Utilization of Pineapple (*Ananas comosus*) Peels in the Production of Single Cell Protein Using *Saccharomyces cerevisiae*

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ABSTRACT

This study aimed to utilize pineapple [*Ananas comosus* (L.) Merr.] peels in the production of single cell protein (SCP) using Saccharomyces cerevisiae via submerged fermentation. This investigation determined the lignocellulosic composition of pineapple peels such as the holocellulose, hemicelluloses, total acid soluble and insoluble lignin, alpha cellulose and total extractives. Other properties of pineapple peels such as the moisture and ash content as well as the total reducing and total sugar were also determined. In the production of SCP, four different media supplements such as pure powdered pineapple peels hydrolyzate, powdered pineapple peels hydrolyzate supplemented with salts, powdered pineapple peels hydrolyzate supplemented with glucose and powdered pineapple peel hydrolyzate supplemented with salts and glucose were used. Results of the study revealed that the pineapple peels showed a high percentage of holocellulose and hemicellulose indicating that it is a good substrate for the production of SCP. It was also found out that there was no significant difference on the percentage yield of SCP using different media supplements. On the other hand, a significant difference was noted on the crude protein content of SCP using different media supplements. Using the best media supplement, it was found out that the moisture, ash and crude fat differed significantly while no significant difference was noted in the crude fiber and crude protein when the commercially available SCP and the SCP produced were compared.

Keywords: *Single cell protein (SCP), Saccharomyces cerevisiae, pineapple peels, submerged fermentation*

1. INTRODUCTION

The utilization of pineapple -[*Ananas comosus* (L.) Merr.] SCP peels in single cell protein production has the potential to solve the worldwide food protein deficiency by obtaining an economical material for foods and feeds. Protein is a critical food group for everyone. Most adults need about 10 to 11 ounces of a protein food each day. Like humans, proteins are also an essential nutrient required for the proper nutrition of all animals. The animal proteins required to supplement vegetable proteins are available in insufficient quantities for the adequate nutrition of swine and poultry [1].

Conventional agricultural production of cereals, pulses, vegetables, fruits, etc., may not be able to meet the demand of food at the rate at which human and animal population is increasing. It has been estimated that if necessary measures are not taken the malnutrition condition will lead to some major crisis in the developing countries. Therefore, it is very important to increase protein production and its availability to the population by utilizing all the available methods of production. The increased world demand for food and in particular feed protein has spurred the search for non-conventional protein sources to supplement the available protein source. The use of molasses for the production of SCP is determined by its availability and low cost, its composition and absence of toxic substances and fermentation inhibitors.[2]. SCP production technologies arose as a promising way to solve the problem of worldwide protein shortage. With increase in population and worldwide protein shortage, the use of microbial biomass as food and feed is more highlighted.

Single cell proteins (SCP) refer to source of proteins which is extracted from single cell organisms like algae, yeast, bacteria, and fungi normally grown on agricultural waste.Single-cell proteins are the dried cells of microorganisms, which are used as protein supplement in human foods or animal feeds [3]. These are produced as substitute for protein rich foods for humans and as well as for animals. Single cell proteins are produced from microbial ferment waste which includes wood, straw, cannery, residue from alcohol production or food processing waste.

Pineapple has the second highest production volume of all tropical fruits in the world [4]. The production of processed items results in massive waste generation, estimated about 40-50% from fresh fruit as peels and core [5]. Since a pineapple peeling is a rich source of cellulose, hemicellulose and other carbohydrates, it has been used as a substrate for growing protein SCP and its production.

A variety of microorganisms and substrate are used to produce SCP. Yeast is suitable for SCP production because of its superior nutritional quality. It is a rich source of protein and B-complex vitamins. It has been used as a supplement in animal feed to compensate for the amino acid and vitamin deficiencies. In addition, yeast is considered a cheaper dietary supplement as it is easily produced on an industrial level from a number of carbon-rich substrate by-products [6] .

