*Science World Journal Vol. 16(No 4) 2021*
*www.scienceworldjournal.org*
*ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)*
*Published by Faculty of Science, Kaduna State University*

Full Length Research Article

# FRUIT WINE PRODUCED FROM PAWPAW (*CARICA PAPAYA*) AND WATERMELON (*CITRULLUS VULGARIS*) USING *SACCHAROMYCES CEREVISIAE* ISOLATED FROM SELECTED FERMENTED FOODS

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**ABSTRACT**
This study was aimed at investigating the suitability of the fruits (pawpaw and watermelon) as substrates for wine production and the efficiency of yeast isolated from fermented foods for alcoholic fermentation of fruits. Yeast cells were isolated using pour plate technique. The probable isolates were confirmed using molecular procedure. The yeast cells were used to ferment pawpaw and watermelon Juice (must) to produce wine. During fermentation aliquot samples were removed daily from the fermentation tank for analysis of pH, temperature, total titrable acidity and total viable yeast counts were determined at 24 h interval. During wine production temperature ranged from 27 °C to 30 °C. There was a gradual decrease in pH (ranging from 4.4 to 3.3) and specific gravities (ranging from 0.99 to 0.84), with increase in titratable acidity (ranging from 1.53 to 2.23%) and alcohol contents (ranging from 0.0 to 13.63 %). Proximate composition revealed that fruit juices had higher percentage moisture content, ranged from 76.72% to 85.94%, ash content of 0.08% to 0.67%, protein content of 4.59% to 5.69%, percentage fat content of 7.35% to 8.75%, and total carbohydrates of 1.46% to 5.99%,while proximate composition of the produced fruit wine revealed percentage moisture content of 83.45% to 85.12%, ash content of 0.17% to 1.05%, protein content of 3.17% to 5.47%, percentage fat content of 7.45% to 830%, and total carbohydrates of 1.08% to 3.80% respectively. Microbial analysis and sensory evaluation of the produced wine was carried out using nine (9) point hedonic scales by 10 panellists, Sensory evaluation (P>0.05) rated the wines acceptability as Pawpaw wine > Watermelon wine > blend of Pawpaw and Watermelon wine. Shelf life assessment of the produced fruits wine were carried out and samples were plated on nutrient agar and sabouraud dextrose agar for enumeration of bacteria, using standard procedures. During shelf life assessment, it was observed that there were no spoilage or growth of microorganisms in wines samples at room temperature (26$^{o}$C) and refrigeration temperature (4$^{o}$C) except *Saccharomyces cerevisiae*. This study revealed that watermelon is the most acceptable wine and could be produced from this fruit with yeast from palm wine.

**Keywords:** Fermented Foods, Saccharomyces Cerevisiae, fruits wine.

**INTRODUCTION**
In botany, a fruit is the seed-bearing structure in flowering plants (also known as angiosperms) formed from the ovary after flowering(Awe, 2011).
Fruits are the means by which angiosperms disseminate seeds. Edible fruits, in particular, have propagated with the movements of humans and animals in a symbiotic relationship as a means for seed dispersal and nutrition; in fact, humans and many animals have become dependent on fruits as a source of food. Accordingly, fruits account for a substantial fraction of the world's agricultural output, and some (such as the apple and the pomegranate) have acquired extensive cultural and symbolic meanings (Awe, 2011).

Pawpaw (*Caricapapaya*) is grown mostly for fresh consumption or for production of latex. *C.papaya* plants produce natural compounds (annonaceous, acetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties (Nwofia and Ojimelukwe 2012; Nwofia and Okwu 2012). The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present which has value as a remedy in dyspepsia and has been utilized for the clarification of beer. The juice has been in use on meat to make it tender, (Ayoola and Adeyeye, 2010). The unripe fruit is used as a remedy for ulcer and impotence. It cleans bacteria from the intestines and hence encourages the absorption of vitamins and minerals, especially vitamin $B_{12}$. The tea prepared with the green papaya leaf, promotes digestion and aids in the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Nwofia and Ojimelukwe 2012). However, ripe pawpaw fruits are very perishable, and large quantities are disposed off yearly due to lack of or poor storage facilities resulting to loss of the vital nutrients contained in the pawpaw fruits (Souza *et al.,* 2008; OECD 2010; Awe, 2011; Nwofia and Okwu 2012; Ugbogu and Ogodo, 2015). However, these losses can be reduced and pawpaw can be made available all year round, by utilizing the fruits for other purposes such as wine production.

Watermelon (*Citrullus vulgaris* L.) is a tropical fruit which grows in almost all parts of Africa and South East Asia (Koocheki *et al.*, 2007). It serves as a good source of vitamins and phytochemicals that have chemopreventive effects against cancer (Perkins-Veazie and Collins 2004; Collins *et al.,* 2005; Oms-Oliu *et al.*, 2009;

Enukainure *et al.,* 2010; Inuwa *et al.,* 2011). In Nigeria, watermelon are fermented, blended and consumed as juice, nectars, fruit cocktails and can also be used as an appetizer or snacks, depending on how it is prepared (Onyeleke and Olaniyan, 2007; Oms-Oliu *et al.*, 2009; Enukainure *et al.,* 2010). The seeds are also reported to possess medicinal properties and are used to treat chronic or acute eczema. It contains high levels of proteins, lipids and is a rich source of carbohydrate and fiber. Arginine, glutamic acid, aspartic acid and leucine are the predominant amino acids in watermelon proteins. Reports are also available on the biological value, true digestibility, protein efficiency ratio and net protein utilisation of watermelon seeds (Wani *et al.,* 2011; Lawal, 2011; Inuwa *et al.,* 2011). Moreover, they are used as a domestic remedy for urinary tract infection, hepatic congestion, catarrh, worm remedy, abnormal blood pressure (Amadi *et al.,* 2003). Watermelon contain large amount of beta carotene and are significant sources of lycopene (Collins *et al.,* 2005). The production of wine from common fruits could help reduce the level of post-harvest losses and increases the variety of wines (Okoro 2007; Alobo and Offonry 2009).

