

# Comparative Study of Bioethanol Production Ability of *Bacillus Subtilis* and *Saccharomyces Cerevisiae* Using Peels of Watermelon and Pineapple

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**Abstract:** Comparative study of bioethanol production ability of *Bacillus subtilis* and *Saccharomyces cerevisiae* using peels of watermelon and pineapple was investigated. The peels of watermelon and pineapple are lignocellulosic wastes which can be utilized as a common and cheap substrate for bioethanol production. The peels were prepared, pretreated and then fermented with *Bacillus subtilis* and *Saccharomyces cerevisiae* using the solid state fermentation method for five days. The fermentation process was monitored with the determination of titratable acidity, pH, temperature and microbial load using standard techniques and microbiological methods. Proximate and mineral composition together with the bioethanol composition were determined before and after fermentation using conventional procedures. The pH of the substrates decreased from (9.16-3.30), the titratable acidity also decreased from (0.018g/L-0.002g/L) and the temperature was fairly constant. The proximate compositions of the substrates varied with an increase in the ash, moisture, fat and crude fibre composition and decrease in the protein and carbohydrate composition of the fermented substrates as compared to the raw samples. The maximum ethanol yield was obtained from pineapple peels fermented with *Saccharomyces cerevisiae* (2.51%±0.43 v/v) while the minimum yield was obtained from the peels of pineapple fermented with *Bacillus subtilis* (1.21%±0.16 v/v). The ethanol yield from the fermentation process indicated that *Saccharomyces cerevisiae* was more efficient in bioethanol production with the peels of pineapple and watermelon than *Bacillus subtilis*.

**Keywords:** *S. cerevisiae*, *B. subtilis*, pineapple peel, watermelon peel, Solid state fermentation

## 1. Introduction

Ethanol is one of the most advanced liquid fuel because it is environmental friendly [1]. Its carbon content has a vegetable origin and as a consequence, when it is released during the combustion process, it does not contribute to the increase of carbon dioxide in the atmosphere, reducing global warming [2]. Biomass is the earth's most attractive alternative among fuel sources and sustainable energy resource. The annual availability of these wastes amounts to 1.05 billion tons. The major part of this is mostly discarded and it is the main source for increasing the pollution in environment on occasions. The mechanical drying of these wastes (watermelon and pineapple peel) gave opportunity to store the substrate all over the year.

One of the most abundant sources of energy in the world is the bio-polymer cellulose, which forms a major component of most plant and algal cell walls. Different acids and bases with the enzyme cellulases are able to hydrolyze this cellulose into its constituent glucose units. The glucose can then be utilized by organisms such as *Saccharomyces cerevisiae* and *Bacillus subtilis* which can ferment the glucose into ethanol. This work deals with the production of bioethanol from watermelon and pineapple peels. The generation of bio-fuels from wastes forms an attractive solution towards both waste management and energy generation. This study is thus aimed at a comparative study on the bioethanol producing ability of *Saccharomyces cerevisiae* and *Bacillus subtilis* by utilizing the peels of pineapple and watermelon.

## 2. Materials and Methods

### Collection of Raw Materials

The substrates used for the fermentation process are pineapple and watermelon peels. The substrates were collected around South-Gate and Stateline fruit Shops, FUTA South-Gate, Akure, Nigeria. The substrates were washed twice with distilled water and then wiped with 70% ethanol, after which they were chopped into small pieces and dried in room temperature. The dried substrates were then powdered using a grinder name- Retsch GmbH, Model: 5657 HAAN.

### Preparation of Substrates

Five hundred grams of each substrate (pineapple and watermelon peel flour) was weighed. This was followed by pre-treatments, first by treating with 2% NaOH. The treated pineapple and watermelon peels flour was subjected to heat treatment by autoclaving at 121°C for 1 hour. After the heat treatment, substrates were washed using distilled water and then neutralized by acetic acid and sodium hydroxide. The substrate was dried at 60°C in oven for 12 hours in readiness for hydrolysis. Enzymatic hydrolysis was carried out in reaction mixture containing 40 g each of pre-treated substrates in 400 ml 0.1M citrate buffer with 20 µl of concentrated crude cellulases enzyme, pH was adjusted to 4.5. The reaction mixtures were incubated on rotary shaker at 30°C, 75 rpm for 24 hours. After the 24 hours of incubation, reaction mixtures were boiled for 2 minutes to denature the enzyme.

### Isolation of Bacterial and Fungal species

*Bacillus subtilis* was isolated from soil and *Saccharomyces cerevisiae* was isolated from baker's yeast. The yeast isolate was maintained by subculturing on potato dextrose agar (PDA) slants, incubated for 48 hours at 27°C and the bacterial isolate was maintained by subculturing on nutrient agar (NA) slants, incubated for 24 hours at 37°C and thereafter stored in a refrigerator.