With the above reasons, the researchers investigated the possibility of utilizing pineapple peels in the production of (SCP) using a yeast, specifically *Saccharomyces cerevisiae.*

Objectives of the Study

The study aimed to utilize pineapple peels to produce (SCP). Specifically, this study sought to answer the following objectives:

1. Determine the properties of pineapple peels in terms of:

   1.1. holocellulose,
   1.2. hemicellulose,
   1.3. total acid soluble lignin,
   1.4. total acid insoluble lignin,
   1.5. moisture,
   1.6. ash,
   1.7. alpha-cellulose,
   1.8. total extractives,
   1.9. reducing sugar, and
   1.10. total sugar?

2. Test if there is a significant difference in the percentage yield of SCP using the following media supplements:

   2.1. pure powdered pineapple peels hydrolysate (P),
   2.2. powdered pineapple peels hydrolysate supplemented with salts (PS),
   2.3. powdered pineapple peels hydrolysate supplemented with glucose (PG), and
   2.4. powdered pineapple peels hydrolysate supplemented with salts and glucose (PSG)

3. Compare the crude protein content of SCP from pineapple peels using the above-mentioned media supplements.

4. Test if there is a significant difference between the properties of SCP from pineapple peels using the best media supplement and the commercially available SCP.

   4.1. moisture content,
   4.2. ash content,
   4.3. crude fat content,
   4.4. crude fiber content, and
   4.5. crude protein content

2. MATERIALS AND METHOD

Collection and Preparation of Raw Materials

Fresh pineapple peels were collected from the local markets of Batangas City. The pineapple peels were washed with water thoroughly to remove dirt and impurities. Ten kg sample of fresh pineapple peels was collected and transported to the laboratory for immediate processing. Large pieces of pineapple peels were cut to smaller sizes to enhance drying at 135^{o}C for 24 hours using a forced draft oven available at the Chemical Engineering Laboratory of Batangas State University Main Campus II.

Dried samples were pulverized using a Wiley Mill available at the Unit Operations Laboratory of Batangas State University Main Campus II. The pulverized pineapple peels were sieved using a 40 mesh screen and the undersized fraction was collected and packed in a sealed container.

Dilute Acid Hydrolysis of Pulverized Pineapple Peels

Fifty milliliter of 10% (w/v) HCl was added to the each peel waste (40 g) in conical flask, respectively. The mixture/solution was placed in a water bath at 100^{o}C for one hour. After being allowed to cool, it was filtered through Whattman filter paper. The filtrates were diluted with distilled water at varying concentrations and autoclaved at 121^{o}C for 15 minutes. The sterile solution/broth thus prepared was used as carbon and nitrogen source for biomass production [7].

Biochemical Analysis of Pulverized Pineapple Peels and Hydrolysate

Upon hydrolysis, the samples were collected and placed in a sealed container, and were submitted to Central Analytical Services Laboratory (CASL) at the University of the Philippines Los Banos College, Laguna. The properties such as holocellulose, hemicellulose, total acid soluble lignin, total acid insoluble lignin, moisture, ash, alpha-cellulose, total extractives, reducing sugar, and total sugar were determined using standard NREL procedures. The methods used for moisture, ash, total extractives, acid soluble lignin, acid insoluble lignin, hollocellulose, alpha and hemicellulose were based from AOAC 925.45 Modified 19th edition, AOAC 923.03 Modified 19th edition, NREL/TP-510-42619, NREL/TP-510-42618, TAPPI 45.

Inoculum Preparation

Cultures of *Saccharomyces cerevisiae* were obtained from the Philippine National Collection of Microorganisms (PNCM) at the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Banos, and maintained on agar slants at 4^{0}C. There were cultured for large scale production.