Wine is any alcoholic beverage produced from juices of variety of fruits by fermentative action of microorganisms either spontaneously or seeding with a particular strain mainly of yeast species to adopt a particular quality of wine. Wine is one of the most recognizable high value added products from fruits. Most commercially produced wines are usually made from fermented grapes; this fermentation process is not done by introducing any chemicals or sugar but by adding different species of yeast to the crushed grapes (Awe, 2011). Yeast has the capability of converting grapes into an alcoholic compound and removing the sugar content in it for the production of different types of wines. Sometimes wines are produced from different types of fruits like; Paw-Paw, mango, Pineapple, Banana, Lemon, Watermelon etc., here the wine so produced bears the name of the fruit or fruit mixture used in its production. It is a rich source of vitamins, many essential amino acids, minerals, fatty acid and others; however other fruits with same characteristics have been discovered and used effectively for wine production (Awe, 2011).

The Nigerian wine industry is still young as the processing and bottling of palm wine is only recent despite the fact that production of palm wines locally was achieved several decades ago. Inyang reported that a major breakthrough in wine making technology by Nigerians which received patent right was the production of cocoa wine in 1983(FAO, 2013). Although, there has been tremendous efforts at exploring different forms of fruits from which wine is made h such that wines like coconut wine, kolanut wine, pineapple wine cashew apple wine and star apple wine have been produced, yet the large scale production of most of these wines remain abysmal. South Africa is among the top ten world producers and exporters of wine (FAO, 2013).

Wine has enormous health benefits similar to those of fruits from which they are derived. A number of these effects have been documented in recent times. For instance, almonds have been found to be more effective in reducing blood levels of low density lipoprotein cholesterol (LDLC) when combined with other foods known to independently lower cholesterol (FAO, 2013). The consumption of citrus fruits like orange and lemon singly and especially when combined offer significant protection against various cancers, diabetes, Parkinson's disease and inflammatory bowel disease. Cucumber, production in Nigeria started at about 17th century ago but its cultivation and marketing has been subsistence level. It was reported that pilgrims brought cucumber in to Nigeria from North Africa during trans- Saharan trade. Though Nigeria is not a major cucumber producer in the world, the crop strives in Northern parts of the country particularly regions above latitude 100 North of the equator (Omamor *et al*., 2009). It is propagated by seed, offshoot and tissue culture. All over the world people have learnt to culture and use essential microorganisms for production of alcoholic beverages and fermented foods.

Fermentation is a metabolic process of deriving energy from organic compounds without the involvement of an exogenous oxidizing agent. In general, that process proceeds under the influence of activities exerted by enzymes and microorganisms (Djegui *et al.,* 2014). Even though the scientific explanation and the identity of these beneficial microorganisms mostly lactic acid bacteria, filamentous moulds and yeasts were unknown to people in the past, they cultured them traditionally for production of foods for consumption. In Europe, America and Africa fermented foods are prepared exclusively using bacteria or bacteria-yeasts mixed culture (Djegui *et al.,* 2014).

Yeasts serve a critical role in the production of many traditionally fermented staple foods and beverages across the world. Alcoholic beverages represent a vast diversity of products ranging from table wines, sake, cider, fruit wines, beer and distilled alcoholic products. Cereals are the main substrates for fermentation (Jimoh *et al.,* 2012; Djegui *et al.,* 2014). Today, beer brewing and wine making are huge, enormously profitable agricultural industries. These industries developed from ancient and empirical knowledge from many different cultures around the world. Today this ancient knowledge has been combined with basic scientific knowledge and applied toward modern production processes (Jimoh *et al.,* 2012).Palm wine is an alcoholic beverage from the sap of various species of palm tree such as palmyra and coconut palm. This is commonly called "emu" and "oguro" in western part of Nigeria. Palm wine may be distilled to produce a strong drink "ogogoro" (local gin) (Adeleke and Abiodun, 2010). Palm wine is an alcoholic beverage that is produced and consumed in different regions of the world, according to the country of origin. The sap of the palm trees, which is originally sweet serves as a rich substrate for the growth of various types of microorganisms (Naknean *et al.,* 2010; Santiago-Urbina *et al.,* 2013). The sap undergoes spontaneous fermentation, which promotes the proliferation of yeasts and bacteria for the conversion of the sweet substrate into several metabolites mainly ethanol, lactic acid and acetic acid (Santiago-Urbina *et al.,* 2013).

Palm wine is an alcoholic beverage from the sap of various species of palm tree such as palmyra and coconut palm. This is commonly called "emu" and "oguro" in western part of Nigeria. Palm wine may be distilled to produce a strong drink "ogogoro" (local gin) (Adeleke and Abiodun, 2010). Palm wine is an alcoholic beverage that is produced and consumed in different regions of the world, according to the country of origin. The sap of the palm trees, which is originally sweet serves as a rich substrate for the growth of various types of microorganisms (Naknean *et al.,* 2010; Santiago-Urbina *et al.,* 2013). The sap undergoes spontaneous fermentation, which promotes the proliferation of yeasts and bacteria for the conversion

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## MATERIALS AND METHOD

### Sources and collection of Samples

Ripe Watermelon (*Citrullus vulgaris* L.) and Pawpaw (*Carica papaya*) were purchased from the local market at (Bakin Dogo), Kaduna State, Nigeria. Fresh palm wine were obtained from the palm wine tappers in kujama, Kaduna State within 1hour of tapping. The fruits and the palm wine were transported to the laboratory in clean cellophane bag sand in an ice box respectively for analysis.

