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Evaluation of Changes in the Microbial Profile, Physico-Chemical and Nutritional Attributes During the Bioconversion of Soursop (*Annona muricata)* Must to Wine

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Abstract
Low alcoholic wines of amiable qualities were produced from soursop (*Annona muricata*). The fermentation process was for a period of 7 days at 26 ± 2 ^{0}C and soursop wines coded I, II, III and IV were produced. The microbial counts were taken during the fermentation process using Nutrient agar and Malt Extract agar respectively. Changes in the physico-chemical and nutritional properties (pH, temperature, specific gravity, alcohol content, titratable acidity, sugar content, carbohydrate content, soluble protein, ash content and fat content) during the control/spontaneous and modified fermentation processes of the soursop must were also monitored. The mean bacterial counts ranged from 0.1×10^4 cfu/ml to 1.7×10^4 cfu/ml respectively. The mean fungal counts varied from 0.1×10^4 cfu/ml to 0.6×10^4 cfu/ml respectively. The differences in the microbial counts were insignificant (P>0.05). Microbial isolates identified included; *Bacillus megaterium*, *B. subtilis*, *B. coagulans, Streptococcus* sp., *Staphylococcus* sp., *Alcaligenes* sp. and *B. polymixa*, *Saccharomyces cerevisiae*, *Trichoderma* sp. and *Penicillium* sp. Wine IV had the lowest total organoleptic attributes while wine I had the highest total organoleptic attributes. This study revealed that low alcoholic wine with appreciable characteristics and acceptability can be produced from ripe soursop fruit.

KEY WORDS: Soursop, bacterial counts, fungal counts, must, organoleptic**.**

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Introduction

Soursop; *Annona muricata,* also called guanabana is a fruit with an acidic taste, closely related to custard apple. It is a small evergreen tree, member of the custard apple family; *Annonaceae*. *Annona muricata* or soursop which originated from tropical Mexico, Central America, Caribbean, South America and sub-Saharan African countries now have spread and is grown in many countries, including some areas in Southeast Asia such as Indonesia, Malaysia, Philippines, and Vietnam (Morton, 1987). Soursop tree grows as high as 6 m (20 ft). It is cultivated for its edible fruits, which are large, juicy, and dark green, and have short, fleshy spines. Soursop fruit seeds are small and have a glossy black colour (Morton, 1987). Soursop fruit contains various types of nutrients beneficial to human health such as vitamins C, B1, and B12. Soursop fruit is also rich in carbohydrates, particularly fructose (Taylor, 1998). The Nutrient value per 100 grams serving includes: Vitamin C (20.6 g), Calcium (14 g), Iron (0.6 g), Calories: (66 g), Dietary Fibre (3.3 g), Protein (1 g), Cholesterol (0 mg), Sodium (14 mg), Sugars (13.54 g), Total Carbohydrate (16.84 g), Total Fat (0.3 g), Saturated Fat (0.05 g), Monounsaturated Fat (0.09 g)

and Polyunsaturated Fat (0.06 g) (Umme et al. 1997). Soursop can be eaten fresh as fruit; made into cakes, ice cream, preserved, beverages and for flavouring. The young soursop, where the seeds are still soft, is used as a vegetable. The fermented fruit is also used to make an apple cider-like drink (Lutchmedial et al. 2004). Post harvest losses is a thorny problem hampering the agricultural development in Nigeria and the estimates for fruits was reported to stand at over 60% (Osuide, 1999). Moreover, nutrient depletion, quality loss, and damage of physiological structures before consumption or converting into secondary products also result. The production of fruit crops in Nigeria is seasonal, thus, there is a need to preserve and store them from time of harvest through the period of scarcity, for the purpose of retaining them as foods and articles of trade. Owing to the discovery of soursop as an anti-cancer agent, (Asprey & Thornton, 1995) the fruit can be preserved by converting it to a stable product like wine (Okigbo, 2009). Sequel to the above, the aims and objectives of this study was to; Determine the microbial flora and the resultant changes in the physicochemical qualities occurring during the fermentation of soursop must to wine, the production of a low alcoholic wine from soursop must using the most favourable starter culture in the fermentation processes.

