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Physico-chemical and Sensory Acceptability of Soursop *(Annonamuricata)* Wine

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Abstract

Physicochemical and sensory acceptability of wine made from soursop (Annonamuricata) were evaluated. A "must" (soursop juice before or during fermentation) sample of the soursop pulp was prepared and replicated. Its replica is referred to as sample B in this work. The "must" samples were treated with 0.543/litre of sodium metabisulphite, inoculated with 5grams reconstituted active baker's yeast and allowed to ferment. The fermentation lasted for 11days. The wine sample produced had a final alcohol content of 12.99%, pH of 3.42, and total acidity of 0.82%. The green wine (young) was aged for 12weeks to reduce the acidity and to develop a characteristic bouquet. It was packaged and presented for sensory evaluation using Don Morris white wine as a standard. The sample wine compared favorably with the standard with no significant difference ($p>0.05$) in odor and taste but with a significant difference ($p<0.05$)in color acceptability. The green wine was also compared with the replica and no significant difference obtained. The wine at 9months old was tested again and 3.35, 0.9826, 13.95% and 2.15% for pH, specific gravity, alcohol content and titratable acidity respectively was obtained. Sensory evaluation at 9months old was also carried out and result obtained was used in evaluating analysis of variance. No significant difference existed in color, odor, taste and acceptability.

Keywords: *Soursop, must, wine, physicochemical properties, sensory properties.*

Introduction

Winemaking can be summarized as the biotransformation of must into wine, which is performed principally by *Saccharomyces cerevisiae* strains during the primary or alcoholic fermentation (Alexandre *et al*,; 2004). Wine is a fermented acid drink (pH 3.5 and below). Wine is made by the normal alcoholic fermentation of juice of sound, ripe grapes modified by the usual celler treatment. (Smith and Hui, 2004; Alias and Linden, 1999)

From the above definitions wine is the fermented drink from grapes. But a large number of fruits are usually fermented for production of wine and other alcoholic beverage. Wines produced from grape (*Vitis* species) are the true wines (Amerine 1981) while wines from other fruits are simply referred to as fruit winesor more specifically designated with the name of the fruit used. Examples are orange wine, banana wine, cherry wine and pineapple wine (Lea, *et al*.; 2003, Awan and Okaka 1983). Indeed a great number of fruits have been listed as having been used for wine production and include cashew fruits, orange, pineapple, masuku, paw paw (Steven and Phillip, 1980,Akubor, 1996) apples, berries, peaches, apricots, (Duarte, *et al* ,;2010). The principle behind wine making is based on the grounds, that wine yeast capable of transforming sugar naturally in fruit juices are utilized to effect fermentation. Fermentation is a biological degradation of glucose and fructose to ethanol, carbon dioxide and a number of other flavoring components (Alias and Linden 1999): Energy is given out as heat.Various types of fruits are grown in different parts of Nigeria to meet the needs of the populace. Though these fruits are in abundance the production of these fruits are seasonal thus putting a limitation on their availability throughout the year since there is no effective processing of them. (Akinyele and Keshinro, 1980).Soursop is one of such fruits. Akinyele and Keshinro (1980) recommended that soursop and other fruits, found to contain considerable amounts of nutrients should be processed either at home or factory level to make them available to the people all the year round. The need to avoid wastes has initiated the search on how to preserve these fruits during the peak harvest season. Having the above objectives in mind the need to study the wine making characteristics of soursop as one of these local fruits (tropical fruits) that is seasonal and evaluation of soursop wine is far more important now than any other thing. Production of soursop wine and its evaluation is a means of preservation and this makes it available all year round, minimizes wastes that would have been incurred during its peak and reduces cost. By its production (Soursop wine) variety is created for consumers of wine, its ability to produce wine like apples, paw paw, pineapple, plums, orange, grape, berries, apricot etc is testified to know whether it can give good stable wine or not. These and many other reasons is the reason behind production of soursop wine and its evaluation in this work since there is no other adequate processing technique available in this country to preserve and make it available when not in season. By its nature, soursop has suffered transportation problems. Much of the soursop in the tropics is consumed locally, as it is difficult to transport the fruits satisfactory over long distance (Steve and Phillips, 1980).