### Isolation and Identification of Yeast from Burkutu

Culturing of the Palm wine was done on Potato Dextrose Agar (PDA). The medium was prepared according to manufacturer's instructions and supplemented with 40mg/L chloramphenicol for selective enumeration of yeast. The isolates obtained were sub-cultured on fresh medium to obtain pure cultures. The yeast cultures were transferred to modified Malt Extract Agar (MEA) containing yeast extract and 2 % glucose and then incubated for 24 hours. Carbohydrate utilization and ethanol tolerance where carried out according to Alobo (2009), they were screened for their ability to tolerate different concentrations of sugar and alcohol by inoculating on MEA supplemented with 10–60, and 5–30 %, sucrose and ethanol respectively. Upon molecular confirmation, the isolate with the highest sugar and alcohol tolerance was selected and used as the starter culture. The identified organism was maintained on MEA slant.

### Methods of processing involved in wine production

**Sorting**: The fruits were sorted in order to obtain wholesome, ripe banana of even sizes. Parameters such as size, shape and colour were used in sorting.

**Washing**: The fruits were washed with sponge and rinsed with portable water till there was no dirt on it.

**Slicing**: The whole fruits were sliced into small bits with the aid of a kitchen stainless knife in order to reduce the size and allow for a larger surface area for easy blending.

**Blending**: The sliced fruits were crushed using a blender.

**Weighing**: The must was weighed to know the amount of sugar, yeast etc. that must be added to the must for fermentation.

**Sulphiting**: Sodium metabisulphite was added to the must.

**Addition of yeast**: Yeast was added to the must after 24 hours.

**Fermentation**: The sugar in the fruit was left to be fermented by the yeast into alcohol and carbon dioxide.

**Racking**: After the fermentation, the wine was siphoned into another jar carefully without touching the aqueous layer.

**Bottling**: The wine was bottled and corked (Nwanekezi *et al.,* 2004).

### Preparation of Must Juice for Separate Fruits and Mixed Fruits Fermentation

The fruits (Pawpaw and Watermelon) were washed thoroughly with distilled water and then peeled. Fruit samples, pawpaw and watermelon were weighed for mixed fruit and single fruits fermentations respectively. This was then chopped into smaller pieces using a clean knife before transferring them quantitatively into laboratory blender for crushing. The crushed samples was transferred into a clean new transparent bucket and mixed with distilled water (1:1 w/v). Exactly 0.656 kg of sugar was added to the must followed by vigorous stirring. Exactly 4 g of sodium metabisulphate ($Na_2S_2O_5$) was dissolved in 400 ml of water and poured in 100 ml aliquots to each of the mixtures and stirred properly. Sodium metabisulphate serve as a sterilizer and prevents fermentation before the addition of the yeast starter. (Alloysius *et. al.,* 2015)

### Development of the Inoculum for Must Fermentation

The inoculum medium was prepared using the following formulation; glucose, 150g; yeast extract, 2g; peptone water, 2g; malt extract, 3g; $MgSO_4.7H_2O$, 1g; $KH_2SO_4$, 1g; $NH_4SO_4$, 4g; NaCl, 1g; Ferrous Sulphate, 1g in 1litre of water. Isolates from Palm wine (PW 1) and Baker's yeast (BY) were in inoculated into 100ml each of the inoculum medium and allowed to stay for 48 hours. Exactly 1.5g of the medium was dissolved in 50ml of distilled water (Adedeji and Oluwalana, 2013).

A 0.5 Mcfarland was prepared where 1 ml of concentrated sulphuric acid ($H_2SO_4$) was added to 99 ml of distilled water in a conical flask or beaker and mix well .In this way a 1 % v/v solution of $H_2SO_4$ is prepared. A solution of 0.5 g of dehydrate barium Chloride salt ($BaCl_2$. $2H_2O$) was dissolved in 50 mL of distilled water. In this way a 1 % w/v of $BaCl_2$ is prepared. A solution of 0.6 mL of $BaCl_2$ solution will be added to 99.4 mL of $H_2SO_4$ solution to make up to 100 mL. The solution was mixed well. This was the stock solution of the 0.5 McFarland turbidity standards. Exactly 2 mL of the solution was transferred into capped tubes and stored at room temperature until use (Damisa and Ataikiru, 2015).

### Fermentation of Musts for Wine Production

The fermenters was washed with detergent and rinsed with sterile distilled water. Two **(**2) litres each of the pasteurized juice (must) was transferred aseptically to the fermenter. Exactly 0.3ml of sodium metabisulphate was added, and a mercury glass thermometer inserted at the top cover of the containers. Exactly 3mL (3 x $10^8$cell) of the prepared inoculum using 0.5 Mcfarland standard were added to the different musts in the fermenter and were agitated to dispense evenly in the fermenter and kept at room temperature. The musts were aerated daily by stirring twice to encourage yeast multiplication. Specific gravity (S.G), pH, temperature, % titrable acidity and viable yeast count of the must were determined daily for seven days during the period of fermentation. Fermentation was terminated after seven days and the wine was sieved to remove the shaft, dealcoholized, bottled, pasteurized and stored. The wines were refrigerated as described by Adedeji and Oluwalana (2013).

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**Physicochemical Analysis of the Musts**

**Determination of pH**
Ten (10) mL of each must was transferred into a sterile beaker, and the pH of the must was determine using a digital pH meter (model pHS -25) as described by Ochai and Kolhatkar (2008).