Materials and Methods

Sources of materials and preparation of starter cultures: Fresh, healthy and mature soursop fruits of various sizes were collected from Otuo in Owan Local Government area of Edo state. Brewer's yeast was collected from a brewery in Benin City and kept in a freezer until it was required for use. Lyophilized baker's yeast was purchased from a shop in Lagos Street, Benin City and fresh palm wine was purchased from palm wine tapers in NIFOR, Edo State. Dry baker's yeast and sediments from fresh palm wine was allowed to stand for 60 minutes and rehydrated. Four grams of lyophilized baker's yeast was kept in a sterile bottle and 4 ml of warm distilled water at 37 ^{0}C was added to make a dilution of 1: 1 and rehydrated for 15 to 20 minutes before inoculation. Yeast was acclimatized to within 10 ^{0}C of the must temperature which was at 26 ± 2 ^{0}C before inoculation.

Soursop juice extraction: Ten fully ripe soursop fruits with an average weight of 563.08 g were washed thoroughly with distilled water and surface sterilized with 70% ethanol. The fruits were peeled with sterile knife to remove the skin and then deseeded. The juice was extracted with an electric juice extractor manufactured by Nakai in Japan (Model: NJ-828). One hundred milliliters of sterile distilled water was added to facilitate grinding and extraction since the juice was very thick. One liter of extracted juice was poured into a sterile aspirator bottle previously rinsed with 2% sodium metabisulphite (Imade, 2012). Must concentrate was reconstituted with one liter of distilled water as 1: 1 w/v ratio (Imade, 2012).

Controlled and spontaneous bioconversion of soursop must to wine: For the first treatment, the soursop must was allowed to ferment naturally. The bottle was covered with cotton wool and allowed to stand for 36 hours for aeration. After this, the bottles were tightly corked and left at a temperature of 26 ± 2 ^{0}C to ferment for 7 days (Imade, 2012). During the second, third and fourth treatments, there was an amelioration of the juice with 40 g/100 ml commercial sucrose along with other nutrients which include ammonium sulphate 0.5 g/l, potassium dihydrogen phosphate 0.18 g/l and sodium metabisuphite 0.2 g/l as preservatives (Imade, 2012). The ameliorated must samples were pasteurized at 71 ^{0}C for 15 seconds and cooled rapidly to 26 ^{0}C before pitching with 5 % of different strains of *Saccharomyces cerevisiae*. Aeration was allowed by covering the bottles with cotton wool and left to stand for 36 hours. They were later tightly corked with the bottle covers and left to stand at a temperature of 26 ± 2 ^{0}C to ferment for 7 days. The various treatments were designated I, II, III, IV as specified below. Fungal enumeration was done at two-day intervals while bacterial count was conducted on a daily basis. The pH, specific gravity, alcohol formed, titratable acidity, sugar content, sugar type, total sugar and ash content were determined on a daily basis. I) Spontaneous fermentation (control); II) Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* (Baker's yeast); III) Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* (Brewer's yeast) and IV) Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* from fresh palm wine

Total aerobic bacterial and fungal flora of the soursop must treatments and control: The aerobic mean bacterial and fungal bioload of the respective must treatments and control were evaluated using

the serial dilution and pour plate methods as described by Harley and Prescott (2002), Sharma (2009) and Peptone water (Oxoid) was used as diluent while general purpose media; Nutrient agar (Biotech) and Malt Extract agar (Oxoid) supplemented with erythromycin (500 mg) was utilized in the preliminary recovery of bacteria and fungi from the fermenting soursop must and wine. Plating was done in duplicates and the resultant discrete colonies were counted and cultural characteristics recorded (Sharma, 2009). Unique bacterial and fungal colonies were sub cultured onto freshly prepared nutrient agar and potato dextrose agar plates. These pure cultures were also streaked onto prepared nutrient agar and potato dextrose agar slants and stored at 5^0C. The sub cultured bacterial isolates were identified by their colonial and cell morphology, gram reaction and a combination of standard biochemical tests described by Cullimore (2000), Aneja (2003), Roberts and Greenwood, (2003). Also, the sub cultured fungal isolates were identified through marcoscopic observation of their colonies. Microscopic examination of the respective spores and hyphal appendages using wet mount method with lactophenol cotton blue and water respectively as mountants respectively was also conducted (Sharma, 2009). The results of the microscopy were compared with illustrations stated in Barnett and Hunter (1972). Several physiological and biochemical attributes of the yeast isolates was evaluated according to methods described by Van der Walt (1970) and Barnett et al. (2000).

Physico-chemical and nutritional analyses of the must treatments and control: The pH and temperature of the fermenting must treatments and control was determined with the aid of a pH/temperature meter (ELE international, U.K). The sugar, alcohol and carbohydrate content of the soursop must treatments were evaluated using procedures described by AOAC (2000). The fat and ash values of the fermenting soursop must treatments were estimated using procedures stated by AOAC (1980). The specific gravity of the must treatments was evaluated using methods described by Imade (2012). The titratable acidity of the respective musts was determined using a procedure stated by Bianco et al. (1978).