Nigeria imports several million liters on fruits based alcoholic beverages annually indicating that her wine industry is underdeveloped. (Aina and Onyekelueze, 1983; Akubo, *et al.;* 2003). So, the exploitation of locally available fruits for industrial venture such as wine making would not only enhance efficient fruit utilization but also encourage the growth development and expansion of indigenous wineries. When this is achieved successfully importation of fruit wine will stop completely resulting in increase in production of local fruit wines, increase in employment opportunities, decrease in cost of living, availability of food and drinks for the populace and profit maximization. The aim of this study is to produce soursop wine and evaluate its sensory and physicochemical properties.

Materials and Methods

Source of Raw Material

Mature soursop fruits used for this study were purchased from Eke Ukwu Owerri, Imo State Nigeria. They were allowed to ripen at room temperature (25^0C). At its optimum and wholesome stage for wine production the fruits were washed, weighed and must (Will *et al.;* 1998) prepared from it. Commercial active baker's yeast (*Saccharomyces cerevisiae*) used in fermentation and other chemicals of analytical grade were bought from a chemical shop in Owerri Nigeria. Most of the equipments used were supplied by the University laboratory.

Preparation of Must

The ripe soursop fruits were washed in clean portable water and finally rinsed with portable water. 1-3% w/w of sodium metabisulphate was added to wash water to destroy spoilage microorganisms. It was peeled, seeds removed and 3.0kg of fruits ground to pulp. Thick juice obtained was made lighter by making up the ten liter mark that contains the pulp with clean portable water. Its specific gravity was measured using S.G bottle and adjusted to 1.090 by adding granulated sugar. 0.0543/L of sodium metabisulphite was added to inhibit the growth of undesired microorganism. Urea was added at a level of 0.2g/L as yeast nutrient. The must was then boiled to sterilize it and allowed to cool before inoculation with yeast (AOAC, 2005).

Starter Culture Preparation

The active baker's yeast (10.0g) was reconstituted in 200ml of water. The mixture was allowed to stand for about 25minutes after which it was transferred into 400ml of already prepared must and left to stand until growth was obvious and fermentation in progress. It was allowed to ferment for about 6-12hrs. The starter was then used to pitch the whole brew in an open bucket (Duarte, 2009).

The Following parameters were monitored before and during the fermentation process:

Specific gravity
pH
Total titratable acidity
Temperature
Alcohol content

Must Specific Gravity

The must specific gravity was determined using a density bottle (AOAC, 2004). The bottle was properly washed and kept in the oven to dry. The bottle and stopper were cooled in the dessicator and weighed accurately.
The must and distilled water were cooled to 15.5^0C, the bottle was filled with distilled water and stopper inserted and weighted. The bottle was then thoroughly cleaned and the process repeated with the fermenting must. The values obtained were used for calculation using the formula.

Specific gravity = $\frac{W_2}{W_1}$

Where;
W_2 = *weight of the must*
W_1 = *weight of equivalent volume of water*

Simply put

Specific gravity = $\frac{B \quad C}{A-C}$

A - Weight of distilled water at 15.5^oC and density bottle
B - Weight of the must at 15.5^oC and density bottle
C - Weight of empty density bottle

Must pH

The must pH was determined using AOAC, (2004) procedure. The pH meter electrode was thoroughly rinsed with distilled water and reading adjusted to zero mark. The meter was then standardized in buffer 4 and 7 solution at 25^{o}C. Each 25ml of the must was pipette into a beaker and the pH electrode (probe) was dipped into the must and the reading allowed stabilizing before reading off.