**Determination of Titratable Acidity**
The titratable acidity was determined during the fermentation based on Association of Analytical Communities International (2000) method 962.12. The sample was degassed by agitation.
The pH of the water was adjusted by adding 1mlphenolphthalein indicator for each 200ml of water. 0.1 N NaOH was used to neutralize the water to a distinct but faint pink colour (desired endpoint). 5mL of the degassed sample was pipette into a 250mL conical flask, 100mL of distilled water was added to the flask. The flask was swirled to release any remaining $CO_2$. 0.1 N NaOH was titrated against the content of the flask until the pale pink colour endpoint which persists for30 seconds is achieved. Titratable acidity was calculated as follows:

% Tartaric Acid $= \frac{(mi\ alkali)\ X\ (normallity\ of\ alkali) X\ 7.5}{weight\ of\ sample\ (mls\ of\ samlpe)}$

**Determination of Specific Gravity (S.G)**
This was carried out using relative density bottle as described by Yabaya *et al.,* (2016), where the bottle was washed with tap water and dried. The surface of bottle was further clean with ethanol and allowed to dry again. Using an electronic balance, the empty weight of the bottle was determined ($M_0$). The weight of the bottle plus 5ml of the wine sample ($M_1$) was determined, follow by the weight of the empty bottle plus5ml of distilled water ($M_2$), and the specific gravity then calculated from these values as follows:

$$specific\ gravity = \frac{weight\ of\ volume\ sample\ (M_1 - M_0)}{weight\ of\ an\ equal\ volume\ of\ water\ (M_2 - M_0}$$

**Measurement of Temperature**
120$^0$C mercury bulb thermometer was inserted to the side arm of the fermentation tank through a sterile rubber cork. The periodic temperature changes during fermentation were recorded (Yabaya *et al.,* 2016).

**Determination of Total Sugar of the Wine**
The concentration of soluble sugars was determined with a refractometer. The prism was dried with a blotter and lens paper. Several drops of the fruit must (juice) and wine was applied to the lens using a cotton swab and readings in the degree Brix were obtained (Adedeji and Oluwalana, 2013).

**Determination of Percentage Alcohol Contents of Wine**
One hundred (100) ml of produced wine was placedintoa100 ml capacity graduated cylinder. This was refrigerated for 15 minutes until the temperature of the liquor reached 15$^0$C. The alcohol meter was allowed to float freely on the sample and then the alcohol content was recorded. The reading was expressed as % alcohol (v/v). The purified alcohol was calculated using the formula:

$$Purified\ alcohol\ (L) = \frac{Volume\ of\ Alcohol\ (L)\ x\ Alcohol\ percentage\ (\%)}{100}$$

(Chim *et al*., 2015)

**Proximate Analysis of Must and Wine**
The must and the wine produced were subjected to proximate analysis. These analysis include: % moisture content, % ash content, % crude protein, % fat content, % total carbohydrate and % fibre.

**Determination of Percentage Moisture Content**
Metallic dishes were dried in the oven at 80$^o$C for 20 minutes, and allowed to cool in desiccator and weighed. Five (5) g of different fruits and produced fruit wine were placed in dishes and weighed. The dishes with the sample were dried in an oven at 80$^o$C for 24 hours to achieve a constant weighed and quickly transferred to the desiccator to cool. After cooling, it was weighed immediately with minimum exposure to the atmosphere. The loss of weight of the must and wine during drying were moisture content (AOAC, 2016).

$$\%\ moisture = \frac{W_2 - W_3}{W_2 - W_1}\ X\ 100$$

Where $W_1$= initial weight of empty crucible
$W_2$= Weight of crucible + sample before drying
$W_3$= final weight of crucible + sample after drying

**Determination of Total Ash Content**
Ten (10) grams of fruits samples and 10 ml for the produced fruit wine were introduced into a small dry crucible of known weight. The sample in the crucible was charred on furnace. The charred sample was ashed in a muffle furnace at 550$^o$C for 2 hours. The ashed materials was removed from the furnace and cooled. The materials were placed in the desiccator and weighed (AOAC, 2016)

$$\%\ ash\ content\ (dry\ basis)\ = \frac{W_3 - W_1}{W_2 - W_1}\ X\ 100$$

Where $W_1$ = weight of empty crucible
$W_2$ = weight of crucible + sample before drying and or ashing
$W_3$ = weight of crucible + ash

**Determination of Crude Protein**
The fruits and produced wine were digested with concentrated $H_2SO_4$, concentrated NaOH (40%), $K_2SO_4$ and $CuSo_4$. 5ml of the digested sample were placed into a micro-kjeldahl distillation apparatus and excess concentration NaOH was added to make the solution strongly alkaline. Ammonia was distilled into 5ml of boric acid indicator in a titrating flask. Above 45mL of the distillates was collected. Titration was done with 0.01m HCL. The end point of the titration was light green (AOAC, 2016).

% protein = % N × F

Where F = conversion factor 100 (% in food protein)

Where $\%\ N\ = \frac{VS\ VB \times Nacid \times 0.01401 \times 100}{W}$

$V_S$ = vol. (ml) of acid required to titrate sample
$V_B$ = vol. (ml) of acid required to titrate blank
Nacid = normally of acid (0.1)
W = weight of sample in grams

**Determination of Total Fat Content**
Exactly 250 mL clean boiling flask was dried in an oven and transferred into dessicator to cool. Empty filter paper was weighed and labeled, $W_1$. Two (2) grams of sample was weighed into labeled thimbles (filter paper), $W_2$. The boiling flask was filled with

petroleum spirit or N-hexane. The soxhlet apparatus was assembled and was to reflux for 8 h. It was removed and transferred to an oven to dry. It was transferred from the oven into the dissicator and allowed to cool, and was weighed, $W_3$.

$$\% Fat = \frac{W_2 - W_3 \, X \, 100}{W_2 - W}$$

(AOAC, 2016).

