



Comparative Analysis Of Single Cell Protein (SCP) Produced From *Saccharomyces Cerevisiae* By Utilizing Fruit Wastes

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Abstract: Comparative analysis of single cell protein (scp) produced from *Saccharomyces cerevisiae* by utilizing fruit wastes was conducted in Abuja. Malt extract agar was used to isolate *Saccharomyces cerevisiae* from fruit wastes which include watermelon, banana, orange, pineapple and pawpaw respectively using spread plate method of inoculation. After homogenization, 1g of the fruit wastes sample was dissolved in 10 ml sterilized distilled water. The sample suspension was diluted up to 10^3 . About 0.2 ml of the samples was inoculated on already prepared Malt extract agar plates. The inoculated plates were incubated at ambient temperature ($25 \pm 20^\circ\text{C}$) for 72 hrs and were subsequently sub cultured to obtain pure isolates. A total of twelve *Saccharomyces cerevisiae* belonging to one strain were isolated from the five (5) different fruits with four (4) *Saccharomyces cerevisiae* (33.33 %) isolated from Banana fruit, three (3) from orange (25 %). Two (2) *Saccharomyces cerevisiae* (16.67 %) each were isolated from Pawpaw and Pineapple respectively while only one *Saccharomyces cerevisiae* was isolated from Watermelon equivalent to 8.33 % being the least. The fruits juices were filtered with the use of a Muslin cloth. The juices were first combine in ratio 1:1 and then inoculated with 10^3 cells/ml of 48 hrs old culture of *Saccharomyces cerevisiae* isolate and then incubated for 5days to ferment. After fermentation, the dry weights were measured and the protein estimation was determined. The dry weight for the combinations of pawpaw and banana was the highest (210 mg), followed by the combinations of pineapple and banana (205 mg). Based on the fermentation caused by *Saccharomyces cerevisiae*, the highest biomass (dry weight) was recorded for banana being 220 mg. Although the biomass obtained was significantly high, but there is no significant difference between the biomass obtained from various combinations of fruits wastes ($P < 0.0$). The maximum Single cell protein content by *Saccharomyces cerevisiae* was 52.3 mg from the combination of pawpaw and banana fruit wastes. Also 52.2 mg of protein was obtained from the combination of orange and banana fruit waste, followed by the combination of pineapple and watermelon with protein content of 51.7 mg. The minimum protein content with *Saccharomyces cerevisiae* was obtained to be 25.2 mg on the combination of pawpaw and orange fruit waste. The best fruit waste combination that produced maximum single cell protein was determined to be that of pawpaw and banana which is significantly higher ($P > 0.05$) than all other combinations. The fermentation of the fruits waste by *Saccharomyces cerevisiae* has given good result for single cell protein production, which is an indication that *Saccharomyces cerevisiae* is effective in utilization of carbon source from the fruits wastes.

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1.0 Introduction

Microbial proteins or single cell proteins are dried microbial cell culture or purified protein gotten from microbial cell cultures of bacteria, yeast, algae or filamentous fungi, with prospect to be a source of protein for both plants and animals (Nasseriet al., 2017; Byrne, 2016). Single Cell Protein are sold as dehydrated and purified microbes that are used as a

source of protein for human and animal feed because of their high protein concentration, high vitamin content and low-fat content (Azam et al., 2018; Sarathadeviet al., 2017a).

The large-scale production of SCP has tremendous effect on the advancement of present-day biotechnology. Other fields involved in the research and development of single cell proteins production

includes; microbiology, biochemistry, genetics, chemical and process engineering, food technology, agriculture, animal nutrition, ecology, toxicology, medicine and veterinary science and economics (Ageitoset *al.*, 2017; Sarathadeviet *al.*, 2017b). Applications of single cell proteins includes; animal nutrition as fish breeding and fattening calves, poultry, pigs, in the foodstuffs area as it's uses include aroma carriers, vitamin carrier, emulsifying aids and to improve the nutritive value of baked products, in soups, in ready-to serve meals as well as in diet recipes, and in the technical field as in paper processing, leather processing and as foam stabilizers (Byrne, 2016).

