

## BIOTECHNOLOGY INFLUENCE FOR THE PRODUCTION OF ETHYL ALCOHOL (ETHANOL) FROM WASTE FRUITES

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### ABSTRACT

The use of waste fruits of pineapple (*Ananas comosus* L. Merr) banana (*Musa accuminata* Colla) and pawpaw (*Carica papaya* L.) as biomass for production of wine was investigated. Ripe, soft pineapple, banana and pawpaw fruits weighing 5.8, 3.3 and 5.8 respectively were used for microbial analysis and nutritional composition. Fungi and bacteria were isolated from the peels, pulp and juice. Ash, pH, moisture, lipid, total available carbohydrate, crude protein, iron, sodium, potassium, calcium, specific gravity, alcohol content was also determined. Seven testers evaluated the wine base on colour clarity, aroma and taste. Bacteria isolated from the fruits were *Acetobacter aceti*, *Clostridium butyrium*, *Bacillus* sp. and *Micrococcus* while the filamentous fungi include *Aspergillus flavus*, *A. niger*, *Penicillium chrysogenum* and *Fusarium* sp. The yeast involved were *Saccharomyces cerevisiae*, *S. lactis* and *Pichia* spp. Ethanol production from the fruits ranged from 25 to 87ml on the tenth day. The nutritional composition of pineapple, banana and pawpaw juice ranged for moisture content 8.5 to 91.1%, Calcium 8.4 to 12.2%, phosphorus 6.8 to 8.7%, protein 0.44 to 1.43 and fat 0.04 to 0.5%. Pineapple juice had the best organoleptic quality followed by banana then pawpaw juice. However, the most ethanol distillate was produced from pawpaw.

### INTRODUCTION

Inadequate developments of postharvest technologies consequently bring about postharvest losses (Farzana, 2006). Postharvest losses thus are the thorny problems hampering the agricultural development in Nigeria (Osuide, 1999). Estimates of the postharvest losses in the developing world resulting from mishandling, spoilage and pest infestation are put at 25%, hence, quarter of what is produced never reaches the consumer for whom it was grown, and the effort and money required to produce it are lost forever (Smith *et al.*, 2003). 33% of the major food crops, fruits and vegetables produced in Nigeria are wasted as a result of postharvest deterioration (Enyinnia, 1998). In addition, it was estimated in 1986 that there was one billion naira loss in Nigeria produce as a result of postharvest disease (Enyinina, 1998). Moreover, current estimates of postharvest losses stand at about 40% for vegetables, roots and tuber crops and over 60% for fruits (Osuide, 1999). Furthermore, inadequate development of postharvest technologies does not affect perishable crops only quantitatively but also qualitatively (Enyinnia, 1998). The qualities considered include: appearance, the flavour, the texture, the nutritional value and the safety

(Frazana, 2006; Safra and Yeshua, 2003). Food security remains a challenge in Nigeria where insufficient attention to the effect of postharvest physiology reflects on food quality (Okigbo, 2003). Nutrients depletion, quality loss and damage of physiological structures before consumption or converting into secondary products are the major effects (Aniche, 2003, McDonald *et al.*, 2006).

Tropical fruits indigenous to Nigeria such as pineapple (*Ananas comosus* L. Merr.), banana (*Musa acuminata* Colla) and pawpaw (*Carica papaya* L.) are potential sources for wine production (Akubor *et al.*, 2003; Ifeanyi, 2004).

Grape wine is perhaps the most common fruit juice alcohol. Because of the commercialization of the product for industry, the process has received most research attention and is documented in detail. However, available literature shows fruit wines has been produced from some tropical fruits (Akubor *et al.*, 2003; Ifeanyi, 2007). Peterson (1971) also stated that available records on sources of fruit wines show that wines are made from non-grape source e.g. herbs, roots, flower, apples, pears, cherries, various types of barriers honey, molasses and fermentable carbohydrates. The production of pineapple, banana and pawpaw wines follows the same basic steps as applicable to grape wine. (Encyclopedia Britannica, 2006)

Brazil leads in ethanol production (Berg, 2001; Lynch, 2006). Brazil accounts for 53% of the embryonic global ethanol trade. Europe is a distance second with a market share of 12%. Brazilian companies are investing \$9billions in dozens of new sugar mills to double boost ethanol production which aiming to double exports by 2010 .The eventual goal is to spreads new ethanol industries in countries from Japan to Nigeria (Lynch, 2006).

In general, according to Berg (2001), fuel ethanol, besides its environmental value, is and will remain first and foremost an instrument to support farmers, as they will profit from fuel ethanol programmes.

Based on the foregoing, there is the urgent need for a research to study if naturally fermenting fruits particularly those considered as losses by the sellers can still serve useful purpose such as wine production, ethanol generation amongst others, and as a means to salvage fruits (food) wastage, reducing the bulk of solid waste and cleaning the environment.

This research study therefore reported on ethanol production from the naturally fermented juices of pineapple, banana and pawpaw at regular intervals.

