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Research Paper

Microbial and physico-chemical changes in wine produced using isolate from *Annona muricata, Psidium Guajava* and *Syzgium malaccense*

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Abstract. In this study, wine was produced from *Psidium guajava*, *Annona muricata* and *Syzgium malaccense* using microbial isolates common to the fruits which were used as the substrates and comparing the wines produced with that produced using *Saccharomyces cerevisae*. The microbial and physicochemical changes in the wines produced were compared with those produced using baker's yeast. Isolation of fungi and bacteria was carried out on Saboraud Dextrose Agar and Nutrient Agar respectively using pour plate technique. The identification of the isolates was done using appropriate morphological, cultural and biochemical characterization. The mean bacterial and fungal counts ranged from 48.63 to 83.53 cfu/ml and 29.80 to 42.00cfu/ml, respectively. *Proteus* sp. and *Fusarium* sp. were common to the three fruits. There was no significant difference in the wines produced with baker`s yeast but the ones produced using indigenous isolates differed significantly. However, wine produced from *Syzgium* sp using indigenous isolates gave the highest yield of alcohol. Wines produced from each fruit using the common isolates; *Fusarium* sp. and *Proteus* sp. compared favourably well with those produced using baker`s yeast in terms of microbial counts, pH, specific gravity, titratable acidity, optical density, temperature and alcohol content.

Keywords: wine, isolates, *Annona muricata*, *Psidium guajava*, *Synzgium malaccense*, physico-chemical.

INTRODUCTION

Wine is an alcoholic beverage made from fermented grapes or other fruits (Okafor, 2007). Wine contains 85 to 90% water, comprises ethyl acid, organic acids such as tartaric, acetic, citric, lactic and succinic acids. The mineral composition of palm wine is special as it contains potassium, calcium, magnesium, sodium, iron, sulfur, phosphorus, phenol, glucose, pectin (Okafor, 2007). It is produced by the action of yeast, bacteria and other organisms through the process of fermentation which is a relatively simple, natural, efficient and inexpensive low energy food preservation process. Juices and wines produced from tropical fruits have increasingly gained global importance due to their characteristic exotic aroma and color. Wines play a lot of indispensable roles in the life of man which ranges from social functions, religious rites, rituals as well as economic benefits to producers and merchants (Hallgenten, 2006). Moderate consumption of alcohol and wine is associated with a decrease in death due to cardiovascular event such as heart failure (Lundberg et al., 2008). They have been found to be effective antibacterial agents (Strepper et al., 2009) against organisms such as streptococci (Daglia et al., 2007).

Fruits that are readily available for the production of wines include grapes, pineapple, water melon, guava. Soursop, malay apple, lychee are some of the fruits that have not been widely utilized in wine making (Abbo et al., 2006). The usage of the fruits depends on the type of drink intended (Aniene and Kunkee, 2005). The sugars present in these fruits form the most common substrate of fermentation. Typical examples of fermentation products are ethanol, lactic acid, lactose and hydrogen.

More exotic compounds such as butyric acid and acetone can be produced by fermentation of some foods using molds (Wang et al., 1980). Isitua and Ibeh (2010) produced wine using banana and pineapple waste as substrates. Temperature, pH, are some of the factors that affect fermentation. Fresh fruits are highly susceptible to rapid microbial, enzymatic, chemical and physical deterioration. Wine production and processing minimize these undesirable reactions while still maintaining and in some cases enhancing the inherent quality of the fruit. Wastage of the fruits especially at their peak of production season necessitates the need for alternative preservation and post-harvest technologies that can reduce the level of post-harvest losses. This can be found in wine production (Alobo and Offonry, 2009; Okoro, 2007).

Soursop and guava are seasonal while the fruits of malay trees present in very few homes in Nigeria are aesthetic plants which drop and are left to rot. There is therefore a need for the preservation of these fruits which could be in form of wine production. They can also serve as a raw material for wine making using microorganisms inherent in them.