Yeasts are the most accepted and used microorganism for single cell protein production because of their lower nucleic acid content, large size, high lysine content and ability to grow in acidic pH. Yeast single-cell protein (SCP) is a high nutrient feed substitute (Sarathadeviet *al.*, 2017b). Examples include *Candida* (Sarathadeviet *al.*, 2017b), *Hansenula*, *Pichia*, *Torulopsis* and *Saccharomyces*. Hence, the focus on yeast single cell protein instead of bacterial and algal single cell protein. Fruit wastes rich in sugar content and other basic nutrients could support microbial growth, thus, making fruit waste useful substrates in the processing and production of single cell proteins. A lot of research has been carried out over the years for reprocessing and reuse of different fruit wastes such as pineapple, pawpaw, orange, banana and watermelon for the conversion of valuable and nutritive products. Therefore, there is need to comparatively analyse single cell protein (scp) produced from *Saccharomyces cerevisiae* by utilizing fruit wastes.

2.0 Materials and Method

2.1 Study Area

The study was carried out in Maitama in Abuja Municipal area council, Federal Capital Territory, Abuja Nigeria.

2.2 Sample collection

A total of twenty (20) fruit waste samples were randomly collected from four (4) fruit vendors with five (5) different fruits types each. Fruit waste samples were collected from fruit vendors at farmers market Maitama. The fruits waste samples were collected between 10 am to 12 noon, using sterile beakers. The fruit wastes include watermelon, banana, orange, pineapple and pawpaw respectively. The fruits were collected using hand gloves and then transferred into sterile beakers, and transported to the laboratory for analysis.

2.3 Isolation of *Saccharomyces cerevisiae*

The isolation of *Saccharomyces cerevisiae* was carried out using the spread plate technique. The fruits wastes were pulverized and the juice was extracted. One milliliter (1 ml) of the sample was aseptically transferred into 10 ml of sterile distilled water as the stock culture. Tenfold serial dilutions of the stock culture were made using sterile water as diluents. Then 1.0 ml of the dilution sample was aseptically pipetted into a sterile test tube containing 9.0 ml of sterile distilled water. The contents were mixed thoroughly. Other ten-fold dilutions were similarly made up to 10^{-3} and some 0.2 ml was inoculated on the Malt Extract Agar using the spread plate method.

The plates were allowed to stand undisturbed for 15 minutes and then incubated at ambient temperature ($25 \pm 2^{\circ}\text{C}$) for 72 hours. Colony developments were observed after the incubation period. The colonial density were calculated as the count multiplied by the dilution factor and the mean count obtained was recorded and expressed in colony forming units per milliliter (cfu/ml) of the sample analyzed.

2.3.1 Preparation of Pure Cultures of Yeast isolates

The young colonies of yeast isolates were aseptically picked up and streaked on fresh sterile Malt Extract Agar plates to obtain pure cultures. The pure cultures were grown at ambient temperature ($25 \pm 2^{\circ}\text{C}$) for 72 hours and stored at 4°C .

2.3.2 Identification of Yeast Strains

The yeast isolates were characterised based on their morphological, biochemical properties according to Kurtzman *et al.* (2017). Among the characteristics used were colonial characteristics such as size, surface appearance, texture and colour of the colonies. Appropriate references were then made using mycological identification keys and taxonomic description.

2.3.2.3 Morphological characteristics

The yeast strains were checked for their morphological characteristic features such as textures (mucoid, fluid or viscous, butyrous); elevation (flat or raised); colour (yellow, orange and red); surface (glistening or dull, smooth, rough, and sectored) and margin (entire, undulating, lobed, and filaments) were investigated. The cells of a young actively growing culture from 2~3 days at 25°C were stained by lacto phenol-cotton blue and examined microscopically to determine the shape of cells, budding or fission formation.

2.3.4 Biochemical characteristics

The ability of the yeasts isolates to utilize and grow aerobically on carbon energy source was studied. Several carbon sources (D-glucose, D-galactose, lactose, maltose, sucrose and D-xylose) were used in this study. The growth of colonies on negative control plates (without carbon sources) was compared with plates supplemented with carbon sources after 24-48 h of incubation. On the other hand, fermentation abilities of yeast isolate to ferment 2% sugar solutions of (glucose, lactose, maltose, raffinose, galactose, sucrose, and xylose) were tested.