**Determination of Total Fibre Content**

Exactly 2g (W) of the sample was placed in a beaker containing 1.2mL of $H_2SO_4$ per 100mL of solution and boiled for about 30minutes, the residue was filtered and wash with hot water, the residue was transferred to a beaker containing 1.2g of NaOH per 100ml of solution and boiled for about 30minutes, the residue was washed with hot water and dried in an oven and weighed ($W_2$), the weighed sample was incinerated in a furnace for about 550$^o$C, removed to cool, and weighed ($W_3$).

$$\% Fibre = \frac{W_2 - W_3 \, X \, 100}{W}$$

(AOAC, 2016)

**Determination of Total Carbohydrate Content**

The Total carbohydrate content of the sample was obtained as described by Moronkola *et al*., (2011), where the results from fat, protein, moisture and ash content analyses were sum-up and the carbohydrate content was calculated as follows:100% (% moisture + % protein + % fat + % ash) (AOAC, 2016).

**Microbiological Analysis of the Produced Wine**

**Determination of Total Yeast Colony Count**

Potato dextrose agar (PDA) was used for enumeration of yeasts. Well homogenised wine samples were serially diluted with 0.1%pepetone water up to $10^{-4}$. Aliquots (0.1ml) from each dilution were transferred aseptically plates. The medium was poured into the plates and swirled gently to the left and to the right and allowed to solidify. The plates were then incubated for 72 h at 28$^0$C. Counting (CFU/mL) was carried out using colony counter (Adedeji and Oluwalana, 2013).

**Bacterial Enumeration from the Wine**

Nutrient agar was used for enumeration of bacteria. Serial dilution of the wine was carried out and inoculated using pour plate techniques. The plates were incubated at 37$^0$C for 24 h (Adedeji and Oluwalana, 2013).

**Total Coliform Count of Bacteria (TCC)**

MacConkey broth was used for the detection of coliform bacteria by the multiple tube technique. The medium was distributed in 9 ml quantities standard test tubes with inverted Durham tube and was then autoclaved for 15 minutes at 121$^0$C.Well homogenized samples was serially diluted ($10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$) with 0.1% peptone water. Exactly 25mL from each dilution was aseptically inoculated into triplicate of 225mL MacConkey broth in standard test tube and incubated for 48 hours at 37$^0$C (Adedeji and Oluwalana, 2013).

**Dealcoholization of the Produced Wine**

The fermented product (wine) was dealcoholized using a rotary evaporator to remove residual water and impurities. The rotary evaporator was operated at a temperature of 65$^0$C for 45 minutes (Chongkhong and Lolharat, 2013).

**Sensory Evaluation of produced Wine**

The sensory evaluation was determined using the method described by Chim *et al*. (2015) to evaluate the fruits wine. Exactly 15 mL of the prepared fruit wine was dispensed in a clean short glass and placed on a table. A panel of ten (10) judges consisting of students and lecturers of the Department of Microbiology, Kaduna State University were used. Sensory properties such as clarity, aroma, flavor, taste (sweet, bitter and sour) and general acceptability of the produced fruit wine was determined using quality scoring test with 9-point hedonic scale. (Range of Scores were: 1, Like Extremely: 2, Like Very Much: 3, Like Moderately: 4, Like Slightly: 5, Neither Like Nor Dislike: 6, Dislike Slightly, 7, Dislike Moderately 8. Dislike Very Much, 9. Dislike Extremely (Chim *et al*., 2015).

**Shelf Life Evaluation of the Produced Wine**

Nine (9) bottles containing two individual/blend fruit wine were stored in the refrigerator (4$^0$C) and at room temperature (**26**$^o$C) (Omojasola *et al*., 2012). Exactly 25mL of the different wine was extracted from each bottle using a sterile syringe into sterilized tubes containing 225mL each of distilled water and thoroughly mixed by shaking the test tubes. The wine from each bottle was then serially diluted and 1ml of the diluent transferred into sterile petri dishes using pour plate method. The plates were carefully swirled, allowed to solidify. The cultured NA plates in duplicate per bottle were then incubated at 37$^0$C for 48 h while PDA plates were kept at a temperature 28$^0$C for 72 h. This was repeated after every seven (7) days for 28 days. Fungal isolates were identified using lactophenol stain and microscopy using mychological atlas (Vwioko*et al*., 2013).

**RESULTS**

All isolates from Palm wine (Pw 1) were found to be creamy with flat elevation. They have bud and are circular in shape. They showed positive utilization to sucrose from 5% to 25%; and showed positive tolerance to ethanol at positive tolerance at 14%. During the seven days fermentation period, Table 1 shows there was significant difference ($p<0.05$) across the rows as pH decreased for Pawpaw wine from 4.15 – 3.49, Watermelon wine from 4.0 – 3.3 and blend of Pawpaw and Watermelon wine from 4.4 – 3.6, produced using yeast isolated from Palm wine. Table 1 also shows there was an increase in temperature for Pawpaw, Watermelon and mixture of Pawpaw and Watermelon wine from the first day (29.3$^0$C, 29.5$^0$C and 29.9$^0$C) to subsequent days, but a decrease was observed on the seventh day of fermentation for Pawpaw, Watermelon and mixture of Pawpaw and Watermelon wine respectively, there was significant difference across ($p<0.05$) the rows. Table 1 shows there was a significant difference across the rows at ($p<0.05$) as titrable acidity increased from 1.59 to 2.23, 1.53 to 2.16 and 1.56 to 2.08 for Pawpaw, Watermelon and mixture of Pawpaw and Watermelon wine produced using *Saccharomyces cerevisiae* isolated from Palm wine. Table 1 also shows there was an increase in total viable yeast count during the first few days of fermentation of Pawpaw, Watermelon and blend of Pawpaw and Watermelon wine from 20 – 69, 0 – 21 and 17 – 138 x $10^4$ CFU/ml. A decrease was observed during last days of fermentation for Pawpaw, Watermelon and blend of Pawpaw and Watermelon wine. Table 1 shows the Specific gravity decreases from 0.93 – 0.84,

