# Microbiological and Physico-chemical Analyses of Wine produced from *Citrulluslanatus* (Watermelon) and *Psidiumguajava* (Guava) blend using *Saccharomyces cerevisiae*.

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Abstract: Citrullusl anatus (Watermelon) and Psidium guajava (Guava) are tropical fruits with short shelf-lives because of the temperature and relative humidity conditions in tropical countries like Nigeria. Production of wine from these fruits could help to reduce the extent of post-harvest loss and increase the variety of wines. Juice from the fruits was extracted and after treatment were mixed using the following ratio WMJ:GJ, 100:0, 0:100, 50:50, 40:60, 30:70, 20:80 and 10:90; to be labelled A, B, C, D, E, F and G respectively. Wine A and B served as control. Fermentation was done for 7 days with daily analyses of microbiological and physico-chemical parameters. Results revealed yeast and mould counts ranging from 236 x  $10^5$ to 290 x  $10^5$ cfu/ml, while there was no viable plate count and coliform growth in the final products. These results fall well below acceptable limits in the formulated wine which is safe for human consumption. During fermentation, consistent increases in titratable acidity and alcoholic content were observed while there were gradual decreases in pH, reducing sugars and specific gravity. Vitamins A and C contents as well as the alcoholic content of the final wines were moderate. The pH values of all the wines were acidic and ranged from  $4.05 \pm 0.14$  to  $4.72 \pm 0.10$ . Acidity was within the acceptable limit. Quality assessments reflected that wine product coded 'C' was of higher quality and could be stored at  $28 \pm 2^{\circ}$ C for a minimum period of six months without marked changes in quality.

Keywords: Wine, Guava, Fermentation, Watermelon, Yeast.

#### Introduction

ruit juices are fermented to produce wine, an alcoholic beverage. Fruits such as banana, cucumber, pineapple and other fruits have been utilized in wine production (Obaedo and Ikenebomeh, 2009; Chilaka et al., 2010). Homemade wine production has been practiced with various fruits such as apple, pear and strawberry, cherries, plum, banana, pineapple, guava, oranges, watermelon, cucumber etc. using natural fermentation or commercial strains of Saccharomyces cerevisiae which convert the sugar in the fruit juices into alcohol and organic acids, that later react to form aldehydes, esters and other chemical compounds which also help to preserve the wine (Duarte et al., 2010). Generally, all wines are produced using wine yeast- Saccharomyces cerevisiae var. ellipsoideus; (Amerine et al., 1980) which is also imported. However, Okoro (2007) and Okeke et al (2015) have shown that palm wine yeast Saccharomyces cerevisiae has the potential of wine yeast andit is also alcohol tolerant.

Citrullus lanatus (Watermelon) is a fruit which belongs to the family of cucumbitacea and native to tropical Africa. Its global consumption is greater than that of any cucurbit due to its reported health benefits (Kerje and Grum, 2003). This fruit contain a thick rind (exocarp) and fleshy center (mesocarp and endocarp). The fruit is round with a reddish mesocarp having a lot of seed and is mostly common in the south.

<sup>®</sup>Corresponding author: londonpatrick7228@gmail.com Akpoghelie, P. O Copyright © 2018 Nigerian Society for Microbiology There are various species with different coloured endocarp for example red flesh, yellow flesh and orange flesh. Watermelon juice is a very rich source Vitamins, minerals and carotenoids some of which include lycopene, phytofluene, phytoene, betacarotene, lutein and neurospnene. Lycopene makes up the majority of the carotenoids of watermelon. Carotenoids have antioxidant activity and free scavenging property thereby help in reducing the risk of cancers, cardiovascular diseases, arteriosclerosis, diabetes and arthritis and protects against macular degeneration (Collins et al., 2005)

Psidium guajava (Guava), which belongs to the myrtaceae family, is a native of tropical America and is widespread throughout the tropical and subtropical areas (Chopda and Barrett, 2001). Guava can be considered as a "superfruit" due to its nutrient content. Guava fruit not only has exotic flavour but also a rich source of relatively low methoxylated pectin (50%) amounting to more than 10% of the dry weight. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fibre and fatty acids. Guava fruit is higher in vitamin C than citrus (80mg of vitamin C in 100mg of fruit) and contains appreciable amounts of vitamin A as well. Due to its strong flavor and aroma; it is used as an additive for many products (Correa et al., 2010).

