



## **Incidence of *Saccharomyces cerevisiae*, Proximate Composition and Single Cell Protein Produced from Fruit Wastes**

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**Abstract:** The study on incidence of *Saccharomyces cerevisiae*, proximate composition and single cell protein produced from 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 technique 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. 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, four (4) were isolated from banana waste, three (3) from orange waste, two (2) each from pawpaw and pineapple respectively while only one was isolated from watermelon being the least. The protein content for the selected fruit wastes showed that the crude protein content was higher in pawpaw ( $18.09 \pm 0.02\%$ ) and lower in pineapple ( $5.11 \pm 0.47\%$ ) and, there was no significant difference ( $P < 0.05$ ) between the proximate values of each of the fruits wastes obtained. The extracted fruits juices were filtered with the use of a Muslin cloth. The juices were separately inoculated with  $10^3$  cells/ml of 48 hrs old culture of *Saccharomyces cerevisiae* isolate and then incubated for 5 days to ferment. After fermentation, the dry weights were measured and the protein estimation was determined. The result showed that the dry weight of banana waste was the highest (210 mg), followed by pawpaw with 200 mg. Pineapple had a dry weight of 190 mg and the dry weight of orange was 170 mg while that of watermelon was 100 mg. Based on the fermentation caused by *Saccharomyces cerevisiae*, the highest biomass (wet and dry biomass) was recorded for banana being 270 and 210 mg respectively. The single cell protein obtained from the fruits wastes using *Saccharomyces cerevisiae* showed that banana had a significantly higher ( $P > 0.05$ ) quantity of single cell protein of 26.2 mg than pawpaw and watermelon with 20.7 mg and 20.2 mg respectively. Similarly, Orange produced 15.5 mg of single cell protein which was higher than that of pineapple having produced only 12.6 mg of single cell protein, being the least. It is evident that the fruit wastes have significant proximate values which rapidly promote the growth of *Saccharomyces cerevisiae* cells for the production of single cell protein.

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**Keywords:** *Saccharomyces cerevisiae*, fruit wastes, single cell protein

### **1.0 Introduction**

Yeasts are unicellular eukaryotes that belong to the Kingdom of Fungi and play various roles in affecting the quality and safety of food products. They are ubiquitous, and commonly spoilage fruits, vegetables and other plant materials, in addition to, an association with soil and insects (Bekatorou *et al.*, 2019). Decaying fruits are an important microhabitat for several yeast species (Stamer and Lachance, 2018). Colonization of fast growing, fermentative, low assimilative profiles of apiculate yeasts: (*Kloeckera*, *Hanseniaspora*, and *Saccharomyces*) initiate severe deterioration of fruits (Jager *et al.*, 2018). Oranges and mangoes usually rotted by spoilage of a wide variety of fermentative or weakly

non-fermenting yeasts. Basidiomycetous genera such as *Cryptococcus* and *Sporobolomyces* yeasts were dominated on leaves of mango trees (Jager *et al.*, 2018). In addition, ascomycetous yeasts associated the wounded parts of the phylloplane (Stamer and Lachance, 2018). Fruit juices and soft drinks constitute suitable environment for growth of most microorganisms. Actually, beverages are excellent substrates for supporting the growth of yeasts, where the highest amount of nitrogenous compounds and vitamins promote occurrence of yeasts (El-Sharoud *et al.*, 2019).

In the year 1967 Single Cell Protein was produced commercially and currently different microbes and substrates are made use of in large-

scale single cell protein production. Examples of microbes used includes; algae (*Spirulina* spp and *Chlorella* spp) which utilizes carbon dioxide, yeasts (*Candida lipolytica* and *utilis*) utilize ethanol, filamentous fungi (*Fusarium graminearum* and *Chaetomium celluloliticum*) which utilizes starch and cellulose waste respectively, and bacteria (*Methylophilus methylitropous* and *Brevibacterium*) that utilizes hydrocarbon and methanol respectively (Nasseri *et al.*, 2018). These microbes have the ability to ferment large quantities of waste that are used as substrate to produce protein (Nasseri *et al.*, 2018).

## 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.

#### 2.3.1 Proximate analysis of the fruits wastes

The fruit wastes were analysed for protein, carbohydrates, crude lipid, moisture content, ash contents, and crude fibre according to the method of analyses described by Association of Official Analytical Chemists (AOAC, 2010).