This research therefore is aimed at preserving the qualities of soursop, guava and Malay apple through wine production using an isolate common to the three fruits and determining the microbial and physicochemical changes in the wine production over time.

MATERIALS AND METHODS

Sources and collection of samples

Fresh, healthy soursop and guava fruits of various sizes were purchased from Abraka, Nigeria and the Malay fruits plucked from the tree in Abraka. The fruits were authenticated in the Department of Botany, Delta State University, Abraka, Nigeria.

Enumeration, isolation and characterization of isolates

The fruit samples were washed with sterile water and ethanol. The washed fruits were crushed in a mortar and one gram of each was measured into test-tubes containing 10 ml distilled water.

A small portion (0.1 ml) of serially diluted samples were pour plated on Nutrient Agar (for bacterial isolates) and Saboraud dextrose Agar (for fungal Isolates) and incubated at 37°C for 24 to 48 h and 30°C for 2 to 3 days respectively. Plates with distinct colonies were counted and recorded as colony forming units (CFU/ml).

Identification of isolates was done using morphological and biochemical characterization. The fungal isolates were observed macroscopically for colour, shape, pigments, surface appearance and mounted on a drop of lactophenol blue and viewed under the microscope. Observation was made for the sexual and a-sexual reproductive structures and compared with the cultural and morphological characteristics of known taxa.

Preparation of standard inoculum of isolates for wine production

The standard inoculum was prepared by inoculating a loopful culture each of *Fusarium* sp. and *Proteus* sp. into 250 ml conical flask containing 100 ml Potato Dextrose broth and Nutrient broth respectively under the same conditions. The flasks were kept on a rotatory shaker (120 rpm) at 28 ± 2°C for 4 days. At the end of the incubation period, cells were harvested by centrifugation at 4000 rpm for 30 min. The harvested cells were mixed with 50 ml saline solution.

Optical density was measured at 660 nm and appropriate dilutions were made in order to obtain uniform cell concentrations. From the liquid culture, 2 ml was used to inoculate 100 ml aliquots of the fruit juice.

Preparation of wine

The method of Idise (2012) was adopted. Soursop fruits, guava fruits and Malay apple fruits were washed with distilled water and ethanol. The skin and seeds of soursop and the seeds of Malay apple were removed. The fruits were blended separately using an electric blender (Moulinex). About 500 g of the blended samples were filtered using a clean sterile white muslin cloth. The blended fruits were diluted by adding 100 ml of water (fruit juice). The diluted fruit juices were divided into 2 parts each. Two millilitres of the standard inoculum was inoculated into one part while the other was inoculated with *Saccharomyces cerevisae* (baker's yeast). The inoculated juices were plugged with cotton wool and allowed to stand for 24 h after which they were tightly corked and left to ferment at 28 ± 2°C for 15 days. Sampling for fungal and bacterial counts and isolation was done at 3-day interval for the 15 days.

Physical examination of wine

The wines were monitored for taste, flocs sediments and colour changes at 3 days interval for the 15-day period.

Physicochemical analysis of wine produced

The temperature of the wines produced were measured using mercury thermometer while the pH was determined using a pH meter.

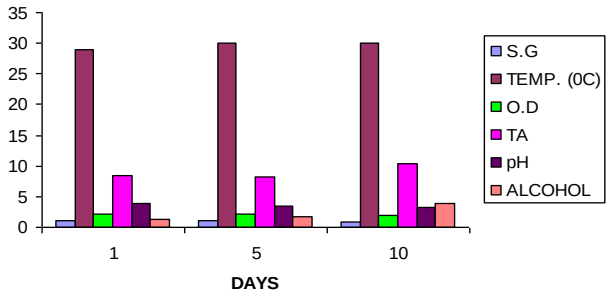

Figure 1. Titratable acidity, pH, temperature, optical density, alcohol content and specific gravity of soursop wine fermented with indigenous isolates against fermentation days.

Total titratable acidity

This was done by titrating 0.1 NaOH against 20 ml of sample using 10 drops of phenolphthalein as indicator. A prominent pink colour indicated the end point. Titration was made in triplicate and the volume of NaOH used recorded.