Determination of Titratable Acidity

The titratable acidity of the wine was determined using AOAC, (2004). Apparatus/Reagents used are as follows:

1. *Burette*
2. *Conical flask*
3. *Measuring cylinder*
4. *0.1NNaOH solution*
5. *5% phenolphthalein indicator.*

Using the the method described in AOAC (2004)15ml of the sample was put into the conical flask, 0.5ml (3drops) of 5% phenolphthalein indicator was added into the same conical flask and shaked gently to ensure thorough mixing of the must sample and the indicator. The content of the conical flask was titrated with 0.1N sodium hydroxide against a white black ground. The titration was carried on till the first appearance of a faint pink colour was observed and the burette read off. 0.2ml more of alkali was then added to the pink solution. This gave permanent pinkish red colour indicating over titration. The first burette reading was taken as the end point. The total acidity was calculated thus:

Each 1ml NaOH= 0.1% acidity as tartaric acid
i.e

$$\frac{\text{Number of mls of NaOH used}}{10} = \text{Total titratable acidity}$$

Simply put

$$\% \text{ Acidity} = \frac{0.064 \times \text{Normality of NaOH} \times \text{Titre value} \times 100}{\text{mass of sample}}$$

Titre value x 0.06404 = % tartaric acid.
0.06404 = ml equivalent weight of tartaric acid.

Must Alcohol Content

Alcohol content was determined only by getting the specific gravity of the wines each day with their corresponding temperature. Using the alcohol determination chart/specific gravity, individual alcohol content for the wines was determined following the Institute of Brewing (IOB 1977) and Association of official Analytical Chemists (AOAC 2004).

Must Temperature determination

Must temperature was determined daily throughout the fermentation period using a thermometer with centigrade.

Fermentation of Soursop Must

Preparation of Fermentation Medium/Pitching

Two sets of must prepared, both comprised only the peeled fruit ground into a puree but the difference is that they were started or prepared different days that is three days gap to ensure accurate result and observation to rule out doubt. 3.0kg of each set of fruits was weighed out and after pulping they were made up to the 10 litre mark with portable water. Both set were prepared as earlier described in section 3.2. Each sample was put in a clean fermenter (open bucket) covered and allowed to stand for about 3hrs creating room for the metabisulphite to act by destroying undesirable microbes, prevent oxidation of valuable nutrients and flavor molecules by oxygen and thus placing the wine yeast to a growth advantage when pitched. Yeast nutrient in the form urea (0.2g/L) was added. The must was pitched with the reconstituted baker's yeast and allowed to ferment. It stayed in the open fermenter until fermentation was obvious. This is to allow oxygen for yeast growth. (AOAC 2005, Torok, 1996).

Fermentation Process

The fermentation process lasted for 11days at room temperature (28 – 30^{o}C). The nature of fermenter used is an anaerobic fermenter. With this period, bubbles of CO_2 were observed. The fermenting tube which was passed into a clear bottle containing clean portable water allowing the CO_2 produced during fermentation to escape while preventing entrance of O_2. This stage began when yeast was vigorously converting sugar to alcohol. The contents of the fermenter were stirred thoroughly during the first 7days of primary fermentations. This was to facilitate temperature equilibrium and encourage aeration which was necessary for initial yeast growth (Mounigan, 2006).

Post Fermentation Treatment

At the end of fermentation when sugar has been converted to alcohol and toxicity of alcohol to yeast has killed yeast in the wine. The wine was racked. This process involves siphoning the green wine into sterile containers with tight seals using a clear rubber

racking tube. This was done by inserting the tube into the wine mounted on a bench and the other end being introduced into an empty sterile container placed on another lower platform. After racking, because the wine is not clear of most of the debris, it was further pressed to remove all the yeast present and other smaller particles that did not deposit below. By these two means (Racking/Pressing) the yeast in the wine was harvested.

After racking the wine was kept for 8weeks maturation at a refrigerated temperature of about 5^0C for further racking and aging the resultant wine was pasteurized at 65^0C for 10 minutes and stored until needed for sensory evaluation.