Organoleptic assessment of soursop wine: A 20-member panel was set up to evaluate the soursop wine based on look (clarity and color), smell (aroma and flavor) and taste (sweetness, astringency and general quality of the wine). All these parameters were scored according to the 5-point scale ranging from 5: like extremely, 4: like very much, 3: like much, 2: like moderately and 1: dislike extremely (Chowdhury and Ray, 2005).

Statistical analysis: Analysis of variance (ANOVA) was used to determine if the differences in the microbial counts was significant (α=0.05). This was conducted with the aid of SPSS version 17.

Results

The total bacterial and fungal counts from the soursop must variously treated are presented in Tables 1 and 2. The mean bacterial counts ranged from 0.1×10^4 cfu/ml for treatment I (day 0) to 1.7×10^4 cfu/ml for treatment IV (day 7) (Table 1). The mean fungal counts varied from 0.1×10^4 cfu/ml for treatment I (day 0) to 0.6×10^4 cfu/ml for treatment IV (day 7) (Table 2). The differences in the microbial counts were insignificant ($P>0.05$) (Tables 1 and 2). Seven bacterial isolates; *Bacillus megaterium*, *B. subtilis*, *B. coagulans, Streptococcus* sp., *Staphylococcus* sp., *Alcaligenes* sp. and *B. polymyxa* were characterized and identified from the various must treatments (Table 3). Three fungal isolates; *Saccharomyces cerevisiae*, *Trichoderma* sp. and *Penicillium* sp. were identified from the treated musts (Table 3). A range of values; 4.6 to 3.8 and 0.022 mg/l to 0.056 mg/l were recorded for pH and titratable acidity of the respective must treatments (Figures 1 and 2). The specific gravity and alcohol content of the various must treatments had values which ranged from 1.021 to 1.003 and 0.000% to 5.978% respectively (Figures 3 and 4). The sugar (%Brix) and carbohydrate (%CH_2O) values of the must treatments ranged from 16.0094 %Brix to 13.777%Brix and 7.950 % CH_2O to 3.960 % CH_2O respectively (Figures 5 and 6). The wine produced from the spontaneously fermented soursop must had the lowest total organoleptic attributes (12) whilst the wine derived from the ameliorated soursop must pitched with *S. cerevisiae* sourced from fresh palm wine had the highest total organoleptic attributes (23) (Table 4).

Table 1: Total mean Bacterial counts (cfu/ml) of fermenting soursop must with various treatments and control at 26 ± 2 ^{0}C.

Time (days)	Treatments/mean counts (×10^4 cfu/ml)			
	I	II	III	IV
0	0.1[a]	NG	NG	NG
1	0.8[a]	1.6[a]	1.5[a]	0.6[a]
2	1.3[a]	2.0[a]	2.1[a]	1.4[a]
3	1.9[a]	2.4[a]	2.9[a]	1.6[a]
4	2.4[a]	5.2[a]	3.0[a]	3.9[a]
5	7.5[a]	5.9[a]	6.4[a]	4.4[a]
6	4.0[a]	4.9[a]	3.6[a]	3.9[a]
7	1.5[a]	1.8[a]	2,0[a]	1.7[a]

NOTE: Means on same column with similar superscript are not significantly different (P>0.05) from each other. NG: No Growth

Treatments: I Spontaneous fermentation (control); II Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* (Baker's yeast); III Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* (Brewer's yeast); IV Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* sourced from fresh palm wine yeast.

Table 2: Total mean fungal count (cfu/ml) of fermenting soursop must with various treatments and control at 26 ± 2 ^{0}C.

Time (days)	**Treatments/mean counts (×10^4 cfu/ml)**			
	I	**II**	**III**	**IV**
0	[a]0.1[a]	NG	NG	NG
3	[a]3.4[a]	[a]2.5[a]	[a]3.0[a]	[a]1.8
5	[a]6.5[a]	[a]6.5[a]	[a]4.2[a]	[a]2.7
7	[a]5.6[a]	[a]2.4[a]	[a]2.6[a]	[a]0.6

NOTE: Means preceded by the alphabet "a" are not significantly different (P>0.05) from each other

NG: No Growth

Treatments: I Spontaneous fermentation (control); II Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* (Baker's yeast); III Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* (Brewer's yeast); IV Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* sourced from fresh palm wine yeast.

Table 3: List of microbial isolates isolated from the respective fermenting must treatments.