Palm wine is a refreshing alcoholic beverage widely consumed in Nigeria, Asia and America. It is obtained from the sap of palm trees such as oil palm (Elaeis guiniensis) and Raphia palm (Raphia hookeris and R. vinifera) (Okafor, 2007). It contains a suspension of live yeasts and bacteria. Studies have reported the

potential of palm wine as a source of yeast isolate for fermentation industries (Okeke et al., 2015). Palm wine yeast are dependable when it comes to ethanol production and can even withstand ethanol concentration of up to 18%, making them highly tolerable to ethanol concentration levels. It has been shown that palm wine yeast has the potential of wine yeast in wine making (Okoro, 2007).

Presently, the share of fruit juice based wine beverages is presently quite small as compared to synthetic carbonated drinks (Adedeji and Oluwalana, 2013). Gradually there is a distinct shift towards fruit juice wine based beverages for obvious advantages of the higher nutritional, medicinal and calorie values over the synthetic aerated beverages. Therefore in view of the above benefits and perishability of the fruits; its conversion into a value added product like wine will be very useful.

# Materials and Methods Samples Preparations

Mature ripe Guava and watermelon were purchased from Uselu market in Benin City, Edo State, Nigeria. Fresh palm wine from Raphia hookeri were

obtained from the palm wine tappers in Benin City within Ih of tapping. The fruits and the palm wine were transported to the laboratory in clean cellophane bags and in an ice box respectively for analysis. The fruits were washed thoroughly with distilled water. Exactly 500g each of the fruit samples, guava and watermelon were weighed. This was then chopped into smaller pieces using a clean knife before transferring them quantitatively into a wet mill blender for maceration. The macerated sample was transferred into a sterile bucket and mixed with distilled water (1:1 w/v). Exactly 4.0g of sugar was added to the must followed by vigorous stirring. Exactly 4 g of potassium metabisulphite (KMS) was dissolved in 400 ml of water and poured in 100 ml aliquots to each of the mixtures and stirred properly. Potassium metabisulphite serve as a preservative and prevents fermentation before the addition of the yeast starter. Physico-chemical and microbiological analysis were carried on aliquots of the extracted juice obtained and the musts were mixed in the combination of guava and watermelon as shown in Table 1. The products were sterilized at 110°C for 3

Table 1: Blending Proportion of Samples.

Sample Code	Samples	Guava Juice (g)	Watermelon Juice (g)
A (Control)	GJ0:WMJ100	0	100
B (Control)	GJ100:WMJ0	100	0
C	GJ50:WMJ50	50	50
D	GJ60:WMJ40	60	40
E	GJ70:WMJ30	70	30
F	GJ80:WMJ20	80	20
G	GJ90:WMJ10	90	10

Note: GJ - Guava Juice, WMJ - Watermelon Juice.

# Isolation of Saccharomyces cerevisiae from palm wine

Culturing of the fresh palm wine was done on Potato Dextrose Agar (PDA) and incubated at room temperature for 24 h. Twelve isolates were obtained and sub-cultured. The yeast cultures were transferred to modified Malt Extract Agar (MEA) containing yeast extract and 2 % glucose and then incubated for 24 h. Five of the isolates were identified as *S. cerevisiae* based on their cultural characteristics, microscopy and their pattern of sugar fermentation as described by Ogodo *et al.* (2015). The identified organism was maintained on MEA slant.

#### Propagation of Saccharomyces cerevisiae

The isolated organism was propagated prior to fermentation by culturing them on Malt Extract Broth (MEB) and incubating for 48–72 h at 28.0°C ± 2.00. The broth cultures of the organism were centrifuged at

3500 rpm for 5 min. The sediments were collected and used for must fermentation.

## Preparation of yeast starter culture

The yeast starter culture was prepared from 200 ml of the must for fermentation, 4 g of sugar, 3.7 ml (approximately 108cfu/ml) of yeast and 300 ml of distilled water. The mixture of all these were treated with yeast nutrients and allowed to stand for 24 h. Approximately 200 ml of water was boiled and allowed to attain 37°C and 200 ml of each mixture of the must (guava and watermelon) respectively treated with sugar was added. Exactly 5 g of citric acid was added to each of the preparations and then stirred for proper mixing. Exactly 2 g each of the yeast nutrient namely Potassium phosphate, Ammonium sulphate and Magnesium sulphate was dissolved in 100 ml of distilled water and poured to each of must mixture. Exactly 3.7 ml representing approximately 108cfu/ml (measured using McFarland standard) of the yeast (S. cerevisiae)

isolated from palm wine after centrifugation was added to each of the mixture, stirred properly and allowed to stand for 24 h before use.