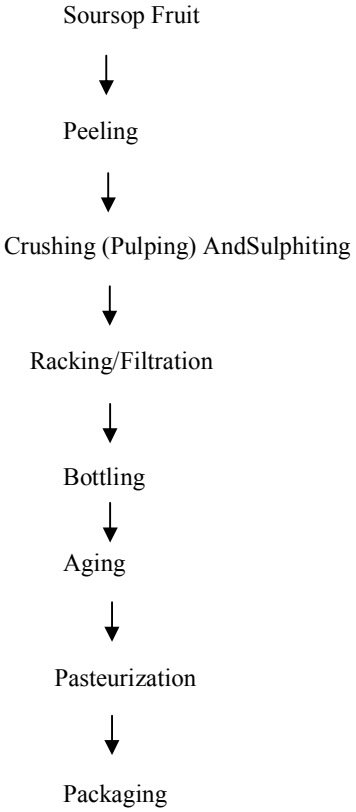

Fig 1: Flow chart for soursop wine production process

Sensory Evaluation
Sensory evaluation of wine samples was conducted in the food processing laboratory of department of Food Science and Technology, Imo State University Owerri, Nigeria using a 10 - member semi-trained panelist who are familiar with wines. The color, taste, odor and overall acceptability were rated using the nine point hedonic scale where 1 and 9 represented dislike extremely and like extremely respectively (Ihekoronye 1985). Samples were presented in identical containers (glass cup) coded with 3-digits random numbers. They are 231, 012 and 119 for standard- Don Morris white wine, soursop wine (12weeks old) and soursop wine (9months old). In a balanced serving order each sample was served in each position first, second and third and equal number of times. Samples were presented simultaneously as it is easier to administer and also allowed panelists to re-evaluate the samples if desired. Each panelist expresses his degree of liking or disliking on the 9 points hedonic scale as described by Iwe(2002).

Statistical Analysis
The data generated was subjected to analysis of variance and means separated using fisher's LSD at $p<0.05$

Results and Discussion
Specific Gravity, pH, Alcohol content Titratable acidity and temperature of fermenting must
Titratable Acidity
From table 1a the acidity of the fermenting must increased from 0.28g/100ml in the first day to 0.82g/100ml at the 11^{th} day as tartaric acid. This increase in acidity is probably as a result of certain organic acids produced during fermentation (Kunkee, (1991).Amerine and Ough (1980) reported that during fermentation, major acids formed include lactic, marlic, succinic and acetic acids. The final acidity of 0.82% is favourable for a check against spoilage organisms. Since table wines have titratable acidity in the range of 0.6-0.9% (Pozo-Bayón *et al.;* 2012). When the wine was nine months old, the acidity was 2.15% due to malolytic fermentation during aging as stated in table 1b.Presence of these acids in wine is necessary because without them the wine would taste unpalatably flat and spoil with a poor colour and flavour. (Reddy *et al,;* 2005)

Table 1 (a): pH, specific gravity, alcohol content, titratable acidity and temperature of the fermenting must.

	Fermentation duration (Days)										
Parameter	1	2	3	4	5	6	7	8	9	10	11
pH	4.25	4.13	4.03	3.83	3.72	3.63	3.55	3.50	2.46	3.42	3.42
Titratable acidity	0.28	0.33	0.38	0.45	0.55	0.63	0.71	0.77	0.80	0.82	0.82
Specific gravity	1.0980	0.9974	0.9961	0.9930	0.9900	0.9884	0.9873	0.9860	0.986	0.9838	0.9838
Alcohol content/vol.	0.00	1.87	2.84	5.06	7.64	9.34	10.23	12.27	12.68	12.99	12.99
Temperature	26.5	27.5	28	28.5	29	28.5	28.3	27.8	27.5	27.3	27.3

Table 1b: pH, specific gravity, alcohol content and titratable acidity of soursop wine at 3months and 9months

	Green wine (12 weeks maturity)	**Mature wine(9 months)**
pH	3.42	3.35
Alcohol content	12.99	13.95
Specific gravity	0.9838	0.9826
Titratable acidity	0.82%	2.15%

pH

The pH was on the decrease as expected from 4.25 to 3.43. This should be a result of its inverse proportionally with acidity. But it is important to point out that is no direct relationship between pH and total titratable acidity because of varying buffer capacity of the fermenting liquor (Sander *et al*.; 2006, Lea *et al.;* 2003). So, little variation in fixed acidity is explained by the fact that yeast produced little acids during fermentation, (Amerine and Singleton, 1989). After aging for nine months pH further decreased to 3.35. This is very important for microbial stabilization of wine, aroma and taste developments.