0.96 – 0.0.91 and 0.99 – 0.86 for Pawpaw, Watermelon and blend of Watermelon and Pawpaw wine respectively. Table 2 shows the $^{o}$Brix of the musts (Pawpaw, Watermelon and blend of Pawpaw and Watermelon) had a $^{o}$Brix of 26.12, 24.4 and 26.2 before fermentation. After fermentation was stopped the $^{o}$Brix value decreased to 12.78, 13.60 and 15.05 $^{o}$Brix for wine produced from Pawpaw, Watermelon and mixture of Pawpaw and Watermelon. There was significant difference at ($p<0.05$). Table 2 also shows the alcoholic content of the produced Pawpaw, Watermelon and Pawpaw and Watermelon wine were observed to be 13.63%, 11.40% and 12.82% respectively. Table 2 also shows there was no significant difference ($p>0.05$) in the moisture, ash and protein content, but a significant ($p<0.05$) difference in lipid and carbohydrate content was recorded. The percentage moisture content obtained for Pawpaw, watermelon and blend of Pawpaw and Watermelon were 85.94, 80.94 and 76.72 for fruit must while the produced wine had 83.45, 84.64 and 84.52% of moisture respectively. The percentage ash content for the musts were 0.0.67, 0.08 and 0.20 and produced wines were 0.46, 1.05 and 0.51. Percentage protein content for the musts were 4.59, 5.04 and 5.69 while the produced wines were 5.36, 5.47 and 5.58% respectively. The percentage lipid content of the musts were 7.35, 8.20 and 8.75 and that of the wines were 8.30, 7.96 and 8.50%, percentage carbohydrate content of the musts were 1.46, 5.99 and 2.83, that of the produced wines was , 2.01, 1.08 and 2.65 respectively. The result of microbial analysis is shown in table 3. The result revealed that there was no bacterial and coliform growth while total viable yeast count ranged from 0.2 x $10^{-4}$ - 3.20 x $10^{-4}$CFU/mL for wines produced using yeast from Palm wine and 0.5 x $10^{-4}$ – 3.7 x $10^{-4}$ CFU/mL for wine using Baker's Yeast; before pasteurization and no count was recorded after pasteurization. The sensory perception of produced wine from fruits are presented in Fig.1, which showed the different views of the ten (10) Panellists on the fruit wine produced. There was no significant difference at ($P>0.05$) between different fruits and the perception of different qualities; colour, aroma, clarity, flavour, taste (sweet, sour and bitter), and general acceptability of the produced wines. Table 4 showed the shelf life study of wine kept at room temperature (26±1$^{o}$C) and refrigerator (4$^{o}$C). The data generated showed that there was no contamination after production of the fruit wine and after four weeks of shelf life. But there was evident of inoculated organisms during the duration (7days) of shelf study.

**Table 1.** Variation in Physicochemical Parameters of Produced Wine within the Days of Fermentation

| Parameters | Wine Samples | | |
|---|---|---|---|
| | P. PW1 | W. PW1 | P/W. PW1 |
| **pH** | 4.15 – 3.49 | 4.0 – 3.3 | 4.4 – 3.6 |
| **T$^{o}$C** | 29.3$^{0}$C | 29.5$^{0}$C | 29.9$^{0}$C |
| **TYC $10^{4}$ CFU/ml** | 20 – 69 | 0 – 21 | 17 – 138 |
| **TTA** | 1.59 – 2.23 | 1.53 – 2.16 | 1.56 – 2.08 |
| **SG** | 0.93 – 0.84 | 0.96 – 0.0.91 | 0.99 – 0.86 |

**Keys**: TTA: Total Titratable Acidity, SG: Specific Gravity, T$^{o}$C: Temperature, TYC: Total Yeast Count, P.PW1: Pawpaw Must + Yeast from Palm Wine, W.PW1: Watermelon Must + Yeast from Palm wine, P/W. PW1: Pawpaw/Watermelon Must + Yeast from Palm wine

**Table 2.** Proximate Composition of the must and the produced wine

| Parameter (%) | Watermelon + SC | Pawpaw + SC | Pawpaw and Watermelon + SC |
|---|---|---|---|
| Moisture | 84.59±0.26$^{a}$ | 83.45±0.26$^{a}$ | 84.52±0.15$^{a}$ |
| Ash | 0.52±0.00$^{a}$ | 0.46±0.01$^{a}$ | 0.51±0.01$^{a}$ |
| Lipid | 7.96±0.08$^{a}$ | 8.30±0.57$^{a}$ | 7.45±0.64$^{a}$ |
| Protein | 4.16±0.62$^{a}$ | 5.36±0.16$^{a}$ | 4.92±0.16$^{a,b}$ |
| Fibre | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Carbohydrate | 2.79±0.43$^{a}$ | 2.84±0.47$^{a,b}$ | 2.61±0.35$^{a}$ |
| Alcohol | 11.40 ±0.05$^{a}$ | 13.63 ±0.54$^{a,b}$ | 12.08 ±0.68$^{a}$ |
| Sugar (Brix) | 13.60±0.09$^{a}$ | 12.78 ±0.14$^{a}$ | 15.05 ±0.04$^{a}$ |

**Table 3.** Microbial Analysis of the Produced Wine

| Wine Samples | TYC ($10^{-4}$) | TVC (Bacterial) ($10^{-4}$) | TCC |
|---|---|---|---|
| P PW | 3.2 | Nil | Nil |
| W PW | 0.2 | Nil | Nil |
| P/W PW | 1.8 | Nil | Nil |