#### Fermentation

The fermentation was initiated by the addition of the starter culture. The must was stirred every 12 h with subsequent reading of the specific gravity, pH, titratable acidity and alcohol content for 4 days. After 4 days, the wine was racked into the secondary fermenter. The secondary fermentation was done in an air tight container in which a tube was passed into a clean bottle containing clean water. The essence was to monitor the course of fermentation. This was allowed until completion of fermentation as was evidenced by lack of the appearance of bubbles in the container within 7 days. Daily analyses of physico-chemical parameters were done. When fermentation stopped, the wine was promptly racked off the lees ensuring minimum exposure to oxygen. After secondary fermentation, the wines were clarified. The clarification/fining were done using bentonite (a clarifying agent) for a period of 3 months. Filtration was done after the wines had completed clarification using muslin cloth, sieve and syphon tubes sterilized by 70 % alcohol. The wines were syphoned into the sieve containing four layers of muslin cloth. The residues were removed and the filtrates were allowed to mature for a period of 6 months.

# Physico-chemical Analysis pH measurements

Ten milliliter of the fermenting broth was transferred into 250 ml conical flask .The pH of the slurry was determined using a digital pH meter after calibration using pH 4.0 and pH 7.0 buffers.

#### Titratable acidity determination

Twenty five milliliters of each sample mixture was measured using a measuring cylinder. It was poured into a pre-washed 250ml Erlenmeyer flask. 5 drops of phenolphthalein indicator was added and was titrated against 0.05N NaOHfrom colourless to permanent pink.

Titratable acidity, (mg/l) was given as  $\frac{N \times titre\ value\ \times constant\ (2000)}{Volume\ of\ sample}$ 

# Specific gravity determination

A known volume was taken using a measuring cylinder from each mixture. Hydrometer of API standard was inserted into the extracted juice. The API of each mixture was then obtained and was converted to specific gravity using the API conversion table.

#### Total Sugars determination

Soluble sugars concentration on each of the samples was determined using a handheld Bellingham and Stanley refractometer at 28°C. This instrument consists of metallic and plastic exterior which protects the lenses, prisms and mirrors inside. At one end of the instrument is a hole for viewing the results while the opposite end consists of a slide with a lid where the sample was placed. The sugar level was determined by noting the point on the scale where the two colours of blue and white intersect on the index. Readings given on the index were dependent on the sugar content of the sample.

#### Alcohol Content

The alcohol content of the fermented media was determined by the gravimetric method using the specific gravity of the samples before and after fermentation. The final specific gravity was subtracted from the initial specific gravity and the value obtained was multiplied by 100 to give %alcohol content% alcohol =  $\frac{S.Gi-S.Gf}{0.793} \times 100$ 

S.G<sub>i</sub>= Specific Gravity before fermentation S.G<sub>i</sub>= Specific Gravity During fermentation

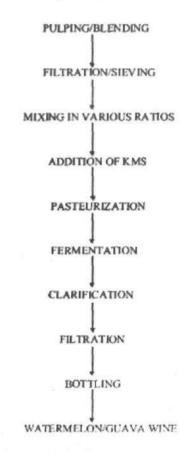


Figure 1: Flow chart for the production of watermelon and guava wine

#### Microbiological analysis Total viable bacterial counts

One milliliter of 10<sup>-1</sup> – 10<sup>-3</sup> serial dilutions of each sample were aseptically drawn with the aid of sterile Pasteur pipette and dispensed into sterile labeled Petri dishes. 15 ml of sterilized nutrient agar (cooled to 45 °C) was poured into each Petri dish and carefully swirled to homogenize. The plates were allowed to cool and set, thereafter incubated at inverted position at 37°C for 48 h for bacterial counts. The colonies on the plates were counted to obtain the colony forming unit and recorded in cfu/g.