Determination of sugar content and specific gravity

The determination was done using a brix-precision hydrometer (Mode: tel-tre).

Percentage alcohol

The percentage alcohol was determined by drawing 10 ml of the fermenting wine into a beaker and measured using the alcoholmeter.

Optical density

The sample was poured into a cuvette and placed in the spectrophotometer and the optical density was read at 500 nm.

RESULTS AND DISCUSSION

Soursop and guava fruits had the highest bacterial count of 4.08 × 10^5 cfu/g and 4.06 × 10^5 cfu/g, respectively. Apple and soursop had the highest fungal count of 4.07 × 10^4 cf/lg and 3.05 ×10^4 cf/lg respectively with guava having the lowest count of 2.85 × 10^4 cfu/ml (Table 1).

The bacterial isolates were *Escherichia coli*, *Staphylococcus aureus*, *Bacillius* sp., *Proteus* sp., *Klebsiella* sp. (Table 2) *w*hile the fungal isolates were *Penicillium, Aspergillus, Fusarium, Mucor, Rhizopus* species and yeast (Table 3). *Proteus* sp. and *Fusarium* sp. were common to all the isolates that were selected for the wine production (Table 4).

The soursop wine had flocs present by the 5th day in both the yeast and isolates fermented wine while Guava and malay apple developed after 24 h. The production of the flocs might be due to improper storage of the wine.

Robins (2003) reported that wines rapidly deteriorate if kept in inadequate conditions. Storing in direct sunlight or incandescent light can adversely react with phenolic compounds in wine (Robinson, 2006). Excessive or insufficient exposure of the wine to oxygen, faulty filtration and stabilization may also cause flocculation. The temperature of fermentation might have affected the product because of increased production of unwanted enzymes by the fermentation agent and growth of possible organisms that thrive at the warm temperature (Robinson, 2006). Subjecting the wine to an increase temperature might have reduced the unwanted organisms producing the unwanted enzymes.

The decrease in pH of all the wines may be attributed to the production of acids. The titratable acidity was found to increase as the pH decreases showing that more acids were produced as substrates were utilized by the microorganisms. Sugars during fermentation can be converted into ethanol, CO_2 and lactic acid (Okafor, 2007). This result conforms to the report of Aneine and Kunkee (2005) that there was a decrease in the pH of wine as fermentation proceeded.

All the microbial counts as shown in Table 5 were found to decrease by the 10th day. This may be due to the products of fermentation particularly the organic acids being inhibitory to the growth of the organisms resulting in their death and hence the low count. It can also be attributable to microbial succession from indigenous organisms and yeasts to lactic acid bacteria. The microbial count increased as the specific gravity decreased which showed that the sugar (nutrient) present was used for metabolic activity (fermentation). As the specific gravity decreased, the alcohol content increased (Figures 1 to 6).

The specific gravity decreased as the sugar content was being utilized by the organisms to produced alcohol resulting in an increase in the alcoholic content and a consequential decrease in the specific gravity. Okafor (2007) reported the decrease in specific gravity of palm wine as the microbial count increased. The increase in

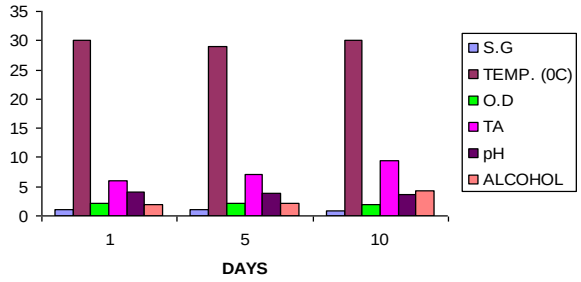

Figure 2. Titratable acidity, pH, temperature, optical density, alcohol content and specific gravity of soursop wine fermented with baker's yeast against fermentation days.

