growth by reducing the lag phase. This study demonstrated that orange and pineapple peels can be utilized as a substrate for the production of SCPs, and that the procedure can be enhanced with using nitrogen and glucose sources.

In conclusion, submerged fermentation of orange and pineapple fruit peels resulted in a high SCP synthesis from *Saccharomyces cerevisiae*. The study showed that the substrate and medium composition determined the amount of SCP produced. The addition of glucose provided the organisms with a readily available carbon source, boosting SCP production. These fruit peels could be used to make protein-rich feed and food. Instead of being discarded, these peels ought to be properly used as a medium or substrate for creating cellular biomass for edible yeast, as they can be employed as food and feed supplement at a low cost.

SCP manufacture has piqued the curiosity of researchers and enterprises from all over the world. The abundance of promised advantages that these proteins provide has led to the emergence of various businesses that claim to have the ability to commercialize SCP. However, finding a sustainable, renewable protein-enriched component is a significant hurdle for the industry. While having beneficial properties, SCP also has certain disadvantages that need to be taken into account, such as manufacturing costs and growth requirements. Most importantly, however, is that SCP's main anti-nutritional

feature is the increased content of nucleic acid compared to other protein sources. To get the most benefits from SCP, there is still much work to be done on this issue. However, by enhancing microbe strains, monitoring the fermentation process throughout manufacturing, and utilizing cutting-edge down-streaming techniques, some of the issues mentioned above can be avoided. Furthermore, choosing active organisms and a suitable substrate can assist overcome the constraints mentioned above, making SCP use desirable.

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