For the preparation of solid culture medium, 100 mL of solution containing 1 gram of yeast extract, 2 grams of peptone, 2 grams of dextrose and 2 grams of agar was heated using a hot plate with magnetic stirrer until it boiled. Upon boiling the solution, the culture medium was transferred into a pre-autoclaved beaker to sterilize the culture media at 1.5 kg/cm2 for 30 minutes. Once sterilized, using a test tube plate, the solution was allowed to cool and solidify in a slanting position. A portion of the *S. cerevisiae* was scraped from the stock culture and was introduced into the agar solution. Scraping was done aseptically which meant that the harvesting loop was placed in an alcohol lamp until it became red hot then using it to collect the yeast culture. Then, the tip of the loop with the specimen was brushed to the agar solution. The brushing was done in a zigzag motion. While transferring the specimen, the test tube containing the agar solution was placed adjacent to the flame. After introducing the yeast, the test tube was sealed immediately using a cotton plug and aluminum foil then incubated at room temperature [7] .

Media Supplementation

Four trial media with different supplements were prepared in an Erlenmeyer flask sterilized at 1210C for 15 minutes and with 5% PPPH concentration [8].

Table 1
Media Composition and Its Supplements

Composition	PS	PSG	PG	P	PS
$(NH_4)2SO_4$	2 g	2g	-	-	2 g
KH_2PO_4	1 g	1 g	-	-	1 g
$MgSO_4.7H_2O$	0.5 g	0.5 g	-	-	0.5 g
NaCl	0.1 g	0.1 g	-	-	0.1 g
$CaCl_2$	0.1 g	0.1 g	-	-	0.1 g
Distilled Water	950 mL	950 mL	950 mL	950 mL	950 mL
Glucose	-	2 g	2g	-	-
Fruit Hydrolysate	5% v/v	5% v/v	5% v/v	5% v/v	5% v/v

Fermentation and Harvesting of Single Cell Protein

Submerged fermentation was carried out in 250 mL Erlenmeyer flask with the four trial media. In all the media, initial pH was adjusted to 5.5 using 1N HCl and/or 1N NaOH. Each medium (98 ml) was transferred into 250 ml Erlenmeyer flask and sterilized at 121^{0}C for 15 minutes. Inoculum of 2 ml from suspension of *S. cerevisiae* was aseptically transferred into each medium[9]. Fermentation was carried out through incubation at 28^{0}C for 7 days.

Determination of Percent Yield

To express the efficiency of the reaction, the percent yield of the reaction was calculated as follows:

$$Percent\ Yield = \frac{mass\ of\ SCP\ produced}{mass\ of\ media\ nutrients} \times 100\%$$

Production and Proximate Analysis of Single Cell Protein

After fermentation, biomass was separated from culture broth by vacuum filtration and was washed with sterile water. Before taking the weight of the biomass, the samples were oven dried at 105^{0}C for one hour and then cooled to room temperature. The samples then were analyzed at BIOTECH, University of the Philippines Los Banos, College Laguna for the testing of the proximate analysis including moisture content, ash content, crude fat content, crude fiber content and crude protein content [9]. The methods used for moisture, ash, crude fat, crude fiber and crude proteins were AOAC 925.45, AOAC 923.03, SOXTEC Application note 1982, AOAC 978.10 and AOAC 981.10 , respectively.

Statistical Treatment of Data

One-way analysis of variance (ANOVA) was used to assess if there was a significant difference in the percent yield and crude protein content of single cell protein using the different media supplements. Moreover, t-test was used to determine if there were significant differences in the properties of the single cell protein produced from the best media supplement and the commercially-available SCP.

3. RESULTS AND DISCUSSION

After the experiments and testing the properties and product, the following were the corresponding analysis and interpretation of data concerning the utilization of pineapple peels in the production of single cell protein using *Saccharomyces cerevisiae.*

Properties of Pineapple Peels

Determining the properties of pineapple peels was important in the study to know their potentiality as a substrate in the production of SCP. Table 2 presents the mean values obtained for each property.