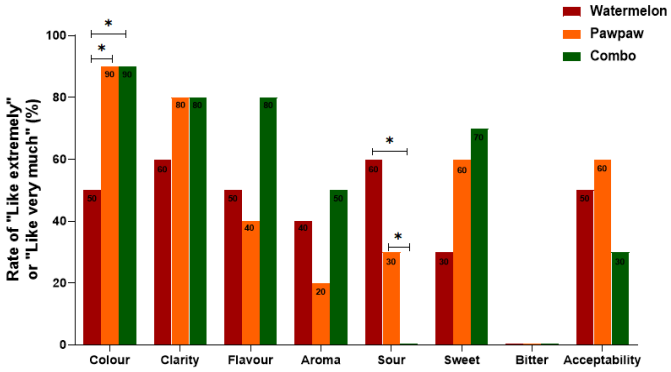


**Fig 1.** Sensory Properties of Produced Wines

**Table 4.** Shelf Life Assessment of the Produced Pawpaw, Watermelon and Blend of Pawpaw and Watermelon Wine

| | Room Temperature(26±1$^{o}$C) | Refrigerator(4$^{o}$C) |
|---|---|---|
| **Shelf life (Days)** | **PDA** | **PDA** |
| **Pawpaw wine** | | |
| **7** | *Saccharomyces cerevisiae* | - |
| **14** | *Saccharomyces cerevisiae* | - |
| **21** | *Saccharomyces cerevisiae* | *Saccharomyces cerevisiae* |
| **28** | *Saccharomyces cerevisiae* | *Saccharomyces cerevisiae* |
| **Watermelon Wine** | | |
| **7** | *Saccharomyces cerevisiae* | - |
| **14** | *Saccharomyces cerevisiae* | - |
| **21** | *Saccharomyces cerevisiae* | - |
| **28** | *Saccharomyces cerevisiae* | *Saccharomyces cerevisiae* |
| **Blend of Pawpaw and Watermelon Wine** | | |
| **7** | *Saccharomyces cerevisiae* | - |
| **14** | *Saccharomyces cerevisiae* | - |
| **21** | *Saccharomyces cerevisiae* | *Saccharomyces cerevisiae* |
| **28** | *Saccharomyces cerevisiae* | *Saccharomyces cerevisiae* |

**DISCUSSION**

Morphologically, *Saccharomyces cerevisiae* clustered was found to be circular shape, whitish cream color, elliptical morphology, and formed pseudohyphae. The presence of the yeast isolate in palm wine could be attributed to the high carbohydrate content in the grain used in its production. This result is related to the findings of Umeh *et al.,* (2015) who also isolated and identified *Saccharomyces cerevisiae* from fresh burkutu and found it potent for use in wine making. The isolates fermented glucose, maltose and sucrose, but could not assimilate xylose, lactose and manitol. This correlates with the report of Olowonibi (2017), they were suspected to be *Saccharomyces cerevisiae.*

Pawpaw, Watermelon and Pawpaw/watermelon were used to produce fruit wine using *Saccharomyces cerevisiae* isolated from Palm wine. Even though aroma and flavour are mostly determined by the fruit variety, as already mentioned, a careful selection of wine yeasts and alcoholic fermentation conditions can also have a profound effect on the wine aroma and flavor, as well as on the overall wine quality. Wine yeasts produce metabolites known to influence sensory characteristics of wine, *e.g.* higher alcohols, esters, volatile acids, carbonyl compounds, volatile phenols and sulfur compounds (Swiegers *et al.,* 2005).

Due to the differences in fruit composition, yeast strains used for alcoholic fermentation of fruit wines have to adapt to different environments, *e.g.*, sugar composition and concentrations, the presence of organic acids, *etc*. The majority of fruit wine elaboration is based on the use of *S. cerevisiae* strains that allow for rapid and reliable fermentation, reducing the risk of sluggish or stuck fermentation, and microbial contamination (Duarte *et al*., 2010; Berenguer *et al*., 2016).

Fluctuations in temperature of the must were observed during the period of fermentation. This could be as a result of biochemical changes occurring during the metabolism of the substrates by the fermenting organism. Temperature of the final mixed fruit wines ranged from 27 to 30 °C (Chilaka *et al*., 2010).

During fermentation, there are several factors that were taken into consideration. The most notable is that of the internal temperature of the must (Keller, 2010). The biochemical process of fermentation itself creates a lot of residual heat which can take the most out of the ideal temperature range for the wine (Fundira *et al*., 2012). Thus, fermentation is an exothermic process so the temperature must not exceed 29.4°C for red wines or 15.3°C for white wines. Otherwise, the growth of yeast cells will stop. Therefore, a lower temperature is desirable because it increases the production of esters, other aromatic compounds, and alcohol itself. This makes the wine easier to clear and less susceptible to bacterial infection (Akubor *et al.,* 2013).

The pH of the produced wine ranges from (4.4 –3.3) throughout the fermentation periods and in the final product. Similar observations have been reported for other tropical fruit wines such as tundu wine (Sahu *et al*., 2012), sweet potato wine (Ray *et al.,* 2011), sapota fruit wine (Panda *et al.,* 2014a, b) and banana wine (Obaedo and Ikenebomeh 2009). Studies have shown that during fermentation of fruit, low pH is inhibitory to spoilage organisms but increases conducive environment for the growth of desirable organisms. Also, low pH is known to give fermenting yeasts a competitive advantage in natural environment (Reddy and Reddy 2005; Chilaka *et al.,* 2010).

Acidity plays a vital role in determining wine quality by aiding the fermentation process and enhancing the overall characteristics and balance of the wine. Lack of acidity will mean a poor fermentation (Berry, 2000). The changes in the % titratable acidity of the wines produced within the period of fermentation shows the occurrence of Malo-lactic fermentation (Idise, 2011). These results agree with the report of Child (2002), Okafor (2007), (Idise, 2011), Agbor *et al* (2011) and Noah *et al* (2013).

The result of the analysis at various stages of the wine production shows that the percentage reducing sugar content falls from 26 to 12.4% for Pawpaw wine, 24.4 to 13.6% for Watermelon wine and 26.2 to 16.4% for mixture of Pawpaw and Watermelon wine.

Sugar is the main substrate for fermentation of fruits juice into alcohol, (Keller, 2010). Although other food nutrients such as protein and fats can be broken down by some microorganism in some cases where sugar is limited, as long as sugar is present, yeast cells will continue the process of fermentation until other factors that affect the growth of yeast become unfavourable (Dickinson, 2013).