Alcohol Content

There was gradual increase in alcohol content within the first 9days as could be seen from table 1a. It later became constant from the 10^{th} day to the last day of fermentation. The reason is that most of the fermentable sugars have been converted to alcohol. Also toxicity of increased alcohol content produced made the yeast inactive for more production (Gambelliand Santaroni, 2004). Its alcohol content was 12.99% but when aged for nine months.Its final alcohol content was 13.95% mostly due to further conversion of residual sugars to alcohol is in agreement with normal alcoholic content of table wines which ranges from 10-14% (Ferreira *et al,;* 2000, McCall, 1988).

Specific Gravity

Specific gravity decreased due to conversion of sugar to alcohols since alcohols have less Specific gravity than sugar. The decrease was from 1.980 on the first day to 0.9838 on the 11^{th} day. After aging for nine months it further decreased to 0.9826. This was as a result of further conversion of more complex sugars during aging. This is known as malolytic fermentation. This corroborates with Akubor, (1996)and Akubor,*et al.;*(2003) observations when he produced wine using bush mango juice and banana respectively.

Temperature

Temperature of sample increased initially during fermentation due to an increased metabolism of the yeast cells as they converted sugar to alcohol thereby releasing heat in the fermenting system. At the later stage the heat dropped and finally became constant indicating the phase at which the yeast activity was stationary (stationary phase). At this phase the system was still dissipating heat to the surrounding (Molina, 2007; Eka, 1983).

Sensory Acceptability of Soursop Wine

Color

The sensory evaluation was carried out two times. First was when the wine was 12 weeks old and secondly 9months old. This was done by comparing the soursop wine (12weeks old and 9months old) to sample X (the standard – Don Morris white wine) for color, taste, odour and acceptability. The color of the green wine produced is white and clear. This was because of the color of must of soursop pulp which is white and longer period of aging. It was also observed that sedimentation of particles was very fast. This could have been as a result of the occurrence of denser insoluble particles in them which settles to the bottom. The action of the protease enzyme such as papain ensured a breakdown of the proteins, peptides and polypeptides present in the wine. The peptide and polypeptide compounds also formed a complex with the tannin (protein – tannin complex) which settled out when the wine was left to stand for some time. This enhanced clarification of wine during aging. The mean value of color acceptability obtained from the sensory evaluation at 12weeks old was 8.2 ± 0.63,6 ± 1.49 and 5.3 ± 1.89for the standard, soursop wine and its replica respectively as shown in table 2a. The wine sample was compared to sample X – (standard – Don Morris white wine) to

know if there was any significant difference between them. There was significant difference ($p<0.05$) between the standard and the soursop wine and the replicated soursop wine at 12 weeks in color. At nine months old, table 2b shows that the mean values obtained for color from sensory evaluation was 7.7 ± 3.03 and 6.3 ± 1.42 for the standard and the mature soursop wine showing that there is no significant different ($p< 0.05$) in color between the standard and the soursop wine at nine month old. Reason for this is the fact that peptide and polypeptide compounds present in the wine have formed a complex with the tannnis (protein – tannin complex) which settled out during 9months of aging (Akubor, 1996, Akubor, *et al.;* 2003)

Table 2a. Mean scores of the sensory evaluation of soursop samples at 12th week maturity