#### Fungal counts

One milliliter (1 ml) of each dilution were introduced into sterile Petri-dishes and 20 ml of sterile molten PDA amended with streptomycin to inhibit the growth of bacteria was introduced into the inoculum aseptically and swirled to homogenize. The plates were allowed to cool and set. The PDA plates were incubated at 28°Cfor 72 h and the fungi colonies were counted and recorded in cfu/g.

#### Total coliform count

Total coliform count was performed by using most probable number technique (MPN).

#### Statistical Analysis

Analysis of variance (ANOVA) was used as described by Ogbeibu (2014) to analyze the data obtained. Mean values seperation and comparison was done using SPSS version 21.0. Significance was accepted at P < 0.05 and results expressed as mean  $\pm$  standard deviation from the mean

#### Results

The result of the microbial load of the formulated wine is presented in Table 2. The numerical strength of the organisms was analyzed using total plate count, coliform count, yeast and mould count. All the colonies were counted in unit per millimeter (cfu/ml). The result revealed that there was a general increase in yeast count for the products during the period of fermentation. The highest yeast count was observed in the wine C while the lowest was recorded in the wine A after fermentation period. Results of statistical analysis showed that the yeast count for the samples differed significantly. The bacteria count and the total coliform count gave a nil result during fermentation period.

Table 2: The Yeast count of the wine products before and after fermentation (cfu/ml),

Treatment	Before fermentation	After fermentation
Wine A	$2 \pm 0.00$	$236 \pm 15.88$
Wine B	$3 \pm 0.00$	$258 \pm 18.50$
Wine C	$2 \pm 0.00$	$290 \pm 21.72$
Wine D	$2 \pm 0.00$	$272 \pm 17.70$
Wine E	$3 \pm 0.00$	$278 \pm 16.50$
Wine F	$2 \pm 0.00$	$286 \pm 18.68$
Wine G	$4 \pm 0.00$	$261 \pm 18.10$

Values are expressed as mean (×105) ± Standard deviation

The physico-chemical characteristics of the samples determined include the pH, total solids, titratable acidity, total sugars, specific gravity, alcohol content, fat content, crude protein, moisture content, Vitamin C and Vitamin A presented in Tables 3 and 4. Variations were observed for the parameters. The result revealed that the pH decreased for all the samples throughout the period of fermentation. Statistical analysis showed significant difference for the pH of the samples. The pH of the wines was acidic throughout the period of fermentation irrespective of the fruit wine. The blended samples had lower pH in the final products compared to the plain samples. The lowest pH (4.05) was observed for wine C after period of fermentation while the highest pH (4.72) was recorded for the wine A after fermentation period. Titratable acidity values increased steadily for the wine products during the period of fermentation. The plain wine samples recorded lower acidity compared to the blended samples after fermentation. Wine A recorded the lowest value (1.414) after fermentation period while wine C had the highest value (2.918) for titratable acidity after the fermentation period.

Reducing sugars values which differed significantly decreased throughout the fermentation period and ranged between 3.19mg/100g to 5.72mg/100g after

fermentation period. The highest amount of sugars was found in wine A while the lowest was found in wine C after fermentation period. The specific gravity for the samples decreased almost uniformly throughout the fermentation period (3.43% to 2.18%). The values ranged between 0.986 to 1.03. The alcoholic content of the formulated products increased throughout the fermentation period. The alcohol content of the wines after fermentation ranged between 2.62 and 4.30%. The blended wines had higher alcoholic content compared to the plain wine samples. The alcoholic content for all the samples showed significant difference.

The result of proximate analyses for the samples revealed no significant difference and is presented in Table 4. Fat content ranged between 0.25mg/100g to 0.87mg/100g. Fat content was relatively higher in the plain wines than in the blended wines. However, the vitamin A content of the blended samples was higher compared to the plain wine samples. The crude protein content for the samples was highest in wine C (0.29%) and lowest in wine B (0.08%). The percentage moisture content of 94.66% was noticed in wine C while the least (91.05%) was observed in wine D. Ash content for the samples ranged between 0.15% and 0.47%. Crude fibre was not detected in the samples.