The specific gravity of the fruit wines, gradual decreases in values were observed throughout the period of fermentation. The observed reduction in Specific gravity decreases from 0.93 – 0.84, 0.96 – 0.0.91 and 0.99 – 0.86 for Pawpaw, Watermelon and mixture of Watermelon and Pawpaw wine respectively and the resultant increase in alcohol concentration in with fermentation show the efficiency of the yeast cells isolated from palm wine (Okonkwo *et al*., 2005).

Alcoholic content of the wine produced with different fruits from yeast were of different percentages. Watermelon wine had the lowest percentage of alcoholic content of 11.40%, while Pawpaw wine had the highest percentage of alcoholic content of 13.63% and the mixture of Pawpaw and Watermelon wine had 12.82%. The different values of alcoholic percentages could be as a result of the different fruits used for wine production and also starter microbe involved in conversion of sugar to ethanol. (Vairappan and Kishneth, 2013).

The performance and potential of the yeast strain as substituent for the commercial baker's yeast was measured by the amount of alcohol produced. High alcohols are known to be important precursors for the formation of esters, which are associated with pleasant aromas as reported by Clemente-Jimenez *et al.* (2005). The characteristics of *Saccharomyces cerevisiae* has been effectively employed in the production of mixed fruit wine from banana, watermelon and pawpaw which was reported by Ogodo *et al,* (2015).

Fruit wine produced from Pawpaw, Watermelon and mixture of Pawpaw and Watermelon were high in moisture content with a value of 85.12%, 84.66% and 84.56%. It was observed that the moisture content of the fruit was high and this accounts for its high perishable nature and its short shelf life under normal storage condition. High moisture content makes beverages suitable as a refreshing and quench-thirsting product which is a characteristic of good beverage and also agrees with the report of Okeke *et al.,* (2015). The fruits (Pawpaw, Watermelon and mixture of Pawpaw and Watermelon) also contained reasonable amount of total

carbohydrate which invariably account for their high caloric values suggesting the presence energy source for metabolic activity of the yeast. The protein content was 4.7%, 4.16% and 3.83% for Pawpaw, Watermelon and Mixture of Pawpaw and Watermelon. There was decrease in protein content after fermentation and low protein content of the wine is good for maintenance of cellular organization as reported by Awe *et al.* (2013). Fat content was low and this suggests that the wine could provide protection against excess body lipids and it's demonstrate the desirable nutritive quality of the fruit wine produced as reported by Awe *et al*, (2013). Total carbohydrate fruit wine produced from mixed Pawpaw/Watermelon were at 2.61% and fruit wine produced from Pawpaw and Watermelon had total carbohydrate at 2.84% and 2.79% respectively. A decrease in the carbohydrate content of the produced wine was observed, this might be due to decline in sugar content as a result of effective breakdown and utilization of the sugar available in the must by the yeast cells leading to the fermentation of the Must which was reported by Awe and Nnadoze (2015).

An increase in number of yeast cells during the first four (4) and five (5) days of fermentation was observed in the wine produced followed by a decline in day six (6) and (7). The increase in number of yeast cells can be attributed to the effective utilization of the available sugar and nutrient component and daily aereation of the must leading to cell propagation and multiplication which agrees with the findings of Awe and Nnadoze (2015).
Decline in the number of yeast cells in the fermenting must might be due to rapid utilization of the available sugar in the must which lead to the fermentation of the must and increase in alcohol content which might also affect the yeast growth rate.

It was observed from the microbial analysis of the produced wine that there was no microbial contaminant of the wine and this revealed that the wine was produced under hygienic conditions and is safe for human consumption. A similar observation was in agreement with Adedeji and Oluwalana (2013), who reported no contamination of wine produce from blend of water melon and pawpaw. Fermentation was carried out under aseptic condition and these measures put in place may have led to absence of contaminants and also pasteurization another reason for no contaminant.

Sensory evaluation rated the wines acceptability as Pawpaw wine > Watermelon wine and Mixture of Pawpaw and Watermelon wine > Banana wine. Also, the sensory evaluation of the wines in the present study do not differ significantly ($P > 0.05$). These attributes compared favourably with the reports for other tropical wines as reported by Ray *et al.,* (2011) Sahu *et al.,* (2012) and Panda *et al* (2014).
It was observed that there were no spoilage microorganisms for wine produced from Pawpaw, Watermelon and blend of Pawpaw and Watermelon during storage at both room temperature and refrigerator temperature ($4^0$C), but there evident of the test fermentation yeast *Saccharomyces cerevisiae* in all during the period of storage. According to Reddy and Reddy (2009) and Chilaka *et al.* (2010), low pH values, high acidity and high alcohol contents of the wines which are known to inhibit the growth of pathogens and gives fermenting yeast a competitive advantage in natural environment could be the reason for this observation.

**Conclusion**

Yeast strain *Saccharomyces cerevisiae* was isolated from Palm wine, identified and characterized to specie level using molecular procedure.
Fruits wine was produced from pawpaw, watermelon and mixture of pawpaw and watermelon using *Saccharomyces cerevisiae* yielded a good taste. Fruit wine produced from pawpaw revealed more acceptability and had an alcoholic content of 13.63%, while watermelon wine had the alcoholic content of 11.40% and blend of pawpaw and watermelon fruit wine had the alcoholic content of 12.82%.
The flavor, colour and clarity of the wines were all commendable. Yeast isolate from palm wine used in the production of wine is better when compared to the commercial bakers' yeast in terms of the flavour of the wine produced and the alcohol tolerance of the yeast.
Finally, the pawpaw fruit wine produced in this study was acceptable in terms of its sweetness, clarity, flavour and colour. Therefore, watermelon just like many other tropical fruits can be used to produce good table wines

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