## MATERIALS AND METHODS

### Collection of waste fruits

Ripe, soft pineapple (*Ananas comosus* L. Merr.), banana (*Musa acuminata* Colla) and pawpaw (*Carica papaya* Linneaus) fruits were bought from the popular Mile I Market in Diobu, Port Harcourt, Rivers State in the month of April and November, 2006 and were authenticated by E.N.U. Okpon (Taxonomist) of the Applied and Environmental Biology Department, Rivers State University of Science and Technology, Port Harcourt. The peel, juice, and pulp of these fruits were used for the microbiological, proximate composition analysis and ethanol distillation during a three week period at an interval of three (3) days.

### Preparation of Samples

Ripe, soft pineapple, banana, and pawpaw fruits weighing  $5.88 \pm 0.24$ kg,  $3.27 \pm 0.41$ kg and  $5.85 \pm 0.21$ kg respectively were thoroughly washed with distilled water before peeling using a sterile knife. The peels from three fruits were collected in three

different sterile plastic containers already labeled for each fruit. The weights of the pineapple, banana and pawpaw fruits after peeling were also recorded as  $5.17 \pm 0.37$ kg,  $2.55 \pm 0.29$ kg and  $4.8.9 \pm 0.93$ kg respectively. The peeled fruits of pineapple, banana, and pawpaw were each blended separately using a sterilized automatic juice blender (Binatone, Nigeria).

Clean sterile cheese-cloth was used to sieve the juice from the pulp of each fruit. This was collected in three different sterile plastic containers from where measured quantities (pineapple-251ml; banana 141ml and pawpaw-251ml) were dispensed into three sets of sterile eight (8) conical flask already labeled according to the dates for each sample analysis. Conical flasks were stopper with non-absorbent cotton wool and aluminum foil to ensure they were air-tight as to provide an anaerobic condition. This was repeated for the second and third replication.

The pulp of the fruits collected on cheese-cloth was then transferred into three different sterile plastic containers already labeled for each fruits this was repeated for the second and third replication.

### **Isolation and enumeration of bacteria and fungi associated with the peel, pulp and juice of pawpaw fruit.**

One gram (1g) each homogenized peel, pulp and 1ml of juice were thoroughly shaken in separate test tubes containing 9ml of sterile normal saline. The serial dilutions were made up to  $10^{-5}$  folds.  $10^{-3}$  and  $10^{-4}$  dilutions were used for fungal enumeration while  $10^{-3}$  to  $10^{-5}$  dilutions were used for bacteria enumeration.

Aliquots of 0.1ml of  $10^{-3}$  dilution of juice and pulp sample were spread on duplicate plates of Sabouraud dextrose agar and nutrient agar. These were also done for the second and third replication. Each aliquot that was introduced on the surface of the solidified media was spread uniformly over the surface of the agar media with the aid of a sterile glass spreader. This method thins out the organisms by separating the cells from each other, so that they will develop into discrete colonies.

The inoculated plates were labeled according to the dilutions and medium used and were incubated at ambient temperature in the laboratory for 1 to 2 days for bacteria and 3 to 5 days for fungi. The discrete colonies that developed on nutrient agar plates and Sabouraud dextrose agar plates were counted, and the average count of duplicate cultures were recorded as colony forming units (cfu) of the total viable count for heterotrophic bacteria and as spore forming unit (sfu) of viable count for fungi respectively.

The above procedure was repeated for pineapple fruit and banana fruit samples.

### **Identification of Bacteria isolated from the peel, pulp and juice**

With the development of discrete colonies, pure cultures were obtained by subculturing them. This involves using a wire-loop to pick a discrete colony and streaking out on a nutrient agar plate, incubated for 24hours at ambient temperature and the bacteria that grow afterward is transferred to a nutrient agar slant and preserved for further biochemical test.

The pure cultures of bacteria isolated were later identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cheesebrough (1984) and Harrigan and McCance (1976). The following characterizations tests were performed in duplicates: Gram's staining, biochemical tests such as catalase, oxidase, indole, methyl red, Voges Proskauer, citrate, ammonia production and sugar fermentation.

### **Determination of pH of the fruit juice**

Samples of the fermented juice of pineapple, banana and pawpaw were collected at 3 days intervals for pH determination. 10 ml of each sample were collected and their pH was determined using a pH meter (Coring Model 7.USA). Duplicate readings were taken for each sample and the average results were recorded.

### **Proximate composition and analysis of fermented fruit juice**

The parameters determined in the fermented pineapple, banana and pawpaw juice include. Ash content, moisture content, lipid, carbohydrate, fiber, protein, calcium, phosphorus, potassium, sodium, iron, magnesium, specific gravity, volume of carbon dioxide produced and vitamin A and C, viscosity, ethanol content and pH.