Table 2
Properties of Pineapple Peel

Properties	*Amount in Percentage*
Moisture	7.16
Ash	5.20
Alpha cellulose	25.67
Total extractives	22.33
Hemicellulose	25.28
Holocellulose	50.96
Total Acid Insoluble Lignin	21.48
Total Acid Soluble Lignin	4.92
Reducing Sugar	5.04
Total Sugar	12.22

Results of the analysis of pineapple peels hydrolysates confirmed the presence of high amounts of lignocellulosic components such as 50.96% holocellulose, 25.67% alpha cellulose, 25.28% hemicellulose, 22.33% total extractives, 21.48% total acid insoluble lignin, 12.22% total sugar, 7.16% moisture, 5.20% ash, 5.04% reducing sugar and 4.92% total acid soluble lignin.

Holocellullose, a carbohydrate fraction of lignocellulose was found the most abundant in pineapple peels, followed by alpha cellulose and hemicellulose. This was confirmed by the study of DS and K that pineapple peels are rich in cellulose, hemicellulose and other carbohydrates. This supports that pineapple peels contain lignocellulosic materials which are the building blocks of plant cell walls indicating that there is high source of glucose. On the other hand, according to Wyman et al. [10], it is possible to yield fermentable sugars from holocellulose at near theoretical levels under mild conditions during hydrolysis which is an important factor to economic feasibility. This supports the study of Wiselogel et al. [11] that lignocellulose is the major raw material for SCP.

The increase in holocellulose is always accompanied with the decrease of lignin. Pineapple peels have relatively lower lignin content which suggests that these materials can undergo hydrolysis step more easily with the utilization of lesser amount of chemicals. Lignin fills the spaces in the cell wall between cellulose and hemicellulose. The lower total acid soluble lignin contents in the fibers is thus associated with slower decays.

Moisture is the small amount of water present in the sample. In this study, moisture content of pineapple peel was 7.16% which is higher to that of the study by Tinoi and Rakariyathama [12], where pineapple wastes had high moisture content of 13.4%. The higher moisture content could be due to the effect of geographical location since the mentioned study was conducted in Thailand and the sample was also taken in that country.

Lastly, the obtained reducing sugar was 5.04%. The amount of reducing sugar could be due to considerable amount of starch in the peels. Upon hydrolysis, reducing sugar concentration decreased to 0.88% and one of the reasons could be only less sugars went with pineapple peels hydrolysate.

Comparison of the Percentage Yield of SCP from Pineapple Peels using Different Media Supplements

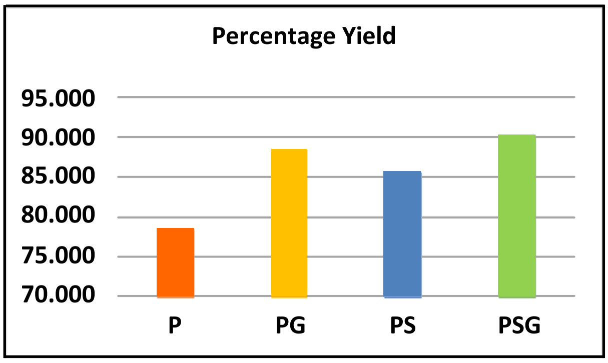

Figure 1. Percent Yield of Single Cell Protein

Results showed PSG had the highest percentage yield of 90.21%. The addition of glucose and salts in the media might have increased the percentage yield of SCP. On the other hand, the lowest percentage yield of SCP was the one that utilized pure pineapple peels only. This is understandable as no other supplement was introduced in the media.

To test the validity of the difference in the above mentioned percent yield of SCP using different media supplements, ANOVA was employed.

Table 3
Comparison on the Percentage Yield of Single Cell Protein from Pineapple Peels upon Varying Media Supplement

	p-values	Computed t-values	Decision on Ho	Verbal Interpretation
Percentage Yield	.081	2.86	Failed to Reject	Not Significant

Table 3 shows that the percentage yield, with a t-value of 2.86, had a p-value of 0.081 which is greater than 0.05 indicating no significant difference on the percentage yield of SCP using different media supplements. Since constant amount of pineapple peel hydrolysate was supplied in each medium, their values were close to each other. The percentage yield of pineapple peels depends on the amount of carbon source supplied in each medium. Increasing the amount increases its percentage yield. The presence of glucose also accounted for the increase of percentage yield. On the other hand, the presence of salts affected the percentage yield of SCP production.