Must treatments and control	Microbial isolates
I	*Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus* sp., *Penicillium* sp., *Alcaligenes* sp. and *Streptococcus* sp.
II	*Bacillus coagulans*, *Saccharomyces cerevisiae*, *Staphylococcus* sp., *B. megaterium* , *Bacillus polymyxa*, *Bacillus subtilis*, *Penicillium* sp. and *Trichoderma* sp.
III	*B. coagulans*, *B. polymyxa, B. megaterium* and *Saccharomyces cerevisiae*
IV	*Alcaligenes* sp., *Trichoderma* sp., *B. subtilis* , *Penicillium* sp., *B. coagulans*, *B. megaterium*, *S. cerevisiae* and *B. polymyxa*

NOTE
Treatments:
I Spontaneous fermentation (control); II Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* (Baker's yeast); III Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* (Brewer's yeast); IV Ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* sourced from fresh palm wine yeast.

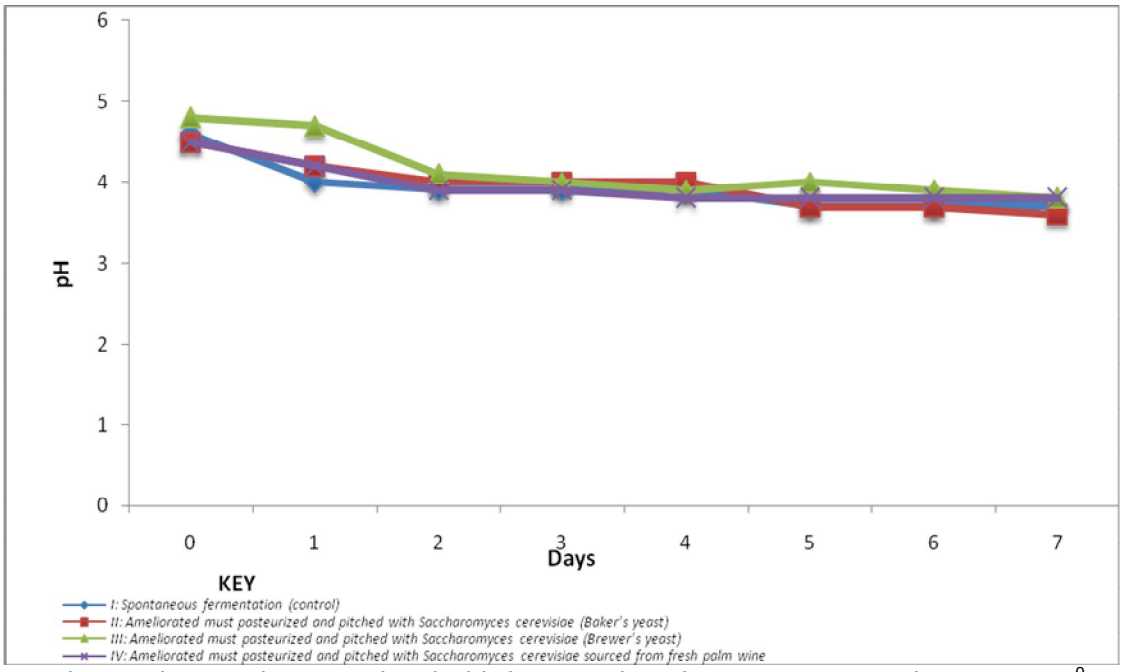

Figure 1: Changes in pH Values associated with fermentation of soursop must to wine at 26 ± 2 ^{0}C.