2.4 Preparation of Fruit Waste Juice

The fruits wastes were washed with sterile distilled water and then macerated separately in a blender (National, MX-795N) for 5 minutes. The fruits juices were filtered with the use of a Muslin cloth. Following incubation, the juices extracts were placed in conical flasks in ten (10) combinations of 1:1 thus;

Pawpaw and Orange ratio 1:1
 Pawpaw Pineapple ratio 1:1
 Pawpaw and Watermelon ratio 1:1
 Pawpaw and Banana ratio 1:1
 Orange and Pineapple ratio 1:1
 Orange and Watermelon ratio 1:1
 Orange and Banana ratio 1:1
 Pineapple and Watermelon ratio 1:1
 Pineapple and Banana ratio 1:1
 Watermelon and Banana ratio 1:1

2.4.1 Production of Single Cell protein

The medium consisting of fruit waste extracts; 50%, ammonium sulphate; 0.3%, potassium dihydrogen phosphate; 0.1%, magnesium sulphate heptahydrate; 0.03% and calcium chloride; 0.03% (w/v) were sterilized and employed as the substrate for the production of single cell protein. The suspensions containing 10^3 cells/ml of 48 hrs old culture of *Saccharomyces cerevisiae* isolate was aseptically introduced into each medium. Cultures were then incubated at $25 \pm 2^\circ\text{C}$ in a rotary shaker incubator at 100rpm for 5 days. Un-inoculated fruits medium serves as the control. All the experiments were carried out in duplicates.

2.4.2 Determination of Biomass and Single Cell Protein Production

After fermentation, the culture liquid was poured into test tubes and then centrifuged at 4000 rpm for 10 minutes. The sediments were collected, washed with

sterile water and the sediments were separately transferred into an aluminium foil and the wet weight was determined. Before taking the dry weight, the sediments were oven dried at 105°C for one hour followed by cooling in desiccators. The dry weights were measured and the protein estimation were determined according to method of Kjeldahl as described by AOAC (2010) thus; 0.5g of each sample was carefully weighed into the kjeldahl digestion tubes to ensure that all materials get to the bottom of the tubes. One (1) tablet of kjeldahl catalyst and 10ml of concentrated H_2SO_4 were added before setting in the appropriate hole of the digestion block heaters in a fume cupboard and heated for 4hrs. The digest was then cooled and carefully transferred into 100ml volumetric flask thoroughly rinsing the digestion tube with distilled water. Five milliliter (5ml) portion of the digest was then pipetted into the distillation apparatus and 5ml of 40 % (w/v) NaOH was added. The mixture was steam distilled for 2 minutes into 500ml conical flask containing 10ml of 2%Boric acid with mixed indicator solution and placed at the receiving top of the condenser. The solution was then titrated against 0.01N HCl in a 50ml burette.

$\% \text{ Nitrogen} = (\text{Titre value} \times \text{Atomic mass Nitrogen}) / (\text{Normality of HCL acid used} \times 4) \times 100$
 Crude protein = $\% \text{ Nitrogen} \times 6.25$

2.5 Data Analysis.

The results were expressed as mean \pm SEM and the statistical analysis was determined using one way Analysis of Variance (ANOVA) from Ms Excel Statistics. Test applied was F-test statistic at $p=0.05$.

3.0 Results

3. Identification of yeast isolated from fruit wastes

The yeast strain was identified on the basis of their morphological characteristic features and biochemical characteristics as presented in Table 1. The observed morphological characteristics showed that the yeast is unicellular, ovoid in shape, cream colour, smooth, larger than bacterial cells and flat edge. The biochemical characteristics result showed that when the isolated *Saccharomyces cerevisiae* was subjected to sugar fermentation test, it shows that the isolate was positive and able to ferment D-glucose, D-maltose, D-galactose and sucrose respectively but, was unable to ferment D-xylose and lactose which gave negative result for fermentation test.