##### 2.3.1.1 Determination of Moisture content

Exactly 20.0g of each fruit waste sample was separately weighed into pre-weighed crucibles and placed in an oven at 105 °C for 3 hours. It was cooled in a desiccator and weighed again. The moisture contents were then calculated by difference in weight using the formular;

$$\text{Moisture Content (\%)} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where  $W_1$  = Weight of sample and crucible before

$W_2$  = Weight of sample and crucible after drying

$W_0$  = Weight of crucible

##### 2.3.1.2 Determination of Lipid Content

Exactly 20.0g of each fruit waste samples were placed in a funnel and 10 ml of petroleum spirit (40-60 °C) was added and allowed to stand, the upper layer (petroleum spirit layer) was collected in pre-weighed beaker and the solvent was allowed to evaporate, the beaker was then reweighed and the weight recorded (AOAC, 2010). The crude lipid was calculated thus:

$$\text{Crude Lipid (\%)} = \frac{W_1 - W_2}{W_0} \times 100$$

Where  $W_1$  = Weight of sample

$W_2$  = Weight of sample and beaker after drying the solvent

$W_0$  = Weight of empty beaker

##### 2.3.1.3 Determination of Ash Content

Exactly 20.0g of each fruit waste samples were weighed into a pre-weighed crucible and then placed in a muffle furnace at 550 °C for 5 hours. These were allowed to cool in desiccators and then weighed. The percentage ash content was calculated thus:

$$\text{Ash Content (\%)} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where  $W_1$  = Weight of sample and crucible before ashing

$W_2$  = Weight of sample and crucible after ashing

$W_0$  = Weight of crucible

##### 2.4.1.4 Determination of Crude fibre

Exactly 1.0g of each fruit waste was weighed into a flask and 200ml of sulphuric acid solution concentration of 1.25% was added and boiled for 30 minutes with periodic rotation. After boiling the solution was filtered and residue washed with hot water to remove acidic residue completely. The washed residue was now transferred into a clean flask and 200ml of sodium hydroxide added and boiled for another 30 minutes with periodic rotation as done previously in acidic solution. The solution was then filtered again and washed with hot water to remove the sodium hydroxide residue completely. The residue was then collected in clean pre-weighed crucible and placed on a hot plate to remove the excess water. It was then placed in hot air oven for 230 °C for 2 hours and allowed to cool for 20 minutes in a desiccator and weighed. The crucible was then place in a muffle furnace for 2 hours at 550 °C and cooled in a desiccator 20 minutes and weighed again. The crude fibre was calculated thus:

$$\text{Crude fire content} = \frac{W_1 - W_2}{W_0} \times 100$$

Where:

$W_1$  = weight of crucible with dry residue (g)

$W_2$  = weight of crucible with ash (g)

Wo = weight of sample (g)

### 2.3.1.5 Determination of crude protein

One gram (1.0g) each of the fruits waste samples were separately placed into a Kjeldahl flask. Three grams (3g) anhydrous sodium sulphate and one (1g) of hydrated copper sulphate (catalyst) were added into the flask. Then 20 ml of concentrated tetraoxosulphate (VI) acid ( $H_2SO_4$ ) was added to digest the sample. The digestion continued under heat until a clear solution was observed. The clear solution was then cooled and made up to 100ml with distilled water and a digest of 5 ml was collected for distillation. Also, 5 ml of sodium hydroxide (NaOH) was placed into the distillation flask and distillation was allowed to take place for 2 minutes. The ammonia distilled off was absorbed into 10 ml 2% boric acid with mixed indicator solution (100 parts bromocresol green 0.1% in 95% alcohol and 20 parts methyl red, 0.01% in 95% alcohol) and placed at the receiving top of the condenser this was titrated with 0.01N hydrochloric acid (HCl) in a 50ml burette. The titre value of the end point at which the colour changed from green to pink was taken. The crude protein was calculated as:

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

$$\text{Crude protein} = \% \text{ Nitrogen} \times 6.25$$

### 2.3.1.6 Carbohydrate

Carbohydrate content was determined by the difference i.e  $100 - (\% \text{moisture} + \% \text{ash} + \% \text{crude fibre} + \% \text{crude lipid})$ .

### 3.3.2 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 ( $25 \pm 2^\circ C$ ) 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.

### 2.3.2.1 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 ( $25 \pm 2^\circ C$ ) for 72 hours and stored at  $4^\circ C$ .

### 2.3.2.2 Identification of Yeast Strains

The yeast isolates were characterised based on their morphological, biochemical properties according to Kurtzman *et al.* (2018). 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  $25^\circ C$  were stained by lacto phenol-cotton blue and examined microscopically to determine the shape of cells, budding or fission formation.