### Fermentation of Substrates

Both fungal and bacterial inoculums were prepared in potato dextrose and nutrient broth respectively. Forty grams of the treated samples were placed into the plastic fermenters (4 plastic fermenter for each day of fermentation), 3ml of the inoculums were then inoculated aseptically into each of the fermenters containing the substrates to begin fermentation.

### Determination of pH and temperature

The pH of each sample was determined using a pH meter daily for the whole fermentation period. The temperature was determined using a mercury thermometer daily for the whole fermentation period.

### Determination of titratable acidity (TTA)

One point five grams of the substrate was dissolved in 30 ml of distilled water and then filtered. Ten ml of the filtrate was dispensed in a conical flask and 2 drops of the indicator phenolphthalein was added to the sample, this was titrated against 0.1M NaOH.

### Determination of proximate composition

Moisture content was determined after oven drying to a constant weight at 105 °C. Ash, proteins, lipids and crude fibers were analyzed according to AOAC methods [3].

### Microbial load determination

The microbial load for each substrate was carried out by using pour plating technique. One gram of each substrate sample was serially diluted with 9ml of sterile distilled water (stock). Zero point one milliliter was drawn from the stock and serially diluted in 4 folds. Using pour plating technique, 0.1ml from dilution  $10^{-4}$  was drawn from each sample and dispensed at the centre of an empty Petri dish. Cooled agar was poured over the inoculum, rocked gently from side to side and allowed to solidify, and incubated in an inverted position at 27°C (PDA) and 37°C (NA).

### Determination of Mineral composition

An amount of 2 g of the fermented fruit peels was dried in an air oven at 105 °C for 3 hours. The dried sample was next charred until it ceased to smoke. The charred sample was then ash in a muffle furnace at 550°C until a whitish or greyish ash was obtained. The ash was treated with concentrated hydrochloric acid, transferred to a volumetric flask and made up to 100 ml before submission to atomic absorption spectrophotometer (AAS).

### Determination of ethanol composition

The ethanol content determination for this research work was carried out after 24 h interval during fermentation and after 96 h. Two grams of each samples from the plastic fermenter was taken and then used for the analysis of ethanol concentration [4]. Ethanol concentration was

determined by measuring its specific gravity after distillation; the specific gravity values obtained were used to determine ethanol concentration from a standard curve prepared using known concentration of ethanol [5].

## 3. Results and Discussion

The results of the temperature (°C), pH and total titratable acidity (TTA) values at different fermentation time are shown in Tables 1, 2 and 3 respectively, the temperature remained fairly constant and pH decreased during fermentation of the substrate while TTA values for some samples increased from the zero day to the second day of fermentation and then decreased and the TTA for the remaining samples decreased. The pH of the substrates decreased from (9.16-3.30) and the TTA also decreased from (0.018 g/L-0.002 g/L). The fermentation lowered the pH of all the samples, for pineapple fermented with *B. subtilis*: 4.17 – 3.30, for pineapple fermented with *S. cerevisiae*: 4.22 – 3.70, for watermelon fermented with *B. subtilis*: 8.91– 6.93 and for watermelon fermented with *S. cerevisiae*: 9.16 – 7.12, which is an indication of greater mobility and activity of the fermenting microorganisms as they feed on the carbohydrates of the peels with subsequent release of organic acids. The acidification increased with increasing period of fermentation due to increased production of titratable acidity [6] [7] [8].

**Table 1:** Temperatures of the fermented samples during fermentation period

Time (hrs.)	Pineapple ( <i>B. subtilis</i> ) °C	Pineapple ( <i>S. cerevisiae</i> ) °C	Watermelon ( <i>B. subtilis</i> ) °C	Watermelon ( <i>S. cerevisiae</i> ) °C
0	30	30	30	32
24	32	31	30	30
48	30	29	29	30
72	29	30	30	30
96	30	30	30	29
120	31	30	30	29

**Table 2:** The pH of the fermented samples during fermentation period

Time (hrs.)	Pineapple ( <i>B. subtilis</i> )	Pineapple ( <i>S. cerevisiae</i> )	Watermelon ( <i>B. subtilis</i> )	Watermelon ( <i>S. cerevisiae</i> )
0	4.17	4.22	8.91	9.16
24	3.86	3.89	7.86	7.81
48	3.38	3.83	7.39	7.68
72	3.34	3.75	7.19	7.42
96	3.32	3.73	7.08	7.20
120	3.30	3.70	6.93	7.12