Comparison of the Crude Protein of SCP from Pineapple Peels using Different Media Supplements

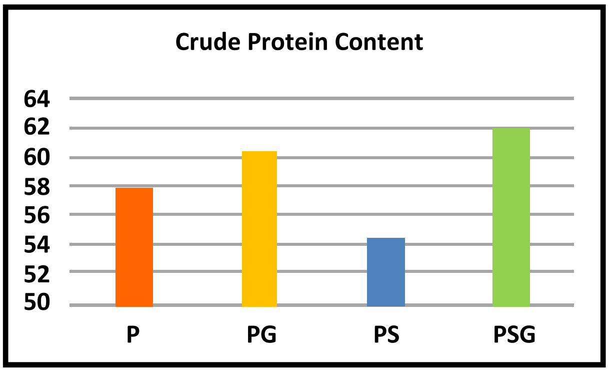

Figure 2. Crude Protein Content

Figure 2 shows the crude protein content of the each media supplements. The crude protein content of P, PG, PS, and PSG were 57.845, 60.31, 54.435 and 61.905%, respectively. The crude protein content of SCP using salt and glucose (PSG) yielded the greatest percentage. It was followed by PG which also contained glucose in its medium. These two media which were both supplemented with glucose showed high percentage of crude protein due to the glucose which enhanced the protein content within the yeast cell. Since P was only pure pineapple peel hydrolysate, it contained less crude protein compared to those media containing glucose. Lastly, PS yielded the lowest percentage because the crude protein content was not affected by the presence of salts, which can unfold proteins and hinder the protein formation. Also, excess salt in a solution can knock the peripheral membrane proteins off a cell.

The result of the crude protein content of SCP using different media nutrients is comparable to 65% yield crude protein content in the study of Nwufo et al. [13] where the effect of different substrates on the properties of SCP revealed that pineapple waste produced the highest quantity of SCP.

From the above results it may be said that *S. cerevisiae* is able to grow in pineapple peels without supplementation of inorganic carbon and nitrogen sources, the addition of which makes SCP production expensive. Under uniform conditions of experimentation, to achieve higher yield of yeast biomass and as a consequence yield higher amount of protein from *S. cerevisiae,* pineapple peels can be a good substrate.

To test the validity of the difference in the above mentioned crude protein content of SCP with varying media nutrients, one-way ANOVA was employed.

Table 4
Comparison of the Crude Protein of Single Cell Protein from Pineapple Peels Using Different Media Supplements

	p-values	Computed F-values	Decision on Ho	Verbal Interpretation
Crude Protein	0.011	16.112	Reject	Significant

α=0.05

Table 4 shows that the crude protein content, with an F-value of 16.112, had a p-value of 0.011 which is less than 0.05 indicating significant difference in crude protein content of SCP using different media supplements. This might be due to the addition of salts and glucose. This confirms the study of Mondal et al. [14], where higher percentage of protein was found in yeast biomass when *S. cerevisiae* was grown on glucose supplemented hydrolysate (GSH) followed by fruit hydrolysate medium only (FHM) and supplemented fruit hydrolysate (SFH) . The low protein content obtained from FHM could result of limited concentration of nutrients particularly carbon source. SFM yielded the lowest protein content because its nitrogen supplementation decreased SCP production. This means that choosing the right media supplement is necessary to yield a SCP with high amount of protein.

Comparison of the Best Media Supplement with the Commercially Available SCP

The media supplements used with the highest protein content and percentage yield was considered as the best medium for the production of SCP. Since PSG had the highest percentage yield and crude protein content, it was used to compare with the commercially available SCP.