### **Ash Content**

The method employed is as described by the Association of Official Analytical Chemist (AOAC), (1984). The total quantity of minerals in a sample can determine by reducing the sample to ash. The open dish were thoroughly washed, cleaned and placed in the oven for about 2 hours to dry and cooled to room temperature in the desiccators. Then 2ml of the sample were accurately weighed into the open dish (crucibles) and paled in the muffle furnace at 600 °C in duplicates and labeled for 6 hours. At the end of the ashing period, when all the volatilisable materials has been burnt off, the samples were removed into the desiccators to cool to room temperature and reweighed. The difference is expressed in percentage.

### **Moisture Content**

The method employed is as described by the Association of Official Analytical Chemist (AOAC), (1984). 2ml of each sample was weighted into a clean dry aluminum dish previously weighed. The dish was then placed into oven and removed from the oven and cooled in a desiccator and reweighed. The procedure was repeated until constant weight was obtained.

### **Determination of lipid**

The method employed is as described by the Association of Official Analytical Chemist (AOAC), (1984). Lipid was determined using the micro-sohxlet extraction unit; 10ml of each sample was wrapped into a fat free filter paper and extracted with 50ml of petroleum ether and fitted into the extraction, and tap water was used to cool the condenser. The extraction commenced at 40-60°C for 6 hours. At the end of extraction, the solvent (petroleum ether) was evaporated by heating at 70°C on a hot plate leaving the lipid extract in the flask. The flask was placed in an oven, the extract dried at 50% for 30 minutes, cooled and weighed and the lipid content calculated by the difference.

### **Total Available Carbohydrate**

The method of Osborn and Voogt (1978) was used. 13ml of 52% perchloric acid was used to hydrolyse 1ml of each sample of pineapple, banana and pawpaw juice in 10ml of distilled water. For complete hydrolysis, the mixture was stirred with a glass rod for 20 minutes in a beaker, filtered into 25ml volumetric flask and made up to the mark with distilled water. 10ml aliquot of the filtered was taken and diluted to 100ml distilled water. 1 ml of each diluted solutions 0.1% standard glucose solution and distilled water (blank) were pipetted into three tubes.

The tubes were then placed in a hot water bath for about 12 minutes for development. The tubes were removed from the bath cooled to room temperature and the absorbance read at 630nm against the blank in spectrophotometer 20.

#### **Crude protein by Microkjeldahl method**

The method employed is as described by the Association of Official Analytical Chemist (AOAC), (1984). This involved that 0.5ml of each sample was weighed into 10ml of Kjeldahl flasks and was digested with 10ml of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and four catalysts of potassium and copper. The process was accelerated by heating under a flame cupboard for 45 minutes to obtain a clear light green coloured solution. The digested sample was followed by distillation using boric acid mixed with indicator and 45% sodium hydroxide (NaOH) to neutralise the acid and consequently release of ammonia gas (NH<sub>3</sub>). The diluted sample was titrated against 0.049N H<sub>2</sub>SO<sub>4</sub>.

$$\% N = \frac{(\text{Titre Value} - \text{Blank}) \times \text{Normality of acid} \times 1.4}{\text{Weight of Sample}}$$

$$\% \text{ Crude Protein} = \% N \times 6.25$$

#### **Determination of iron by potassium thiocyanate method**

The method employed was as described by the Association of Official Analytical Chemist (AOAC), (1984).

Traces of iron are often determined calorimetrically or spectrophotometrically with thiocyanate as reagent. 1.0ml of 100ppm ferric chloride solution was pipetted into different 50ml volumetric flasks. 20ml of the sample was also pipetted in 50ml volumetric flasks. From this point both sample and the standard were treated in the same way. Then 1ml of nitric acid was added. 5ml of 10% potassium thiocyanate was added to both the samples and standards and diluted to the mark with distilled water. The absorbance was read with spectronic 20 at 530nm with zero standards as blank.

#### **Determination of sodium, potassium and calcium in fermented waste fruit juice using the flame photometric method**

The method employed is the Association of Official Analytical Chemist (AOAC), (1984) was employed. When atoms of an element are heated in a flame, some of the heat energy is absorbed by a few of the atoms which become excited; there is a transition by one or more electrons from the ground state to higher energy levels for each element. There are certain permitted shifts given rise to a series of lines, each series being characteristic of the element.

Calculation:

$$\frac{\text{Graph reading} \times \text{solution volume} \times \text{dilution factor}}{10^4 \times \text{Aliquot} \times \text{sample weight}}$$

#### **Determination of phosphorus: ascorbic acid method**

The method described by the Association of Official Analytical Chemist (AOAC), (1984) was employed. 0.1ml of the 100ppm phosphorus stock was pipetted into different 100ml volumetric flasks and 4ml of reagent (potassium antimony tartarate)

added while shaken to mix, the solution was made up to 100ml with distilled water. This contained 0.1 ppm. Then 1.5ml aliquot of the sample was further pipetted into 50 volumetric flasks with addition of 4ml of potassium antimony tartarate, then they were allowed to develop colour.

Calculations:

$$P \% = \frac{\text{Graph reading} \times \text{solution volume} \times \text{dilution factor}}{10^4 \times \text{Weight of Sample (ml)} \times \text{Aliquot (ml)}}$$

### **Determination of Vitamin A and C**

Vitamin A and C were determined using the spectrophotometric method, AOAC (1984).