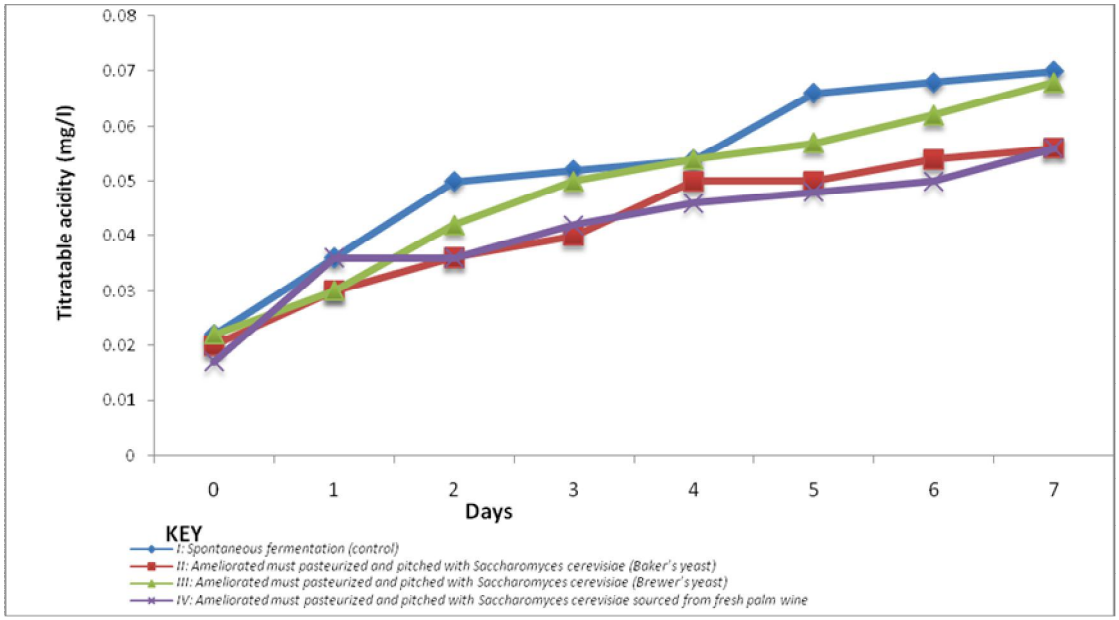

Figure 2: Changes in Titratable Acidity Values associated with ferrmentation of soursop must to wine at 26 ± 2 ^{0}C.

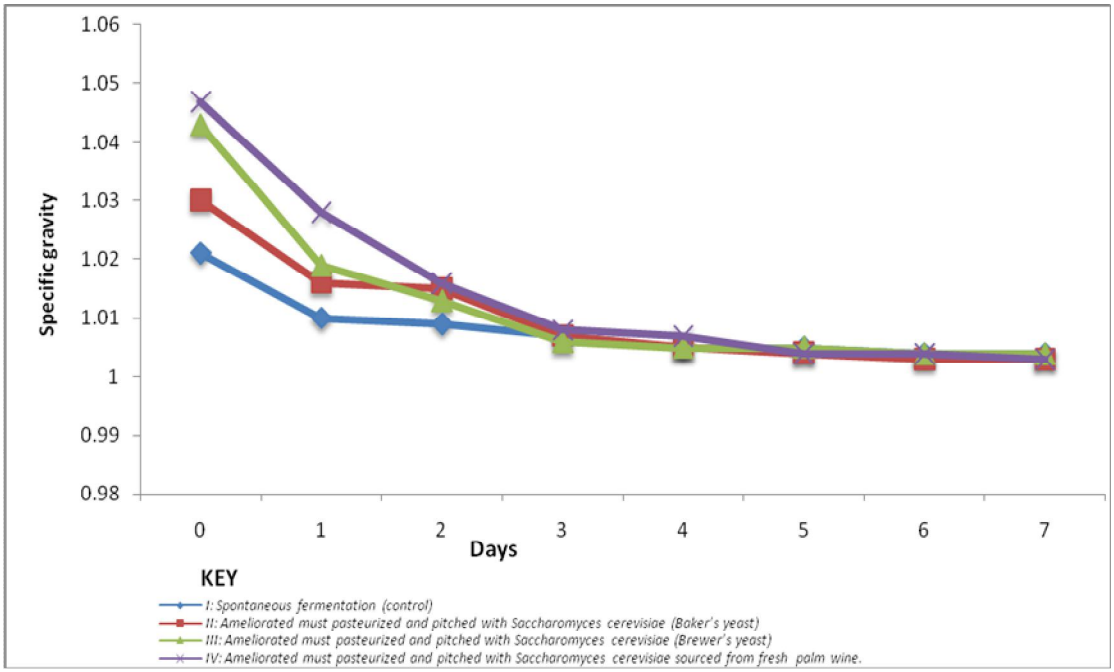

Figure 3: Changes in Specific Gravity associated with fermentation of soursop must to wine at 26 ± 2 ^{0}C.