Table 1: Characteristics of *Saccharomyces cerevisiae* isolated from fruit wastes in Abuja-FCT

| Features | Characteristics |
|----------------------|----------------------------|
| Morphological | |
| Form | Unicellular |
| Shape | Ovoid |
| Colour | Cream |
| Texture | Smooth |
| Size | Larger than bacteria cells |
| Edge | Flat |
| Biochemical | |
| D-Glucose | + |
| D-maltose | + |
| Lactose | - |
| D-xylose | - |
| D-galactose | + |
| Sucrose | + |

Keys; += positive, - = Negative

3.2 Frequencies of Occurrence of *Saccharomyces cerevisiae* Isolated from Fruit Wastes

Table 2 showed the frequencies of occurrence of *Saccharomyces cerevisiae* isolated from fruit wastes. A total of twelve *Saccharomyces cerevisiae* belonging to one strain were isolated from the five (5) different fruits with four (4) *Saccharomyces cerevisiae* (33.33 %) isolated from Banana fruit, three (3) from orange (25 %). Two (2) *Saccharomyces*

cerevisiae (16.67 %) each were isolated from Pawpaw and Pineapple respectively while only one *Saccharomyces cerevisiae* was isolated from Watermelon equivalent to 8.33 % being the least. The frequencies of occurrence of the *Saccharomyces cerevisiae* isolated from the fruit wastes were significantly ($P < 0.05$) higher in banana fruit waste compared to the other fruit wastes.

Table 2: Frequencies of Occurrence of *Saccharomyces cerevisiae* Isolated from Fruit Wastes

| Fruit Waste | Frequencies | Percentages (%) |
|-------------|-------------|-----------------|
| Banana | 4 | 33.33 |
| Orange | 3 | 25.0 |
| Pawpaw | 2 | 16.67 |
| Pineapple | 2 | 16.67 |
| Watermelon | 1 | 8.33 |

3.3 Biomass Obtained from Fruit Wastes

The result of biomass obtained from various combinations of fruit waste is presented in Figure 1. The dry weight of the combinations of pawpaw and banana was the highest (220 mg), followed by the combinations of pineapple and banana (205 mg). However, the combinations of pawpaw and pineapple

as well as orange and banana combinations had a dry weight of 195 mg each and the dry weight of orange and watermelon was 135 mg being the least. Although the biomass obtained was significantly high, but there is no significant difference between the biomass obtained from various combinations and uncombined fruit wastes ($P > 0.05$).

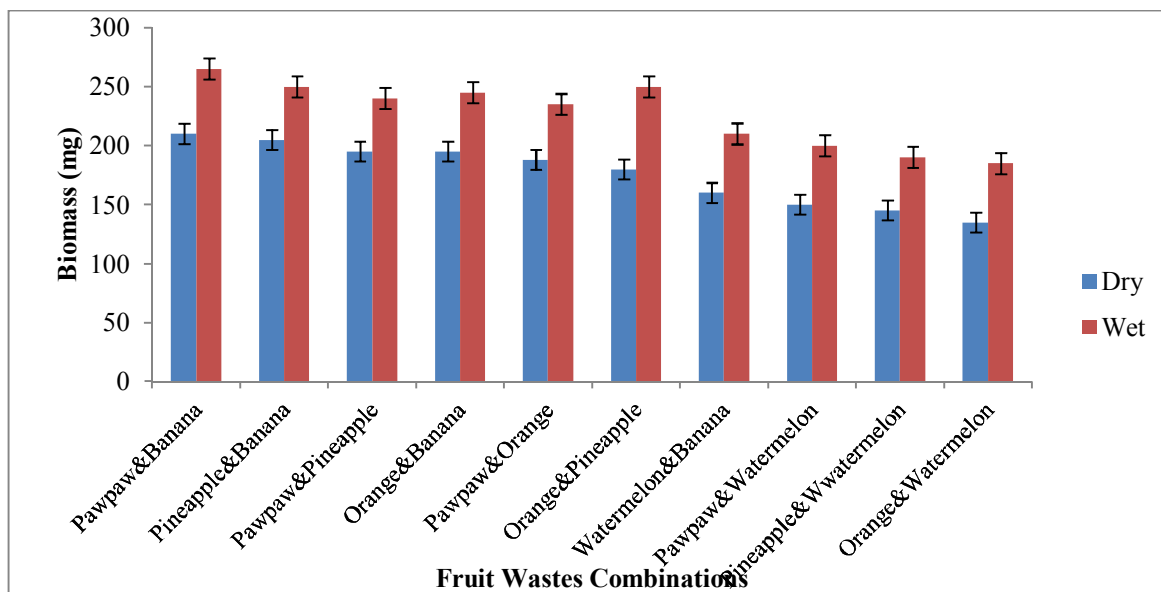


Figure 1: Biomass Obtained From Various Combinations of Fruit Wastes

3.3 Single Cell Protein from Combinations of Fruit Wastes

Table 3 showed the single cell protein obtained from combinations of various fruit wastes. The maximum Single cell protein content with *Saccharomyces cerevisiae* was obtained to be 52.3 mg from the combination of pawpaw and banana fruit wastes. Also 52.2 mg of protein was obtained from the combination of orange and banana fruit waste,