#### **Specific Gravity (S.G.) Using Hydrometer**

The hydrometer (TP-5C) was spanned gently in the fruit juice contained in a graduated cylinder. The hydrometer was twisted to remove most of the air bubbles from its surface, which can invalidate the measurement. The specific gravity of the sample is read directly from the meter. Because the density of a liquid is a function of the temperature, temperature correction values are added to the specific gravity readings to obtain the corresponding values at 60°C, the temperature at which the meter is calibrated.

### **Determination of Viscosity Using Bohlin Viscometer**

A viscometer is simply a device used to measure viscosity. Viscosity describes resistance of the fluid to flow or its thickness. To 40ml of water, add 60ml to the sample stirring continuously to ensure the formation of clear solution air bubbles, leave the solution to stand in a refrigerator (+4°C) for several hours. Using a Bohlin viscometer, measure the viscosity at 25 using a shear rate of 147 seconds.

### **Determination of alcohol production from fermented waste fruit juice using the soxhlet extraction method**

A round bottom flask is filled with the sample (100ml) of each fermented fruit juice. The extractor is fitted to the round bottom flask from the lower pipe. The open end of the extractor is fitted with the lower end of the condenser. The tube is then introduced into the electric source of energy. The tap is then turned on with water flowing through one direction of the tubes and coming out through the other direction of the tube. The regulated heat of this stage is switched on at 40°C to 60°C.

As the fermented juice (sample) heats up, the vapour rise up the tube through the lower tube of the extractor into the extractor and rise up in the inner tube of the condenser. These vapours as touch the sides of the condenser, turn into liquid by the cold temperature of the tap water flushing the sides of the condenser. As the volume of the sample in the tube reduces, the volume in the extractor rise.

The distillate in the extractor is obtained by dismantling the system carefully while the source of heat is turned off.

The volume of the distillates of each pineapple, banana and pawpaw juice sample were measured and recorded per sample. In addition, the distillate obtained are not 100% ethanol, it can also undergo fractional distillation to achieve 96% ethanol.

### **Organoleptic Evaluation**

Seven testers evaluated the wine based on four parameters: colour, clarity, aroma (nose or smell) and taste after five days of fermentation according to Ifeanyi (2004).

### Statistical Analysis

Randomized Complete Block Design (RCBD) and analysis of variance (ANOVA) were used to test any level of significance between the various microbial counts, and between the data obtained from the nutritional (proximate) composition of the three fruits.

## RESULTS

### Culture, isolation and enumeration of bacteria and fungi associated with the peel, pulp and juice of pawpaw fruit

The percentage occurrence of bacteria isolated from the pulp of pineapple showed that *Acetobacter aceti*, *Enterobacter sp.* and *Staphylococcus aureus* had the lowest occurrence of 9.09%, while *Bacillus sp.* had the highest occurrence of 40.9%, (Table 1). *Staphylococcus aureus* had the lowest occurrence of 5.55%, while *Bacillus sp.* had the highest occurrence of 44.4% in banana juice, (Table 1). From the pawpaw pulp, *Acetobacter aceti*, *Clostridium butyricum* and *Flavobacterium sp.* had the lowest occurrence of 8.33%, while *Bacillus sp.* had the highest occurrence of 45.83%, (Table 1).

The percentage occurrence of fungi isolated from the pulp of pineapple showed that *Fusarium sp.* had the lowest occurrence of 5.26%, while *Saccharomyces cerevisiae* had the highest occurrence of 50%, (Table 2). *Hansenula sp.* had the lowest occurrence of 5.71% in banana pulp, while *Saccharomyces cerevisiae* had the highest occurrence of 42.85%, (Table 2). From the pawpaw pulp, *Fusarium sp.* had the lowest occurrence of 4.44%, while *Saccharomyces cerevisiae* had the highest occurrence of 46.66%, (Table 2).

### Ethanol Production from pineapple, banana and pawpaw juice

The result of ethanol production using Soxhlet method from fermented pineapple juice showed that the volume of ethanol produced ranged from 63ml to 82ml. The smallest volume of 63ml was produced on the 1<sup>st</sup> day while the largest volume of 82ml was produced on 19<sup>th</sup> day of fermentation (Table 3). Ethanol production from fermented banana juice showed that the volume of ethanol produced ranged from 25ml to 35ml. The smallest volume of 25ml was produced on the 22<sup>nd</sup> day while, the largest volume of 35ml was produced on the 16<sup>th</sup> day of fermentation (Table 3)

The ethanol produced from pawpaw ranged from 74ml to 91ml. The smallest volume of 74ml was produced on the 16<sup>th</sup> day while, the largest volume of 91ml was produced on 4<sup>th</sup> day of fermentation.