Samples	Color	Odor	Taste
X	8.2 ± 0.63[a]	7.6 ± 1.78	6.1 ± 2.33
A	6 ± 1.49 [ab]	6.3 ± 1.42	6.4 ± 1.26
B	5.3 ± 1.89 [b]	6.1 ± 2.13	6.5 ± 1.65
LSD	2.81	NS	NS

Mean± standard deviations (from Triplicate determinations), Means followed by the same letter along the columns are not significantly different (p≤0.05)
NS ---- Not Significant
X ---- Standard/ Control (Don Morris White Wine)
A ---- Soursop Wine Sample
B ---- Soursop Wine Replicated

Table 2b. Mean scores of 9months matured wine compared to Don Morris White Wine

Samples	Color	Odor	Taste	General acceptablity
X	7.7 ± 3.03	6.1 ± 2.33	7.6 ± 1.78	6.2 ± 2.44
A	6.3 ± 1.42	6.4 ± 1.43	6.1 ± 2.13	6.5 ± 1.35
LSD	NS	NS	NS	NS

Mean± standard deviations (from Triplicate determinations), Means followed by the same letter along the columns are not significantly different (p≤0.05)
NS ---- Not Significant
X ---- Standard/ control (Don Morris White Wine)
A ---- Soursop wine sample
B ---- Is also soursop wine that is replica of A

. . . *A = B so B is no longer mentioned here since they are the same.*

Odor
Sensory evaluation mean values obtained at 12weeks for odor of soursop wine were7.6 ± 1.78, 6.3 ± 1.42 and 6.1 ± 2.13 for standard wine, soursop wine and replica 2 respectively. This showed that there is no significant different ($p<0.05$) in odor between the soursop wine and the standard wine and even between the replicated soursop wines. When this was also carried out at 9months old, mean values of sensory evaluation obtained for odor was 6.1 ± 2.33 and 6.4 ± 1.43 for standard and soursop wine. There is no significant difference in odor between soursop wine and standard (see table 2a and 2b above).

Taste
Table 2a shows the mean values obtained in sensory evaluation of soursop wine for taste at 12weeks old are 6.1 ± 2.33, 6.4 ± 1.26 and 6.5 ± 1.65 for standard (control) wine, soursop wine and soursop winereplicated, there is no significant difference ($p<0.05$) in taste between the standard wine and the soursop wine and even between the replicated soursop wine. At nine months old, sensory analysis was carried out again to find out if there will be any difference in level of significant in taste and mean values of sensory result obtained are 7.6 ± 1.78 for standard wine and 6.1 ± 2.13 confirming that there is no significant difference ($p<0.05$) in taste after 9months old between the soursop wine and the standard wine and it corroborates with Mounigan, *et al.;* (2006) observations insensory acceptance, quantitative descriptive and physicochemical analysis of wines.

Acceptability
At 12 weeks, the acceptability test of the wine was not done becauseit was young and not mature.Immature wine may not compare in acceptability with aged wine. This was done at 9 months old with 6.2 ± 2.44 and 6.5 ± 1.35values as mean values for sensory evaluation result for standard wine and soursop wine. This indicated that there is no significant different ($p<0.05$) in acceptability. On completion of analysis it was seen that a significant difference in color existed at 12weeks between the standard and the soursop wine but after aging for 9months there was no significant difference between them. With respect to odor, taste and general acceptability no significant difference existed between the standard wine and the soursop wine.

Conclusion
The result of this work has shown that Soursop wine making which started with the collection of wholesome soursop fruit, cleaning, crushing, sulphite addition, fermentation, racking, clarification, packaging and pasteurization and finally aging of the soursop wine can be achieved successfully. The green wine produced compared favorably with a table wine bought in the market

thereby calling for action to stop completely the importation of fruit wines. Successful production of soursop wine confirms the feasibility of its production in an industrial scale. This is a good medium for profit making resulting in decrease in cost of living and availability of food and drinks for the populace. It will go a long way in solving the problem of wasting all fruits in the country especially seasonal fruitsand by this means waste management is improved. So, production of good quality, palatable and acceptable wine from soursop pulp is possible.

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