### 2.3.2.4 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. The juices extracted were placed separately in different conical flasks.

#### 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  $105^\circ\text{C}$  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  $\text{H}_2\text{SO}_4$  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.

% Nitrogen= (Titre value x Atomic mass Nitrogen)/(Normality of HCL acid used x 4) x 100  
Crude protein =% Nitrogen x 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

#### 3.1 Proximate Composition of Fruit Wastes

The proximate composition of the fruit waste is presented in Table 1. The protein content for the selected fruit wastes before fermentation showed that the crude protein content was higher in pawpaw ( $18.09 \pm 0.02$  %) and lower in pineapple ( $5.11 \pm 0.47$  %). Other values are watermelon ( $12.42 \pm 0.10$  %), banana ( $10.41 \pm 1.16$  %) and orange ( $9.71 \pm 0.46$  %). Watermelon fruit waste sample had the highest crude fibre of  $26.31 \pm 0.06$  %, while pineapple and orange fruits wastes recorded  $14.80 \pm 0.01$  and  $14.18 \pm 0.17$  % respectively and banana recorded  $11.80 \pm 0.14$  % being lowest value for crude fibre. Pawpaw and watermelon had the highest values of moisture content ( $16.60 \pm 0.67$  % and  $16.47 \pm 0.01$  %) respectively and pineapple had moisture content of  $14.87 \pm 0.07$  % while orange and banana recorded  $8.95 \pm 0.10$  % and  $5.11 \pm 0.13$  % respectively, being the least value of moisture content.

Similarly, the crude lipid content showed that pawpaw ( $18.09 \pm 0.02$  %) had higher concentration of fats as compared to the other fruit wastes. Ash value turned out to be high in banana and pawpaw ( $12.44 \pm 0.31$  % and  $10.21 \pm 0.01$  %) respectively, and low in pineapple, watermelon and orange ( $4.39 \pm 0.21$  %,  $5.03 \pm 0.81$  % and  $5.18 \pm 0.92$  %) respectively. The carbohydrate values obtained for the fruit waste samples ranged from  $32.16 \pm 1.11$  % to  $55.52 \pm 0.93$  % for watermelon and pineapple respectively. There was no significant difference ( $P < 0.05$ ) between the proximate values of each of the fruit wastes.

**Table 1: Proximate composition of different Fruit Wastes**

Fruits Samples	Moisture (%)	Crude lipid (%)	Crude protein (%)	Ash (%)	Crude Fibre (%)	Carbohyd-rate (%)
Banana	$5.11 \pm 0.13$	$8.41 \pm 1.05$	$10.41 \pm 1.16$	$12.44 \pm 0.31$	$11.80 \pm 0.14$	$43.42 \pm 0.55$
Orange	$8.95 \pm 0.10$	$8.71 \pm 0.74$	$9.71 \pm 0.46$	$5.18 \pm 0.92$	$14.18 \pm 0.17$	$53.27 \pm 0.07$
Pawpaw	$16.60 \pm 0.7$	$5.48 \pm 0.92$	$18.09 \pm 0.02$	$10.21 \pm 0.01$	$12.15 \pm 0.07$	$37.47 \pm 0.72$
Pineapple	$14.87 \pm 0.7$	$5.31 \pm 0.72$	$5.11 \pm 0.47$	$4.39 \pm 0.21$	$14.80 \pm 0.01$	$55.52 \pm 0.93$
Watermelon	$16.47 \pm 0.1$	$12.61 \pm 0.63$	$12.42 \pm 0.10$	$5.03 \pm 0.81$	$26.31 \pm 0.06$	$32.16 \pm 1.11$

**3.2 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 2. 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 2: Characteristics of *Saccharomyces cerevisiae* isolated from fruit wastes in Abuja-FCT**