Statistical analysis of the bacteria count revealed the presence of higher significant differences between the peel, juice and pulp of pineapple, banana and pawpaw. There is higher statistical difference 7.6923\*\*, 63.9\*\* and 38.4615\*\*) for the fungal court between the peel, juice and pulp of pineapple, banana and pawpaw. Both bacterial and fungal counts were significant at 1% and 5%. The nutritional compositions were significant at 1% except the volume of carbon dioxide that was significant at 5%. The randomized complete block design was used based on the fact that the predictable sources of variations were more than two ie. The possibility of differences between fruits, differences due to time (days) of sampling and that due to error. The

coefficients of variation for bacterial and fungal counts were less 1.00%. This showed a high level of reliability.

### **Nutritional and functional properties of pineapple, banana and pawpaw juice**

The mean moisture content (%) of the three fruits show that banana juice had the lowest value  $8.5 \pm 1.79$ , pineapple juice had  $78.5 \pm 2.54$  and pawpaw juice had the highest value of  $91.9 \pm 1.12$  (Table 4). The mean ash content (%) shows that pawpaw juice had the lowest value of  $0.05 \pm 0.00$ , followed by pineapple juice,  $0.365 \pm 0.018$ , while banana juice had the highest value of  $0.5 \pm 0.00$  (Table 4). The mean fat content (%) shows that pineapple juice had the lowest value of  $0.04 \pm 0.00$ , followed by pawpaw juice,  $0.41 \pm 0.00$  (Table 4). The lowest mean value of carbohydrate was recorded for pawpaw juice as  $6.78 \pm 1.92$ , followed by pineapple juice,  $11.6 \pm 3.58$  and the highest value of  $15.35 \pm 6.17$  for banana juice, (Table 4). Pineapple juice recorded the lowest value of  $0.17 \pm 0.011$  for the mean fibre content. Banana juice had the highest value of  $0.71 \pm 0.11$  while pawpaw juice had middle of  $0.54 \pm 0.13$  (Table 4). The mean protein value was lowest for pineapple juice ( $0.5 \pm 0.00$ ), followed by pawpaw juice, which had  $0.55 \pm 0.15$ , while banana juice had the highest value of  $1.43 \pm 0.11$  (Table 4).

The mean calcium ( $\text{Ca}^{2+}$ ) value was highest for pawpaw juice ( $12.15 \pm 0.89$ ), followed by pineapple juice ( $11.85 \pm 0.587$ ) and lowest for banana juice with a value of  $8.41 \pm 0.41$  (Table 4). The mean phosphorus ( $\text{P}^+$ ) value was lowest for banana juice with a value of  $3.98 \pm 0.04$ , followed by pawpaw juice which recorded a value of  $6.80 \pm 0.68$  and pineapple juice with the highest value of  $8.65 \pm 0.69$  (Table 4). Pineapple juice recorded the highest mean value for potassium ( $\text{K}^+$ ) as  $16.95 \pm 0.76$ , while pawpaw juice had the lowest value of  $3.35 \pm 0.16$ . Banana juice recorded a middle value of  $4.63 \pm 0.14$ , (Table 4). The mean sodium ( $\text{Na}^+$ ) value was lowest for pineapple juice with a value of  $0.95 \pm 0.07$ , followed by  $2.05 \pm 0.05$  recorded for pawpaw juice, while the highest value of  $2.89 \pm 0.16$  was recorded for Banana juice, (Table 4). Pawpaw juice recorded the lowest mean value of  $0.40 \pm 0.004$  for iron (Fe), followed by  $0.43 \pm 0.04$  recorded for banana juice, while pineapple juice recorded the highest value of  $0.50 \pm 0.007$ , (Table 4). The mean magnesium ( $\text{Mg}^{2+}$ ) value was lowest for pawpaw juice with a value of  $0.20 \pm 0.04$ , followed by  $2.61 \pm 0.12$  recorded for banana juice, while pineapple juice recorded the highest value of  $10.6 \pm 0.07$ , (Table 4).

The mean vitamin A was lowest for pineapple juice with a value of  $1.97 \pm 5.26$ , followed by banana juice with a value of  $19.6 \pm 1.50$ , while the highest value of  $47.5 \pm 5.35$  was recorded the highest value of  $47.5 \pm 5.35$  was recorded for pawpaw juice, (Table 4). The mean vitamin C value was highest for pawpaw juice with a value of  $123.9 \pm 0.06$ , followed by banana juice with a value of  $30.8 \pm 3.39$ , while the lowest value of  $13.5 \pm 4.42$ , (Table 4). The mean specific gravity value recorded for pawpaw juice was lowest with a value of  $0.88 \pm 0.66$ , followed by pineapple juice with a value of  $0.92 \pm 0.04$ , while the highest value of  $0.95 \pm 0.03$  was recorded for banana juice, (Table 4). The mean viscosity value was lowest of  $50.6 \pm 7.28$  was recorded for pineapple juice, followed by banana juice with a value of  $63.3 \pm 22.8$  and the highest value of  $80.6 \pm 14.7$  was recorded for pawpaw juice, (Table 4). The mean value recorded in Table 4 showed that pineapple has the highest value of  $0.88 \pm 0.52$  for volume of carbondioxide, followed by pineapple juice with a value of  $0.75 \pm 0.13$  and the lowest value of  $0.64 \pm 0.35$  recorded for pawpaw juice. The highest mean ethanol value of  $0.89 \pm 0.49$  was recorded for pineapple juice, followed by pawpaw juice with a mean value of  $0.642 \pm 0.350$  and the lowest value of  $0.33 \pm 0.468$  was recorded for



pawpaw juice. The mean pH value was lowest for pineapple juice with a value of  $3.6 \pm 0.37$ , followed by banana juice with a value  $4.35 \pm 0.15$  and the highest value of  $4.41 \pm 0.26$  was recorded for pawpaw juice, (Table 4).