followed by the combination of pineapple and watermelon with protein content of 51.7 mg. The minimum protein content with *Saccharomyces cerevisiae* was obtained to be 25.2 mg on the combination of pawpaw and orange fruit waste. The best fruit waste combination that produced maximum single cell protein was determined to be that of pawpaw and banana which is significantly higher ($P>0.05$) than all other combinations.

Table 3: Single Cell Protein from Various Combinations of Fruit Wastes

| Combinations of fruit wastes | Ratio | SCP (mg) |
|------------------------------|-------|----------|
| Pawpaw and Orange | 1:1 | 25.2 |
| Pawpaw and Pineapple | 1:1 | 30.9 |
| Pawpaw and Watermelon | 1:1 | 40.3 |
| Pawpaw and Banana | 1:1 | 52.3 |
| Orange and Pineapple | 1:1 | 41.3 |
| Orange and Watermelon | 1:1 | 36.7 |
| Orange and Banana | 1:1 | 52.2 |
| Pineapple and Watermelon | 1:1 | 51.7 |
| Pineapple and Banana | 1:1 | 46.1 |
| Watermelon and Banana | 1:1 | 30.5 |

Key: SCP= Single cell protein

4.0 Discussions

Unutilized large quantity of generated fruits waste challenged researchers to have a look at ways to improve their nutritional worth by using them as substrates in the production of single cell protein. The most commonly used yeast in the production of single cell protein is *Saccharomyces cerevisiae*, this may be due to the fact that it is generally accepted in human food industry and animal feed industry.

From this study twelve *Saccharomyces cerevisiae* belonging to one strain were isolated from the five (5) different fruit wastes with four (4) *Saccharomyces cerevisiae* (33.33 %) isolated from banana waste, three (3) from orange waste (25 %). Two (2) *Saccharomyces cerevisiae* (16.67 %) each were isolated from pawpaw and pineapple respectively while only one *Saccharomyces cerevisiae* was isolated from watermelon equivalent to 8.33% being the least. The frequencies of occurrence of the *Saccharomyces cerevisiae* isolated from the fruit wastes were significantly high which is in agreement with the report of De Gregorio *et al.* (2017).

It appears from this study, that a good approach to waste utilization for single cell protein production is combination of different wastes. It was observed that the maximum single cell protein content with *Saccharomyces cerevisiae* was obtained to be 52.3 mg on the combination of pawpaw and banana fruit wastes. These findings are in agreement with findings of Aggelopoulos *et al.* (2018). Aggelopoulos *et al.* (2018) produced single cell protein by combining agricultural waste feedstock containing simple sugars-rich molasses, fibre-rich orange and potato pulps, and protein-rich brewer's spent grains, whey and malt spent rootlets. The most advanced commercial production of single cell protein is the yeast-based process. The combinations of fruit waste containing banana tends to produced the highest single cell protein. This is because Banana waste contained high composition of carbohydrate and protein content which is essentially useful in *Saccharomyces cerevisiae* biomass production; this is in agreement with the observations of Mondal *et al.* (2018) and Lee *et al.* (2019) who reported the successful utilization of fruit waste in producing Single Cell Proteins.

In summary, factors to consider when choosing suitable substrate for single cell protein production should include local availability of the substrate, pretreatment costs of the substrate before using it in fermentation, transportation cost of the substrate and single cell protein yield after fermentation. *Saccharomyces cerevisiae* is effective in utilization of carbon source from the fruits wastes

thereby resulting in single cell protein and the single cell protein increased with the increase in the biomass level of the fruit waste.

4.1 Conclusion

It can be concluded from this study that *Saccharomyces cerevisiae* can be used for the exploitation of agricultural wastes management, and for single cell protein production which will greatly minimize, if not eliminate the immense cost of wastes pollution control.

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