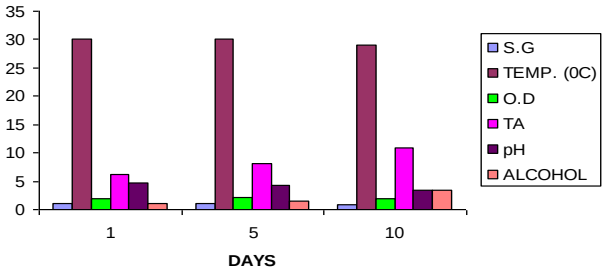

Figure 3. Titratable acidity, pH, temperature, optical density, alcohol content and specific gravity of guava wine fermented with indigenous isolates against fermentation days.

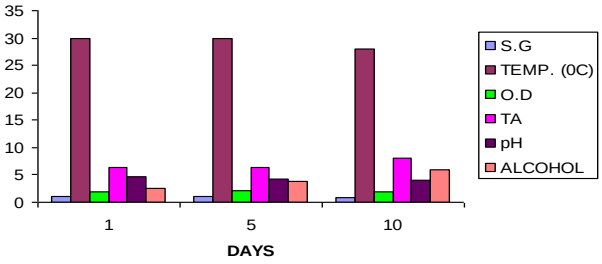

Figure 4. Titratable acidity, pH, temperature, optical density, alcohol content and specific gravity of guava wine fermented with baker's yeast against fermentation days.

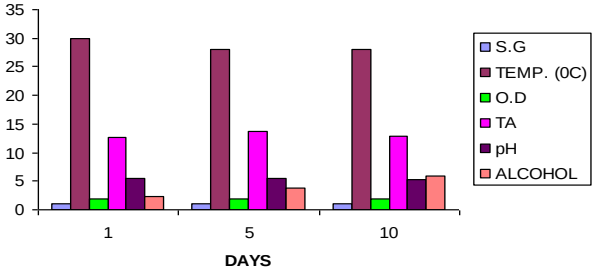

Figure 5. Titratable acidity, pH, temperature, optical density, alcohol content and specific gravity of Malay Rose Apple wine fermented with indigenous isolates against fermentation days.

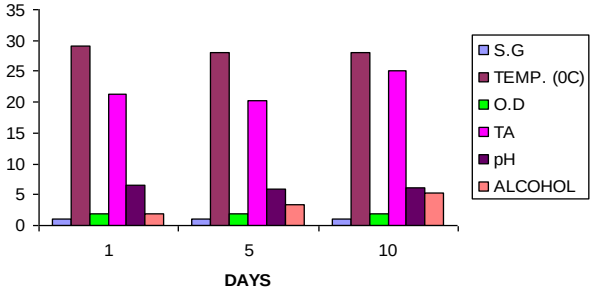

Figure 6. Titratable acidity, pH, temperature, optical density, alcohol content and specific gravity of Malay Rose Apple wine fermented with baker's yeast against fermentation days.

Table 1. Microbial counts of the fruits sample (cfu/g).

Sample	Microbial count	Fungal count
Soursop	8.0×10^4	1.0×10^3
	9.06×10^4	6.0×10^4
	8.0×10^4	6.5×10^4
Guava	1.3×10^4	7.0×10^3
	8.0×10^5	5.0×10^4
	8.0×10^5	5.0×10^4
Malay apple	8.8×10^3	8.0×10^4
	7.5×10^5	1.4×10^3
	7.0×10^5	8.0×10^4

Table 1a. Mean microbial and fungal count of the fruits sample.

Samples	MC	FC	Mean deference
Soursop	83.53 ± 49.97	42.00 ± 29.06	41.53[a]
Guava	53.77 ± 37.10	35.67 ± 20.27	18.10[b]
Malay apple	48.63 ± 33.82	29.80 ± 15.60	18.83[b]

Cell bearing the same alphabet shows no significance difference. Key: MC (microbial count), FC (fungal count).

Table 2. Characteristics of bacterial isolates.