### Organoleptic Evaluation

The aggregate score of the seven testers showed that as regards colour the three fruits were rated equally. On clarity, pineapple is rated the best while pawpaw juice is least rated. The smell of pineapple was rated the best compared to banana and pawpaw. Pawpaw juice had a flat taste when banana juice and pineapple which taste are better and appreciated.

## DISCUSSION

Waste is an inheritable consequence of the food industry (Rodriguez *et al.*, 2003). As concerns over the environment have increased, the protection of the environment can only become possible where there is sufficient knowledge of the range of activities that can defile the aesthetic of the environment. Environmental goods (natural resources and biophysical conditions) have gradually turned into an economic variable its consideration in the context of industry performance leads to redesign the processes of production in line with the so-called environmental technology. Fruits are highly perishable products. Currently, most of the perishable fruits are lost during their journey through the agric-food chain, due to spillage, physiological decay, water loss, mechanical damage during harvesting, packaging and transporting, or due to transportation (Aniche, 2003).

The present investigation has revealed the nutritional and functional properties of pineapple, banana and pawpaw juice; the microbiological quality consistent of the peel, juice and pulp of pineapple, banana and pawpaw. The study also reveals the ethanol production of fermented waste fruits (pawpaw, pineapple and banana) juice.

The microbiological quality of three fruits studied had shown their microbial (bacteria and fungi) load, the types and frequency of occurrence of bacteria and pawpaw peels juice and pulp.

The richness of ripe fruits in species diversity and the population density of yeast could be due to changes in sugar content as the fruit ripens (Faparusi, 1974) or changes in animal visitors to the fruits. Post-harvest handling before fermentation may be responsible for the high values observed. Again, high fungal counts were observed in the peels of the three fruits. This may be due to the rich soil fungal flora of the field where fruits were collected (Okigbo, 2001). This may be attributed to the fact that the peels are the reception for all microorganisms before penetration into the pulp of the fruit. In addition, the pH formed in the peels was tolerable.

Generally, decreased fungal counts were recorded as fermentation period increased. Similar trends in reduction were noted by Gow-chin and Hsin-Tan (1996). However, at some point the counts also increased. This may be due to the fact some fungi had adjusted to the prevailing conditions in the fermenting fruits as typical of synchronous growth. This is also observable in the bacteria count.

The result above revealed that *Bacillus sp.* had the highest frequency of occurrence in each of the three fruits' peel, juice and pulp. Most of the bacteria isolated has implicated in fermentation of different carbohydrate food in Nigeria. They include *Bacillus sp.*, *Flavobacterium*, *Micrococcus*, *Lactobacillus* and *Staphylococcus* species (Odunfa, 1985; Aderiye and Ogunjobi, 1998). *Acetobacter aceti*, *Leuconostoc*

*mesenteriodes*, *Gluconobacter sp.* and *Streptococcus sp.* found in the three fruits are microorganisms commonly found in fermenting fruit and vegetables (Battock and Azam-Ali, 1998).

*Lactobacillus* and *Streptococcus* species identified in the fermented waste pineapple, banana and pawpaw fruits are advertised and listed as probiotic microorganisms (BgVV, 1999). Probiotics are certain living microorganisms; sufficient amounts of which reach the intestines in an active form to exert health (BgVV, 1999; Ezendam and Loveren, 2006). BgVV (1999) stated that depending on the amount ingested and taking into account the best-before date, a regular-in most cases daily-intake of  $10^8$  and  $10^9$  probiotic microorganisms is necessary to achieve probiotic action in the human organisms.

*Clostridium butyricum* also identified in the study is a typical butyric acid bacterium found in soil and intestine of healthy animals and humans. Butyric acid esters are the character-impact flavours in tropical fruits and dairy products (Centeno *et al.*, 2000).

Comparatively, *Saccharomyces cerevisiae*, *Penicillium sp* and *Aspergillus* species are common to the fermented waste fruits (pineapple, banana and pawpaw) with *Saccharomyces cerevisiae* having the highest frequency of occurrence/isolation in the three fruits. *Aspergillus*, *Penicillium*, *Debaromyces* and *Saccharomyces* species have been implicated in fermentation of fruits and other food items (Pelczar *et al.*, 1993; Battock and Azam-Ali, 1998). Kolawole *et al.* (2007) reported that *Aspergillus flavus*, *Aspergillus Rhizopus stolonifer* and *Saccharomyces cerevisiae* were isolated from *burukuto* and *pito* (fermented indigenous alcoholic beverages). Olorunfemi and Adetuyi (2005) actually isolated two different yeasts suspected to be *Saccharomyces* from naturally fermented pineapple (*Ananas comosus*). According to Gow-chin and Hsin-Tan (1996), yeast growth was favoured by the presence of sugar and acid pH. Fruit juice are readily fermented by yeast while acid pH discourages most bacterial growth.