Table 3: Physico-chemical parameters of the final wines

	-3	Wine	4.16±0.15	$1.916 \pm 0.05$	3.79 ± 1.10	$3.30 \pm 0.02$
		Wine F	$4.08 \pm 0.22$	2.002 ± 0.055	$5.25 \pm 1.20$ $1.018 \pm 0.002$	3.43 ± 0.01
		Wine E	4.37 ± 0.13	3.30 ± 1.20	1.013 ± 0.001	3.71 ± 0.02
		Wine D	$4.14 \pm 0.10$ $2.606 \pm 0.025$	3.38 ± 1.20	1.010 ± 0.002	3.82 ± 0.01
	Mean values ± Standard deviation	Wine C 4 05 + 0 14	2.918 ± 0.03	3.19 ± 1.35	$1.005 \pm 0.001$	10.0 ± 0.01
	Mean values Wine B	4.58 ± 0.10	$1.801 \pm 0.02$	3.48 ± 1.10	$3.21 \pm 0.02$	
	Wine A	4.72 ± 0.10	1.414 ± 0.02	1.030 ± 0.002	2.62 ± 0.03	
Parameters		Hd	Litratable acidity Reducing sugar	Specific gravity	Alcohol content	
	1	= F	R	S	V	

Table 4: Proximate and nutritional analysis of wines

	Wine A		Mean values ±	Mean values ± Standard deviation			
Moisture	92.95 + 2 14	Wine B	Wine C	Wine D	Wine E	W.	
Ash	0.47 ± 0.11	0.15 ± 0.19	94.66 ± 3.56	91.05 ± 2.77	91.37 ± 1.07	91 56 ± 4 05	Wine G
Crude fibre	ND	ND TOLEY	0.43 ± 0.22	$0.39 \pm 0.12$	$0.35 \pm 0.16$	0.31 ± 0.10	$91.98 \pm 1.95$
Protein	$0.22 \pm 0.03$	$0.08 \pm 0.02$	0.29 +0.05	ON S	ND	ND	0.22 ± 0.10
rat Vitomin 4	$0.87 \pm 0.11$	$0.53 \pm 0.20$	0.25 ± 0.10	$0.17 \pm 0.02$	$0.15 \pm 0.33$	$0.13 \pm 0.01$	0.11 ± 0.10
Vitamin C	10.26 ± 0.25	12.45 ± 0.10	$15.40 \pm 0.20$	15.60 ± 0.25	0.43 ± 0.18	0.34 ± 0.10	$0.28 \pm 0.11$
ND: Not detected	. 101 ± 101	8.89 ± 1.41	10.00 ± 1.02	9.40 ± 1.55	9.40 ± 1.23	$15.00 \pm 0.15$ 8 26 ± 1 35	15.00 ± 0.10

#### Discussion

In this research work, the choice of the fruits: watermelon and guava were deliberate, pH is one of the main quality parameters that determine the stability of bioactive compounds in fruit wines. The watermelon and guava wine produced revealed low pH values (in the range of 4.05 - 4.72) throughout the fermentation period and in the final product. Similar findings have been reported for other fruit wines such as pawpaw, banana and watermelon wine (Ogodo et al., 2015), tundu wine (Sahu et al., 2012), sweet potato wine (Ray et al., 2011) and banana wine (Obaedo and Ikenebomeh, 2009). Most beverages have their pH ranges between 3.5 and 5.5. The results showed progressive decrease in pH throughout the fermentation period which revealed increasing acidity in the samples as fermentation progressed, as acidity and pH are inversely proportional to each other. This finding was supported by Oluwana and Adedeji (2013) and Abbo et al (2006). Low or high pH values in wines affect the organoleptic or sensory quality of wine as discussed by Bhardwaj and Mukherjee (2010). Wines with lower pH tend to have better sensory qualities than those with higher pH. Low pH and high acidity are known to give fermenting yeasts competitive advantage in natural environments (Reddy and Reddy, 2011). This is reflected by the increase in yeast count throughout the fermentation period for the wine products. Also, low or high pH values can assist in determining the shelf life of wine. Products with low pH tend to have better keeping qualities than those with high pH.Low pH reduces the influence of bacteria that can lead to spoilage (Iherokonye and Ngoddy, 1985). Titratable acidity of the wine products ranged from 1.414 to 2.918% citric acid equivalent of the wine after fermentation period. This is similar to the commercial recommendation of acidity for wine (Srivastava and Kumar, 1993). This was supported by Adedeji and Oluwalana (2013). Titratable acidity values showed increase in the samples as the fermentation progressed while pH value decreased. This is in conformity with Ankush et al (2015) who reported that as the fermentation of beetroot juice and watermelon juice blends progressed, there was an increase in titratable acidity while pH decreased. The acidity is higher than the reports of Ray et al (2011) for sweet potato wine and Ogodo et al (2015) for pawpaw, banana and , watermelon wine.