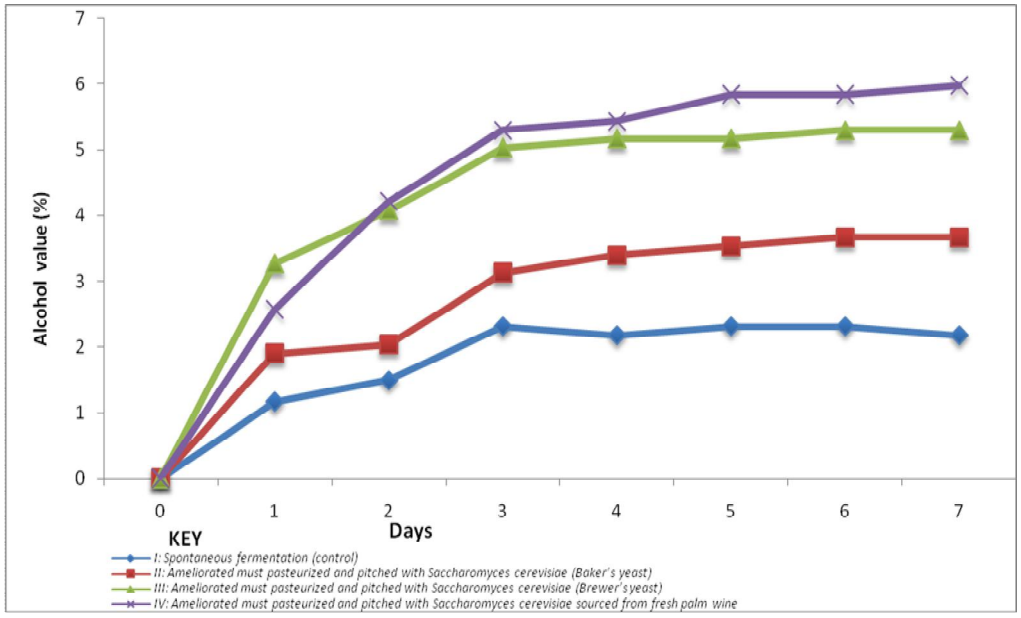

Figure 4: Changes in Alcohol Content (%) associated with fermentation of soursop must to wine at 26 ± 2 ^{0}C.

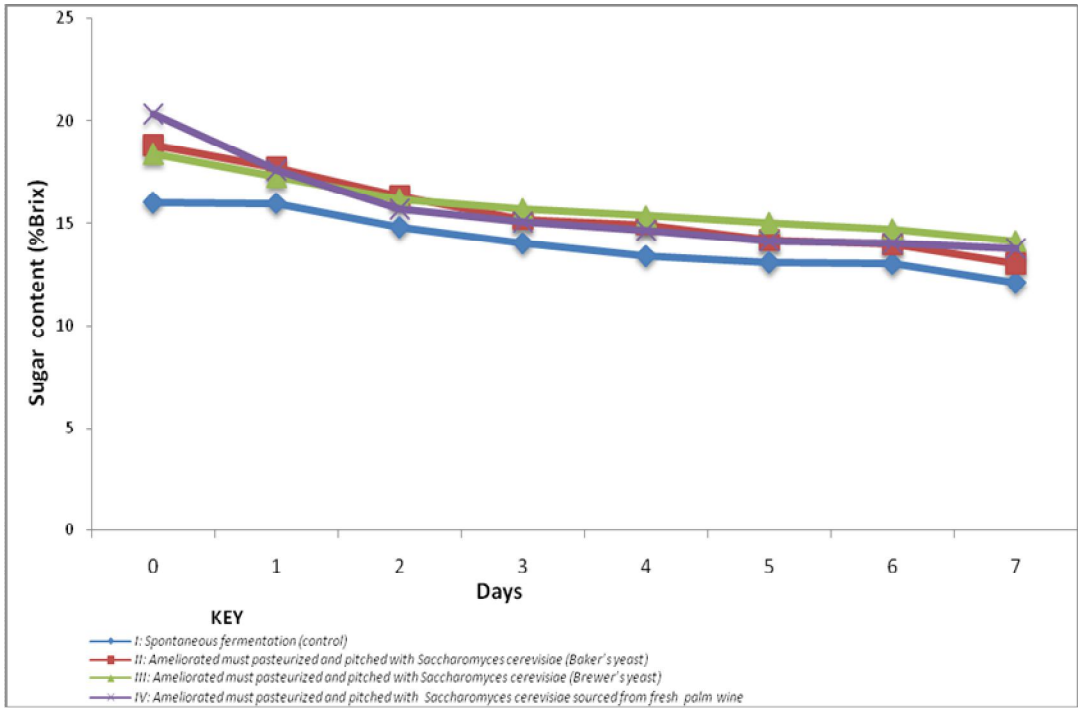

Figure 5: Changes in Sugar Content (% Brix) associated with fermentation of soursop must to wine at 26 ± 2 ^{0}C.