Cultural characteristics	Morphological characteristics			Biochemical characteristics								Identify isolates
	Gram stain	Cell shape	Cell arrangement	Catalase	Citirate utilization	Oxidase	Indole	Glucose	Lactase	Hydrogen sulphide	Motility	
Elevation slightly raised entire/regular margins, colonies are circular, size 3 mm, pinkish in colour	+	Rod	Singles	+	-	-	+	AG	AG	-	+	*Escherichia coli*
Elevation raised, entire margins, colonies are circular, size 1 mm, creamy in colour	+	Cocci	Cluster	+	-	-	-	AG		-	-	*Staphylococcus* sp.
Elevation flat undulate, margins, colonies are irregular, size 5 mm, creamy in colour	+	Cocci	Cluster	+	+	+	+	AG		-	-	*Bacillus* sp.
Elevation raised colonies are swarming, creamy in colour	-	Rod	Single	+	-	-	-	+AG	+AG	-	+	*Proteus* sp.
Colonies appear sticky on plates and are creamy in colour	-	Rod	Chain	+	-	-	-	AG	AG	-	-	*Klebsiella* sp.
Yellow colonies with smooth edges	+	Rod		+	-	-	-	AG	-	-	-	*Mycobacterim* sp.

Key: + = positive, - = negative, A = Acid, AG = Acid and Gas.

Table 3. Characteristics of fungal isolates.

Appearance on agar plates	Growth rate	Microscopic observation	Isolates
Greenish filamentous growth with irregular edges	Growth appeared within 6 days	Septate vegetable spores which produce aerial hyphae on which conidiophores develop	*Penicillium* sp.
Dark filamentous growth spread all over the plates	Growth appeared within 7 days	Septate spores arising from phalides that are radiating from the entire surface	*Apsergillus* sp.
Cotton white colonies on potato dextrose agar	Growth appeared within 7 days	Septate vegetable spores with produce hyphae which conidiophores develop	*Fusarium* sp.
White wooly colonies on agar plates	Growth appeared within 6 days	Non septate vegetative spore presence of stolen bears sporangia at the top containing clusters of light spore	*Mucor* sp.
Brown wooly colonies with profuse growth on agar plate	Growth appeared within 6 days	Septate vegetative bears philates at the apex with conidia at the top	*Rhizopus* sp.
White firm and flat on plates	Growth appeared within 6 days	Neither conidia spores hyphae breaks into arthropspores at the top	Yeast

Table 4. Occurrence of Isolates in the fruits sample.

Isolates	Sample		
	A	B	C
Escherichia coli	+	-	-
Bacillus sp.	+	-	+
Staphylococcus sp.	-	+	-
Proteus sp.	+	+	+
Klebsiella sp.	-	-	+
Mycobacterium sp.	-	+	+
Fusarium sp.	+	+	+
Aspergillus sp.	+	+	-
Penicillium sp.	+	+	-
Yeast	-	-	+
Rhizopus sp.	+	-	-
Mucor sp.	-	+	-

A = Soursop, B = Guava, C = Malay apple, + = Present, - = Absent.

Table 5. Mean bacterial and fungal units of wine produced (cfu/ml).

Sample	Day		Count		Day		Mean BC ± STD	Mean FC ± STD	Mean difference
	BC	FC	BC	FC	BC	FC			
S_1	1.17×10^5	8.65×10^5	3.40×10^5	1.27×10^6	1.33×10^5	4.08×10^5	12.17 ± 1.54	5.59 ± 0.53	6.57^c
Sy	1.54×10^5	2.64×10^6	5.00×10^2	3.45×10^6	0	2.23×10^6	0.52 ± 0.72	27.73 ± 0.51	27.22^a
Gi	1.34×10^5	1.56×10^6	3.34×10^6	8.00×10^6	3.01×10^5	0	12.58 ± 4.74	31.87 ± 3.46	19.28^b
Gy	4.57×10^5	1.63×10^6	7.10×10^5	2.69×10^6	1.53×10^5	1.57×10^6	4.40 ± 2.28	19.63 ± 0.51	15.23^b
Ay	4.09×10^5	5.11×10^6	2.65×10^5	3.08×10^5	2.0×10^2	1.06×10^5	2.25 ± 1.69	18.41 ± 2.31	16.17^b
Ai	2.54×10^6	3.65×10^6	5.31×10^6	5.46×10^6	8.3×10^6	`2.89×10^6	46.21 ± 3.21	40.00 ± 1.08	6.21^c