The major problem associated with the use of tropical fruits in wine production is their low sugar content. The sugar content of the musts was supplemented by the addition of sucrose. The reducing sugar decreased as fermentation progressed for all the wine products. This observation corresponds with the report of Ray et al (2011). The alcohol content of the wines after fermentation ranged between 2.62 and 4.30%. These values are in accordance with results of alcohol concentration for the production of watermelon-pawpaw wine reported by Adedeji and Oluwalana

(2013). This observation correspond with the reports of Ray et al (2011) and Sahu et al (2012) who reported similar values for purple sweet potato wine tendu wine (3.78g/100ml) (1.35g/100ml) and respectively. In general, the percentage alcohol produced was above 2% which is comparable with modern grape wines (Okunowo et al., 2005). High alcohols are known to be important precursors for the formation of esters, which are associated with pleasant aromas, all of which influence the quality of the finished product (Clement-Jimenez et al., 2005). The specific gravity of the final products ranged between 0.986 and 1.030. This was supported by Adedeji and Oluwalana (2013). The specific gravity of the wines produced in this research work reduced as the fermentation days of the wines increased and is due to the type of yeast used in the wine production. Saccharomyces cerevisiae isolated from palm wine has been reported to reduce the specific gravity of fruit wines during fermentation (Ayogu, 1999). The protein content of the beverage ranged between 0.08 and 0.29 percent while the Fat content between 0.25-0.87mg/100g. This protein content was found to be higher than that of Kunun-zaki drink (0.046%) formulated by Agarry et al (2010). The fat contents of the beverage is higher than that of Zobo drink (0.15%) (Egbere et al., 2007). The moisture content of the formulated wine ranged between 91.05-94.66%. High moisture content makes beverage suitable as a refreshing and thirst-quenching product which is characteristic of a good beverage. This is comparably higher than the moisture content of watermelonpawpaw wine formulated by Adedeji and Oluwalana (2013). The formulated wine provides considerable amount of vitamin C. About 27.08mg/100g has been reported to provide more than one-third of the daily requirement of vitamin C (Hou et al., 2011). Vitamin A content ranged from 15.00 to 15.40mg/100g. This value is considerably higher than in most commercial drinks as reported by Collins et al (2005). Microbial examinations are usually used as monitoring indices of food spoilage.

The result of the microbial analysis of guava and watermelon wine revealed the wine quality. The microbiological analysis of guava and watermelon wine revealed that there was no coliform growth in the final product while the yeast and mould count ranged between  $236 \times 10^5$  to  $290 \times 10^5$ cfu/100ml in the final product. The viable plate count for bacteria gave a nil result indicating good quality. This finding which is similar to the work of Ogodo et al (2015) and Adedeji and Oluwalana (2013) may be attributed to low pH and high acidity of the wines which are known to inhibit the growth of pathogens and give fermenting yeast competitive advantage in natural environments as reported by Chilaka et al (2010). This result implies proper hygienic condition observance in the processing of juices and absence of faecal contaminations which presents the beverage as safe for human consumption.

The shelf life evaluation revealed that the wine sample could be kept for six months at room temperature of 28 ± 2°C with very negligible changes in the quality of the product. Therefore, the formulated wine is safe for consumption for a minimum period of six months.

## Conclusion

The research work revealed that the two test fruits (watermelon and guava) are good substrates for wine production. The microbiological and physicochemical characteristics of the wine are acceptable to consumers. The analysis showed that watermelonguava wine is nutritionally rich in protein, fats, and Vitamin A and C. According to microbial results, it can be concluded that the product is safe for human consumption. Yeasts isolated from palm wine has similar potential to wine yeast. Wine C was chosen as the best treatment based mainly on physico-chemical properties and microbial load on comparison with other samples. Therefore, watermelon-guava wine production is possible using yeast isolated from palm wine and could be regarded as a means of preserving the original qualities of the fruit substrates and also serve as after meal drink, ready to serve, refreshing beverage with good nutritional and calorie value for consumers over synthetic aerated beverages which are detrimental to health.

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