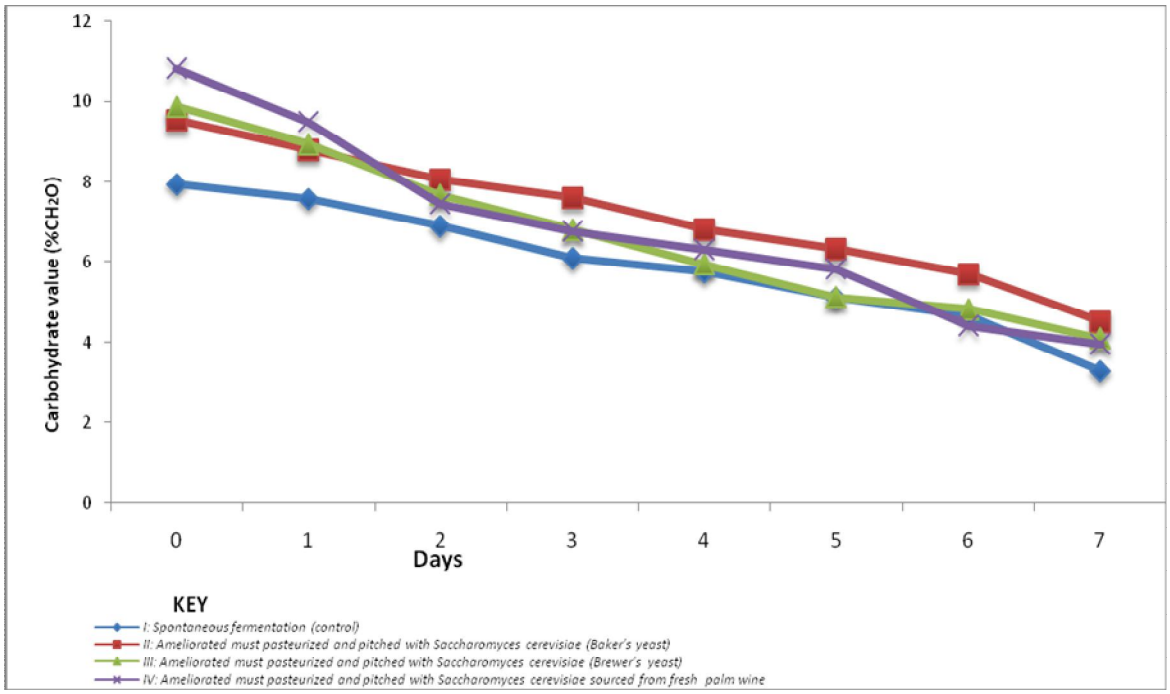

Figure 6: Changes in Carbohydrate Content (% CH_2O) values associated with fermentation of soursop must to wine at 26 ± 2 ^{0}C.

Table 4: Organoleptic assessment of Soursop wines from four different treatments at the end of fermentation

Parameters	**Treatments**			
	I	**II**	**III**	**IV**
Aroma	2	2	4	4
Astringency	1	2	3	3
Clarity	3	4	4	4
Colour	3	3	3	3
Flavour	2	4	3	4
Sweetness	1	3	4	3
Total	12	20	20	23

KEY: 1) Dislike extremely; 2) Like moderately; 3) Like much and 4) Like very much. 5: Like extremely

Discussion

There were variations in the mean aerobic bacterial and fungal counts for the respective must treatments (Table 1 and 2). Also, the microbial counts increased gradually from day 1 and peaked at day 5 for all the must treatments (Table1 and 2). This rise in microbial count from day 1 to day 5 could be a result of utilization of the sugar as well as other accessory nutrients in the must. The decline observed for the microbial biomass from day 6 for the bacterial counts and day 7 for the fungal values (Table 1 and 2) could be a reflection of the exhaustion of dissolved nutrients and accumulation of toxic metabolites such as organic acids within the respective must treatments.

Bacillus subtilis and *B. megaterium* were recovered from all the must treatments (Table 3). This is not surprising as these bacterial species are sporogenic and can survive a wide variety of adverse environmental and physiological conditions. *Saccharomyces cerevisiae* and the moulds; *Penicillium* sp. and *Trichoderma* sp. were recovered from some of the must treatments (Table 3), This observation is at

variance with a report by Okigbo (2009) who isolated *Aspergillus niger, Candida* sp., *Fusarium* sp., *Penicillium* sp and *Trichoderma viridae* from soursop pulp. The moulds isolated from the fermenting must treatments were few (Table 3). This trend might be due to inhibitory effects of gradual increase in both the titrable acidity and alcoholic content of the fermenting soursop must treatments (Figure 2 and 4) coupled with a decrease in both the sugar and carbohydrate content of the soursop musts (Figure 5 and 6).