**Table 3:** The total titratable acidity of the fermented samples during fermentation period

Time (hrs.)	Pineapple ( <i>B. subtilis</i> ) g/L	Pineapple ( <i>S. cerevisiae</i> ) g/L	Watermelon ( <i>B. subtilis</i> ) g/L	Watermelon ( <i>S. cerevisiae</i> ) g/L
0	0.019	0.018	0.004	0.011
24	0.022	0.034	0.004	0.007
48	0.031	0.029	0.003	0.006
72	0.012	0.019	0.003	0.004
96	0.010	0.012	0.003	0.003
120	0.007	0.010	0.002	0.003

The proximate composition of raw, treated and fermented pineapple and watermelon peels appear in Table 4 here moisture contents, fat contents, fibre contents and ash contents were found to increase in fermented sample while protein contents reduced as compared to those of the treated samples and the carbohydrate contents reduced. Fermented pineapple peels with *Bacillus subtilis* showed the highest protein content (19.29±0.13) compared with the unfermented sample (12.65±0.02) while the other samples had low protein content as compared to the raw sample. The increase in protein may be due to the activities of the microbial strains which might have secreted some extracellular enzymes (protein) [9]. Also, fungal fermentation has been reported to increase protein content of biomass. Increase in fat contents were observed for the fermented samples: for pineapple fermented with *B. subtilis*: 30.76±0.17, for pineapple fermented with *S. cerevisiae*: 32.06±0.54, for watermelon fermented with *B. subtilis*:

33.99±2.33 and for watermelon fermented with *S. cerevisiae*: 40.10±3.25. Decrease in the carbohydrate content were observed, for pineapple fermented with *B. subtilis*: 22.51±0.08, for pineapple fermented with *S. cerevisiae*: 18.27±0.43, for watermelon fermented with *B. subtilis*: 26.11±2.02 and for watermelon fermented with *S. cerevisiae*: 15.21±0.37 as compared to the raw samples and these signifies effective ethanol production as the carbohydrates are being degraded and converted to ethanol. Increase in the composition of ash content, moisture content, crude fibre content could be as a result of production of enzymes during growth which is essential for human nutrition. The decrease and increase in the nutritional contents of pineapple and watermelon peels can also be linked to their utilization by microbes and production of metabolites by microorganisms during the fermentation process.

**Table 4:** Percentage proximate composition of raw, treated and fermented samples of pineapple and watermelon peels

Proximate	Pn.R	Pn.T	W.R	W.T	Pn.Bs	Pn.Sc		
Ash content	5.52±0.06	14.33±0.44	22.07±0.36	25.03±0.04	10.15±0.11	18.43±0.04	10.26±1.49	14.82±0.77
Moisture content	3.70±0.14	7.93±0.08	1.15±0.06	9.52±0.06	12.53±0.13	16.58±0.15	16.89±1.39	15.63±0.25
Fat content	26.53±0.02	21.53±0.06	21.25±0.07	18.91±0.06	30.76±0.17	32.06±0.54	33.99±2.33	40.10±3.25
Crude fibre content	2.91±0.01	1.92±0.08	1.40±0.04	0.67±0.24	4.85±0.18	4.00±1.41	3.85±0.49	5.75±2.47
Protein content	12.65±0.02	15.34±0.01	14.65±0.10	24.44±0.17	19.29±0.13	12.49±0.29	12.57±1.49	12.06±0.86
Carbohydrate	48.60±0.06	39.00±0.14	39.65±0.18	21.54±0.08	22.51±0.08	18.27±0.43	26.11±2.02	15.21±0.37

Content

Values are means ± standard deviations of two replicate measurements.

Keys:

Pn.R – raw pineapple sample, Pn.T – treated pineapple sample, W.R – raw watermelon sample, W.T – treated watermelon sample, Pn.Bs – pineapple fermented by *Bacillus subtilis*,

Pn.Sc – pineapple fermented by *Saccharomyces cerevisiae*, W.Bs – watermelon fermented by *Bacillus subtilis*, W.Sc – watermelon fermented by *Saccharomyces cerevisiae*

There was an observable increase in the total number of bacteria and fungi throughout the period of fermentation as shown in Table 5. This is in agreement with the findings of Barth *et al* [10].