The role of the microorganisms (bacteria and fungi) in the process of fermentation showed that waste pineapple, banana and pawpaw fruits contain a fermentable material which was evident from the increase in the acidity of the fermenting juice (Ogunjobi *et al.*, 2005)

Pineapple, banana and pawpaw are produced in Nigeria. They are eaten fresh or processed as fruit juice, since fruits are good sources of minerals and vitamins A and C (Ebuehi *et al.*, 2004).

The mean mineral content (calcium, phosphorus, potassium, sodium, iron and magnesium) level exceeds the mean values reported by Ebuehi *et al.* (2004). For some indigenous and foreign fruit juices marketed in Nigeria. Mineral elements are important because they are essential for regulating and building the living cells and aids in fighting depression Kolawole *et al.* (2006). Calcium is essential for building the living cells that make up the human body balanced, magnesium helps in keeping the muscle relaxed and the formation of strong bones and teeth. It helps to control the blood pressure and nerve transmitter. Iron is an important element that is necessary in the haemoglobin of the red blood cells and myoglobin in the muscle (Thomas, 2002). The finding from this study had reveal that fermented waste fruits do contain mineral contents.

The vitamins (A and C) content of the fermented waste fruit juice was also analysed. Vitamin A level in pineapple decreased from 0.15 mg/100ml to 0.06 mg/100ml on the 22<sup>nd</sup> day of fermentation. The result also reveals that there was a reduction in vitamin C level in pineapple from 16.7 to 5.2mg/100ml. Vitamin A and C decrease 20.5 to 16.3 mg/100ml and 35.6 to 25.0mg/ml respectively for banana juice, while vitamin A

and C decreased from 50.1 to 35mg/100ml and 163 to 25.0ml/100ml respectively for pawpaw juice.

Fruits are good sources of minerals and vitamins A and C (Ebuehi *et al.*, 2004), however the decrease reported may be attributed to utilisation by fermenting microorganisms. Previous works showed that the juice from fruits of black plum infected with *Aspergillus niger* decreased in protein content, pH, ascorbic acid and total sugars in infected fruit (Ekundayo and Okigbo, 1991). However, the values obtained are within the range confirmed by Ebuehi *et al.* (2004). Again, the decrease observed may not be unconnected with the difficulty in the storage of vitamin C, a strong reducing agent, readily losing hydrogen atom to become dehydroascorbic acid and finally hydrolysed losing its vitamin activity to diketoglulonic acid (Lehninger, 1981).

Vitamin C is an important antioxidant and helps protect against cancers, heart disease and stress. It is part of the cellular chemistry that provide energy, essential for sperm production, for making collagen, involved in the building and health cartilage, joints, skin and blood vessels (Lehninger, 1981; Champe and Harvey, 1994).

Ethanol a product of fermentation was recorded for the three fruit juice. Ethanol increase was recorded for the three fruit juice. The mean value recorded shows that pineapple has the highest value of  $0.89 \pm 0.49\%$ , followed by banana juice,  $0.642 \pm 0.35\%$  and the least value recorded for pawpaw juice as  $0.33 \pm 0.468\%$ . According to the classification of wines by Marrison (1959), the fermented juice of three fruits could be classified as light wines or table wines based on alcohol content. There was a decrease in pH for the three fruit juice although, within acidic range suggesting the fermenting micro-organisms must have the inherent capacity to tolerate acidic condition, or they may have been limited during the process. The highest mean pH value of  $4.41 \pm 0.26$  was recorded for pawpaw juice, followed by banana juice,  $4.35 \pm 0.15$  and  $3.6 \pm 0.37$  for pineapple juice. Battock and Azam-Ali (1998) stated that a food with a pH of 4.6 or less is termed as high acid or acid food and will not permit the growth of bacteria species. By acidifying foods and achieving a final pH of less than 4.6, most food are resistant to bacteria spoilage. The pH value of this study conforms to this assertion.

Comparatively, pineapple juice had the best organoleptic quality based on the responses of the testers in terms of colour, clarity, smell and taste, followed by banana juice and pawpaw juice but most ethanol distillate was produced from pawpaw juice.

## CONCLUSIONS AND RECOMMENDATION

The fermentation of peels, juice and pulp of pineapple banana and pawpaw fruits have shown that microorganisms (bacteria and fungi) are associated with the natural fermentation of the waste fruits. *Bacillus sp.* And *Saccharomyces cerevisiae* are the most predominant bacteria and fungi, particularly in the peels. However, the bacteria counts were higher. It also concluded that banana juice is the most significant in terms of energy and nutritional values. Pineapple juice had the best organoleptic quality, while pawpaw juice produced the most ethanol distillate.