Cell bearing the same alphabet shows no significance difference. Key: BC (bacterial count), FC (Fungal count), i (Indigenous isolate), S (soursop), y (yeast, *Sacchaonyces cerevisae*), G (guava), A (Malay Apple) and NG (No growth).

temperature in all the wine may be due to the release of heat during the conversion of sugar to ethanol. The optical density decreased during the fermentation process which is plausibly due to the elimination or reduction of microorganisms that could not withstand the new environment of low pH and high alcohol content.

This confirms with the report of Idise (2012) that as fermentation proceeds there is succession and reduction in the number and type of microorganisms in the fermentation liquor.

Wines produced from these three fruits (soursop, guava and Malay apple) using indigenous organism compared favourably well with that of baker's yeast. The fruits are available and affordable so can be made into wine serving as a means of preserving the fruits and preventing their wastage at the peak of production. It also affords other types of wine this increasing the diversity of wines.

More studies can be done in the area of preservation of the wine produced from these fruits. The organisms can also be applied in wine making genetically engineered to produce safe

mutant strains.

CONCLUSION

The physico-chemical properties of the wines produced from the fermentation of soursop ,guava and malay apple using the isolates compared well with that produced using baker`s yeast. The wine is chemical free and the raw material are affordable and readily available as such can be used in the commercial production of wine on both large and small scales.

REFERENCES

Abbo ES, Olurin T, Odeyemu G (2006). Studies on the storage suitability of soursop. Afr. J. Biotechnol. *5:*108-112.

Alobo AP, Offonry SU (2009). Characteristics of coloured wine produced from *Hibiscus sabdariffa* calyx extract. J. Brewing 115:19-24.

Aniene MA, Kunkee RE (2005). Microbiology of wine making. Ann. Rev. Microbiol. *22*:232-258.

Daglia M, Papetti, A, Grisoli, P, Acti C, Dacorro C, Gazzain G (2007). Antibacterial activity of red and white against oral streptococci. J. Agric. Food Chem. 55(13):5038-5042.

Hallgenten F (2006). Wine Scandel (2nd Ed). London: Sphere Books Ltd. pp. 1-55.

Idise OE (2012). Studies on wine production from pineapple (*Ananas commonsus)*. Int. J. Biotechnol. Mol. Biol. Res. 3(1):1-17.

Isitua CC, Ibeh IN (2010). Novel method of wine production from Bancina (*Musa accuminals*) and Pineapple (*Ananas comosus*) wastes. Afr. J. Biotechnol. 9(44):7521-7524.

Lundberg ML, Ezra A, Amsterdam MD (2008). Alcohol, wine and cardiovascular health. Clin. Cardiol. 31(8):347-51.

Okafor N (2007). Microbiology and biochemistry of palm wine. Adv. Appl. Microbiol. 24:237-255.

Okafor N (2007). Modern industrial microbiology and biotechnology. Enfield, NH, USA: Science Publishers. p. 530.

Okoro CE (2007). Production of red wine from *Hibiscus sabdariffa* and *Carica papaya* using palm wine *Saccharomyces cerevisae.* Niger. Food J. 25:158-164

Robins J (2003). The Oxford companion to wine, (3rd Ed). London: Oxford University Press. p. 840.

Robinson J (2006). *Jancis Robinson's Wine course*. New York: Abbeville Press. pp. 39-41.

Strepper M, Octhe MC, Boohvizen HC, Kok FJ, Kromhont D (2009). Long Term Consumption is related to cardiovascular mortality and life expectancy independently of moderate alcohol intake. The Zutphen study. J. Epidemiol. Commun. Health. 63(2):534-540.

Wang HL, Swain, EW, Hissiltine CN (1980). Phytase of mold used in oriental food fermentation. J. Food Sci. 45:1-14.