**Table 5:** Microbial load of the fermented samples during fermentation period

Time (hrs.)	Pineapple ( <i>B. subtilis</i> ) cfu/ml	Pineapple ( <i>S. cerevisiae</i> ) sfu/ml( <i>B. subtilis</i> ) cfu/ml	Watermelon ( <i>S. cerevisiae</i> ) sfu/ml	Watermelon
0	1.13 x 10 <sup>4</sup>	1.39 x 10 <sup>4</sup>	1.25 x 10 <sup>4</sup>	1.55 x 10 <sup>4</sup>
24	1.78 x 10 <sup>4</sup>	2.65 x 10 <sup>4</sup>	2.14 x 10 <sup>4</sup>	2.35 x 10 <sup>4</sup>
48	2.35 x 10 <sup>4</sup>	2.89 x 10 <sup>4</sup>	2.45 x 10 <sup>4</sup>	2.87 x 10 <sup>4</sup>
72	2.52 x 10 <sup>4</sup>	3.03 x 10 <sup>4</sup>	2.68 x 10 <sup>4</sup>	3.08 x 10 <sup>4</sup>
96	2.70 x 10 <sup>4</sup>	2.75 x 10 <sup>4</sup>	2.44 x 10 <sup>4</sup>	2.96 x 10 <sup>4</sup>
120	2.85 x 10 <sup>4</sup>	2.73 x 10 <sup>4</sup>	2.35 x 10 <sup>4</sup>	2.78 x 10 <sup>4</sup>

The mineral composition as shown in Table 6 varied as the calcium, iron and zinc composition increased with fermentation. Minerals play a key role in various physiological functions of the body, especially in the building and regulation processes [11]. Calcium is an important constituent of bones and teeth and it is actively

involved in the regulation of nerve and muscle functions [12].

The calcium content of pineapple peels increased from 5.54 mg/2g to 65.89 mg/2g after fermentation and that of watermelon peels increased from 41 mg/2g to 82.45 mg/2g. Iron carries oxygen to the cells and is necessary for the production of energy, synthesis of collagen and the proper functioning of the immune system. Iron content also increased from 3.34 mg/2g to 7.20 mg/2g for pineapple peels and from 5.33 mg/2g to 9.82 mg/2g for watermelon peels. Zinc is particularly necessary in cellular replication and the development of the immune response. Zinc also plays an important role in growth; it has a recognized action on more than 300 enzymes by participating in their structure or in their catalytic and regulatory actions [13]. Zinc levels in the peels only increased slightly from 0.42 mg/2g to 1.23 mg/2g in pineapple peels and from 1.79 mg/2g to 1.95 mg/2g in watermelon peels.

**Table 6:** Mineral composition of raw and fermented samples

Substrates	Calcium (mg/2g)	Iron (mg/2g)	Zinc (mg/2g)
Raw Pineapple	5.54	3.34	0.42
Raw Watermelon	41.00	5.33	1.79
Pineapple ( <i>B.subtilis</i> )	45.52	5.83	0.95
Pineapple ( <i>S.cerevisiae</i> )	65.89	7.20	1.23
Watermelon ( <i>B.subtilis</i> )	71.25	9.70	1.84
Watermelon ( <i>S.cerevisiae</i> )	82.45	9.82	1.95

The ethanol composition in Table 7 shows that ethanol yield from peels of pineapple by *Saccharomyces cerevisiae* (2.51%±0.43 v/v) was the highest, followed by peels of

watermelon fermented by *Saccharomyces cerevisiae* (2.31%±0.15 v/v). The yield from peels of watermelon fermented by *Bacillus subtilis* was (1.83%±0.09 v/v) while the minimum yield was obtained from the peels of pineapple fermented by *Bacillus subtilis* (1.21%±0.16 v/v). The highest ethanol content was produced from pineapple peels by *Saccharomyces cerevisiae*.

**Table 7:** Ethanol yield at 24 hours and 120 hours of fermentation

Substrates	Time (hrs.)	
	24	120
Pineapple ( <i>B.subtilis</i> )	0.36±0.02 (%v/v)	1.21±0.16 (%v/v)
Pineapple ( <i>S.cerevisiae</i> )	0.45±0.04 (%v/v)	2.51±0.43 (%v/v)
Watermelon ( <i>B.subtilis</i> )	0.18±0.02 (%v/v)	1.83±0.09 (%v/v)
Watermelon ( <i>S.cerevisiae</i> )	0.41±0.02 (%v/v)	2.31±0.15 (%v/v)

Values are means ± standard deviations of two replicate measurements

#### 4. Conclusion

This work has demonstrated the effect of fermentation on the proximate and mineral composition of pineapple and watermelon peels and the bioethanol producing ability of *Bacillus subtilis* and *Saccharomyces cerevisiae* from these peels. Ethanol yield by *Saccharomyces cerevisiae* was found to be higher than that of *Bacillus subtilis* and the yield was higher in pineapple peels than watermelon peels. The results of this study indicate that maximum ethanol yield can be obtained from pineapple peels by *Saccharomyces cerevisiae*.

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