This study therefore recommend that waste fruits which are considered spoilt hence low price value by the fruit traders should be given consideration by research institutes and food and drugs production companies, because of its nutritional composition. *Saccharomyces cerevisiae* isolated from the waste fruits can be screened for leavening ability. *Penicillium* and *Aspegillus* could be useful for the appropriate

food and drug industries. Probiotic microorganisms may also be isolated from the waste fruits.

Due to the perishable property of surplus fruits during seasons of over production. In tropical countries, it would be convenient to develop methods of preservation that would enable these plant materials to be utilised as animal feeds for longer periods of time.

Research institutes and chemical production companies should intensify effort in producing bio-fuel (ethanol) from the waste fruits. The Government at all levels should encourage this effort as this will provide employment, generate income as well as reduce potential environmental hazards when the fruits ferments in the open or in refuse of waste dumps.

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Table 1: Percentage occurrence of bacteria isolated from the pulp of pineapple, banana and pawpaw.

Bacterial Isolate	Pineapple Pulp (%)	Banana Pulp (%)	Pawpaw Pulp (%)
<i>Acetobacter aceti</i>	9.09	-	8.33
<i>Bacillus sp.</i>	40.9	44.4	45.83
<i>Clostridium butyricum</i>	-	11.11	8.33
<i>Enterobacter sp.</i>	9.09	-	-
<i>Flavobacterium sp</i>	-	-	8.33
<i>Micrococcus sp.</i>	-	16.7	12.5
<i>Lactobacillus sp.</i>	13.63	11.11	-
<i>Staphylococcus aureus</i>	9.09	5.55	-
<i>Streptococcus sp.</i>	18.18	-	16.7

Table 2: Percentage occurrence of fungi isolated from the pulp of pineapple, banana and pawpaw fruit.

Fungal Isolate	Pineapple Pulp (%)	Banana Pulp (%)	Pawpaw Pulp (%)
<i>Aspergillus flavus</i>	7.89	-	8.88
<i>Aspergillus niger</i>	-	14.2	15.55
<i>Debaromyces sp.</i>	5.26	-	-
<i>Fusarium sp.</i>	7.89	-	4.44
<i>Hansenula sp.</i>	-	5.71	-
<i>Penicillium chrysogenum</i>	15.78	17.14	-
<i>Penicillium verucosum</i>	13.15	-	13.33
<i>Rhizopus stonifer</i>	-	8.57	8.88
<i>Pichia sp.</i>	-	11.42	-
<i>Saccharomyces cerevisiae</i>	50	42.85	46.66
<i>Saccharomyces lactis</i>	-	-	4.44

Table 3: Ethanol Production from Fermented Pineapple, Banana and Pawpaw Juice.

Fermentation (Day)	Period	Quantity Distilled (ml)	Quantity Produced (ml)		
			Pineapple	Banana	Pawpaw
1st		100	63	34	76
4th		100	70	27	91
7th		100	64	25	87
10th		100	70	25	87
13th		100	74	31	82
16th		100	76	35	74
19 <sup>th</sup>		100	82	32	58
22nd		100	73	25	86
Total Ex			572	23.4	668
Mean			71.5	29.25	83.5
S.D			6.23	4.23	5.83

Table 4: Comparison of the mean nutritional value of fermented pineapple, banana and pawpaw juice.

Nutritional Composition	Pineapple Juice	Banana Juice	Pawpaw Juice
% Moisture	85.6±1.79	78.5±2.54	91.9±1.12
% Ash	0.365±0.018	0.5±0.00	0.05±0.00
% Fat	0.04±0.00	0.41±0.00	0.5±0.005
% Carbohydrate	11.6±3.58	15.35±6.17	6.78±1.92
% Fibre	0.17±0.011	0.71±0.11	0.54±0.13
% Protein	0.5±0.00	1.43±0.10	0.44±0.15
Ca <sup>2+</sup>	11.85±0.587	8.41±0.41	12.15±0.89
P <sup>+</sup>	8.65±0.69	3.98±0.04	6.80±0.68
K <sup>+</sup>	16.95±0.76	4.63±0.14	3.35±0.16
Na <sup>+</sup>	0.95±0.07	2.89±0.16	2.05±0.05
Fe <sup>2+</sup>	0.50±0.007	0.43±0.004	0.40±0.004
Mg <sup>2+</sup>	10.6±0.07	2.61±0.12	0.20±0.04
Vit A	1.97±5.26	19.6±1.50	47.5±5.35
Vit C	13.5±4.42	30.8±3.39	123.9±0.06
Specific Gravity	0.92±0.04	0.95±0.03	0.88±0.06
Viscosity	50.6±7.28	63.3±22.8	80.6±14.7
Volume of Co <sub>2</sub>	0.75±0.13	0.88±0.52	0.64±0.35
Ethanol	0.89±0.49	0.642±0.350	0.33±0.468
pH	3.6±0.37	4.35±0.15	4.41±0.267