Table 5 shows the comparison of the proximate analysis of the best media (PSG) and the commercially available SCP. The properties SCP from PSG were 12.26% moisture, 6.20% ash, 2.20% crude fat, 1.16% crude fiber, and 61.90% crude protein. On the other hand, the commercially available SCP had 4.86% moisture, 7.88% ash, 1.79% crude fat, 0.58% crude fiber and 60.22% crude protein.

Table 5
Comparison of the Properties of SCP from the Best Media Supplement with the Commercially Available SCP

Properties	SCP from	Commercially Available SCP %
Moisture	12.26	4.86
Ash	6.20	7.88
Crude Fat	2.20	1.79
Crude Fiber	1.16	0.58
Crude Protein	61.90	60.22

**Average Values*

According to the study of Nwufo [15], the high moisture and crude protein content make the pineapple peels a better substrate for the production of single SCP than other substrates considered in the research. Ash content reflects the presence of organic matter in pineapple peels. As organic compounds are natural substrate for microorganisms, so they will lower their content up to a certain level. Crude fat content was also determined wherein both samples showed a very low level of fat contents. It was confirmed that SCP contained low content of fats. Comparing the crude fiber of both samples, PSG had higher than commercially available SCP because pineapple peel is a fiber-rich fruit. The produced SCP from PSG has a higher protein content than the commercially-available one. The increased concentration of pineapple peel hydrolysate enhanced the biomass yield and the protein content formation within the yeast cells.

Table 6 presents the statistical results on the comparison of the properties of SCP from pineapple peels using the best media supplement to the commercially available SCP. The p-values of moisture, ash and crude fat were 0.006, 0.03, 0.008, respectively which are less than 0.05. This show a significant difference between the SCP from PSG with the commercially available in terms of moisture, ash and crude fat. The p-values of crude fiber and crude protein were 0.377 and 0.15 which are greater than 0.05. No significant difference was noted in the crude fiber and crude protein between the SCPs of PSG and commercial SCP.

Table 6
Statistical Results on the Comparison of the Properties of Single Cell Protein from Pineapple Peels using the Best Media Supplement to the Commercially Available

Properties	P - Values	Computed t - Values	Decision on Ho	Verbal Interpretation
Moisture	0.006	113.923	Reject	Significant
Ash	0.03	-21.0	Reject	Significant
Crude fat	0.008	8.3	Reject	Significant
Crude fiber	0.377	1.487	Failed to Reject	Not Significant
Crude protein	0.15	4.16	Failed to Reject	Not Significant

α=0.05

Moisture was significantly different between PSG and the commercially available SCP. This could be because the commercially available SCP had gone through series of drying procedures for it to be accepted as a commercially produced SCP.

The properties of SCP produced from pineapple peels using *S. cerevisiae* yielded higher than commercially available except ash. It confirmed that the pineapple peels can be an effective substrate in the production of SCP; also the chosen microorganism, *S. cerevisiae*, produces high protein content.

4. CONCLUSION

Based from the findings of the study, the following conclusions are hereby drawn:

The pineapple peels show a high percentage of holocellulose and hemicellulose indicating that these are good substrate for the production of SCP. There is no significant difference on the percentage yield of SCP using different media supplements.

There is a significant difference on the crude protein content of SCP using different media supplements. Comparing the SCP produced using the best medium and the commercially available SCP, there is a significant difference in moisture, ash and crude fat content but no significant difference in content but crude fiber and crude protein is noted.

5. RECOMMENDATIONS

Based on the results of the study, the following recommendations are presented:

1. Use of other agricultural wastes as a substrate for the production of SCP
2. Use of other microorganisms that are capable of producing a higher percentage yield and protein content of SCP
3. Determination of the effect of varying the concentration of fruit hydrolysate on the yield and protein content
4. Determination of the effect of varying the amount of microorganism used for SCP production including ratio and proportion of biomass
5. Consideration of methods of removal of impurities for the SCP production

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