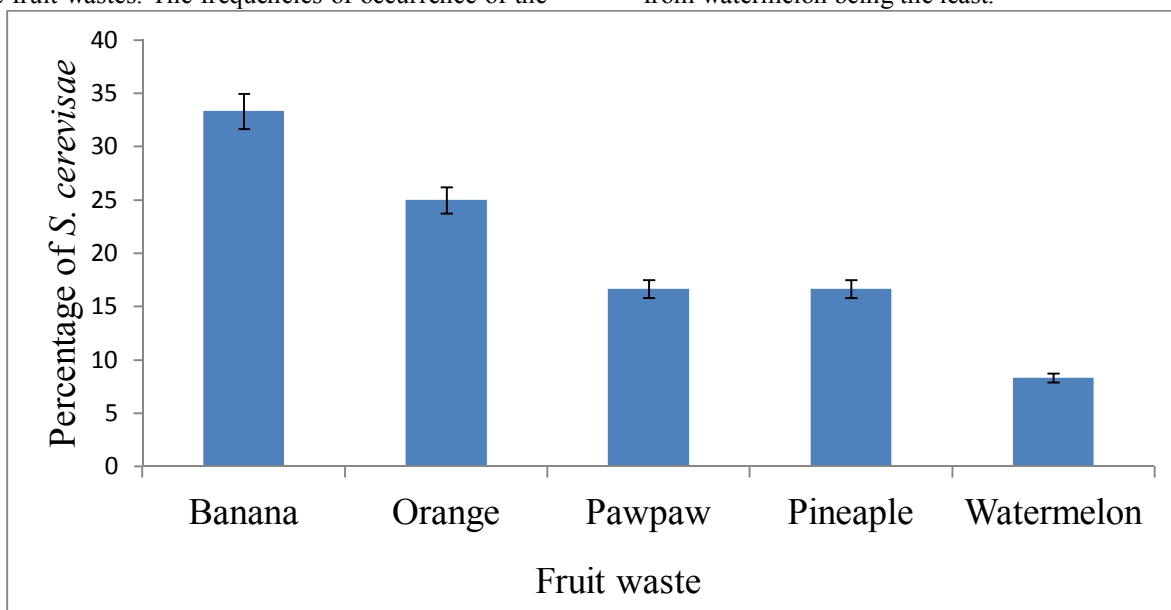
Features	Characteristics
<b>Morphological</b>	
Form	Unicellular
Shape	Ovoid
Colour	Cream
Texture	Smooth
Size	Larger than bacteria cells
Edge	Flat
<b>Biochemical</b>	
D-Glucose	+
D-maltose	+
Lactose	-
D-xylose	-
D-galactose	+
Sucrose	+

Keys; += positive, - = Negative

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

Figure 1 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 fruit wastes. 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, four (4) were isolated from banana waste, three (3) from orange waste, two (2) each from pawpaw and pineapple respectively while only one was isolated from watermelon being the least.

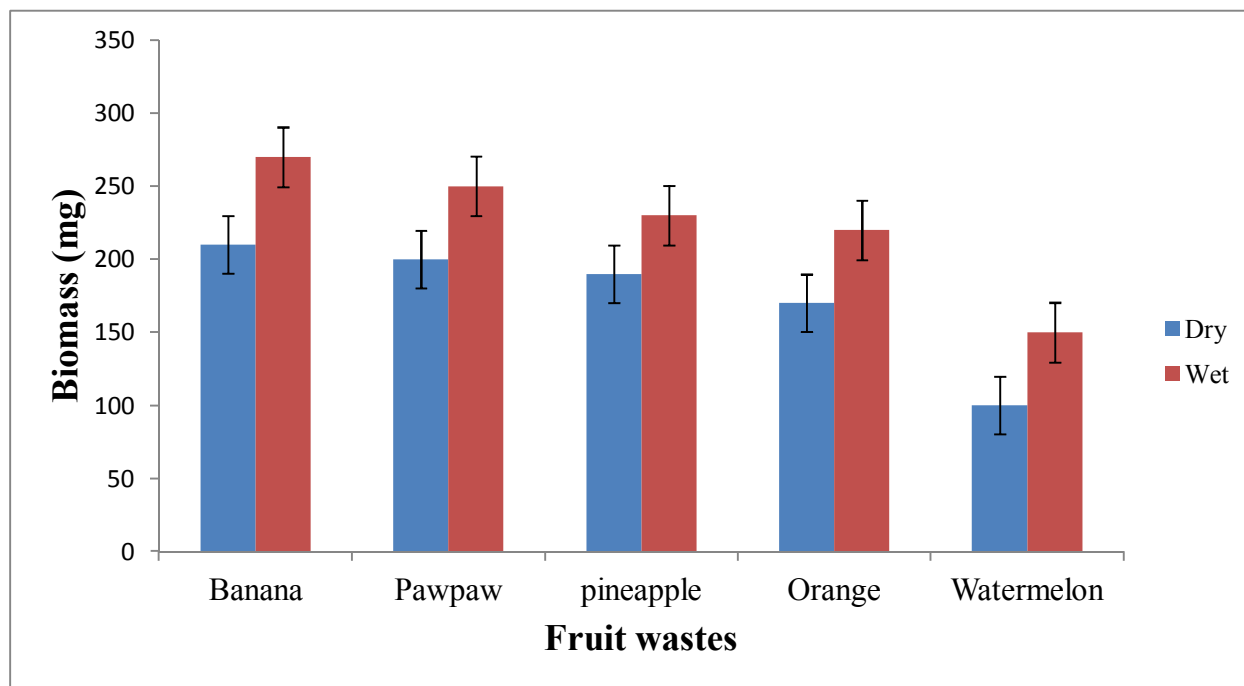


**Figure 1: Frequencies of Occurrence of *Saccharomyces cerevisiae* Isolated from Fruit Wastes.**

**3.1 Biomass Obtained from Fruit Wastes**

The biomass obtained from the different fruit wastes is presented in Figure 2. The result showed that the dry weight of uncombined banana waste was the highest (210 mg), followed by pawpaw with 200 mg. Pineapple had a dry weight of 190 mg

and the dry weight of orange was 170 mg while that of watermelon was 100 mg. Based on the fermentation caused by *S. cerevisiae*, the highest biomass (wet and dry biomass) was recorded for banana being 270 and 210 mg respectively.



**Figure 2: Biomass Obtained from Fruit Wastes.**

**3.2 Single Cell Protein from Fruit Wastes**

Table 2 showed the single cell protein obtained from the fruit wastes using *Saccharomyces cerevisiae*. Banana had a significantly ( $P < 0.05$ ) higher quantity of single cell protein of 26.2 mg than

pawpaw and watermelon with 20.7 mg and 20.2 mg respectively. Similarly, orange produced 15.5 mg of single cell protein which was higher than that of pineapple having produced only 12.6 mg of single cell protein, being the least.

**Table 2: Single Cell Protein from Fruit Wastes**

Fruit wastes (20.0g)	SCP (mg)
Banana	26.2
Pawpaw	20.7
Watermelon	20.2
Orange	15.5
Pineapple	12.6

**Key; SCP= Single cell protein**

#### 4.0 Discussions

This study shows that the proximate analysis of banana, pawpaw, pineapple, orange and watermelon fruit waste have variable amount of carbohydrate, protein, lipid and moisture content which are valuable for microbial growth in the production of single cell protein. Additional measure can be taken to ensure that the medium contains all nutrients and elements required for the cultivation of microorganisms. The yeast *Saccharomyces cerevisiae* contains several suitable characteristics, for example; its ability to utilize a wide range of substrates like hexose, pentose, hydrocarbons, susceptibility to genetic variation, capacity to flocculate and also its high nutritional worth. It appears from this study that there was no significant difference ( $P < 0.05$ ) between the proximate values of each of the fruit wastes. The frequencies of occurrences of *Saccharomyces cerevisiae* isolated from the fruit wastes include four (4) from banana waste, three (3) from orange waste, two (2) each from pawpaw and pineapple respectively while only one was isolated from watermelon being the least.

In this study, 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. The most advanced commercial production of single cell protein is the yeast-based process. However, this present study investigated the bioconversion efficiency of banana, pawpaw, pineapple, orange and watermelon waste into single cell protein by *Saccharomyces cerevisiae*. Banana had a significantly higher quantity of single cell protein of 26.2 mg than pawpaw and watermelon with 20.7 mg and 20.2 mg respectively. Also, Orange produced 15.5 mg of single cell protein which was higher than that of pineapple being the least with only 12.6 mg of single cell protein. The use of Mango, Apple, Banana, Carrot and Orange wastes as potential substrate for microbial growth and single cell protein production was also reported by Mahmoud (2018) which is in agreement with this study. The use of fruit waste and other organic wastes for fungal biomass production aids to combat pollution, malnutrition by providing affordable protein supplements to the less privileged. This study revealed that banana waste generated the highest amount of fungal biomass and single cell protein. The reason may be due to the high carbohydrate content in banana waste than other fruit wastes. This is also in agreement with the findings Khan and Dahot

(2017) who reported that banana waste is the best substrate to use in the production of *Saccharomyces cerevisiae* biomass. Yakoub and Umar (2017) reported that the growth of fungi depends mainly on the nutritional composition of the substrate that can support the organism biomass. Therefore, the banana waste contains high chemical compositions requirement than other wastes thus supported faster growth of the fungus thereby yielding more single cell protein.

#### 4.1 Conclusion

From this study it is evident that the fruit wastes have significant proximate values which rapidly promote the growth of *Saccharomyces cerevisiae* cells for the production of single cell protein.

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