In conformity with the relationships drawn between % brix, Titratable acidity and pH by McCarthy (1994); as the % brix of all the fermenting musts decreased on a daily basis (Figure 5) there was a concomitant increase in titratable acidity (Figure 2) accompanied by a reduction in pH (Figure 1). Low pH promotes balance of wine colour, increases antimicrobial action of sulphur (IV) oxide, and enhances clarification (McCarthy, 1994).

There was an inverse relationship between specific gravity and alcohol content of the fermenting soursop must treatments (Figure 4 and 5). Also, the S. G. of treatment I reduced from 1.021 to 1.005 while the alcohol content increased from 0 to 2.174 % (Figure 3 and 4). This wine had the lowest alcohol content (Figure 4) which could be attributed to the non inclusion of sucrose and other accessory nutrients to the must before fermentation; hence the amount of fermentable sugar present was low. Wine II, III and IV had alcohol content of 3.668 %, 5.299 % and 5.978 % (Figure 4) respectively which is suitable for all lovers of wine as high alcohol intake has deleterious effects on the liver (Iredale, 2003).

Generally, the carbohydrate, pH, brix, and the specific gravity decreased in the wines (fermented must at day 7) when compared to the initial values of these parameters observed for the must treatments at day 0 (Figure 1, 3,5 and 6) . These trends resulted in observable change in color, aroma and clarity thereby giving the wine a pleasant appearance. This could indicate that microbial activity and processing (addition of nutrients and preservatives) affected the physical and chemical composition of the raw soursop juice. In addition, the wine obtained was creamy brown in colour while the must was creamy white. This could be attributable to microbial activities during fermentation process (Abbo et al. 2006).
The wine produced from the ameliorated must pasteurized and pitched with *Saccharomyces cerevisiae* sourced from palm wine had the highest alcoholic content (Figure 4) amongst the wines and also had the lowest percentage carbohydrate value in comparison to the other ameliorated soursop musts (Figure 6).

Also, this wine had the best organoleptic assessment in comparison with the other wines (Table 4). These observations would suggest that *Saccharomyces cerevisiae* sourced from fresh palm wine was the best starter culture necessary for the production of wine from ameliorated soursop must. It was also noticed that the pleasant flavor and aroma of soursop pulp was stronger in the wine produced from spontaneous fermentation. However this wine was the poorest in terms of organoleptic attributes (Table 4). This observation would infer the necessity of the usage of a pitching yeast culture in the production of wine from soursop must. Also, the wine produced from ameliorated soursop must pitched with *S. cerevisiae* sourced from palm had a strong palm wine flavor. However, there was no noticeable soursop, beer or bakery product flavor in the wine produced from ameliorated soursop must pitched with *S. cerevisiae* (brewer's yeast) and (baker's yeast).

Conclusion

This study has shown that low alcoholic wine with appreciable qualities and immense acceptability can be produced from ripe soursop fruit. Although the common starter cultures available influence the overall quality of the wine, it is important to stress however that the quality of the fruit used to a very large extent determines the quality of the wine. Wine IV which was pitched with *S. cerevisiae* from palm wine was rated best by the 20-member panel. Post harvest loss of medically important fruits like soursop therefore can be drastically reduced by using them in the production of fruit wine. With increasing awareness of the health importance of soursop, there is a ready market for this product only if private individuals or government can set up local industries for the production of fruit wines.

References

Abbo, E. S., Olurin, T. and Odeyemi, G. (2006). Studies on the storage stability of soursop (*Annona muricata*) juice. Afr. J. Biotechnol. 5: 108–112.

Aneja, K. R. (2003). Experiments in Microbiology, Plant Pathology and Biotechnology, 4th Edn. New Delhi. New Age Pub. Ltd., p. 606

AOAC (Association of Official Analytical Chemists). (1980). Standard Official Methods of Analysis of the Association of Analytical Chemists, 14th Ed. Washington DC. AOAC International, p. 1020.

AOAC (Association of Official Analytical Chemists). (2000). Official Methods of Analysis of AOAC international, 17th Ed. Geithersburgh, MD. AOAC international, p. 1200.

Asprey, G. F. and Thornton, P. (1995). Medicinal plants of Jamaica. West Indian Med. 4:69–92

Barnett, H. L. and Hunter, B. B. (1972). Illustrated genera of imperfect fungi, 3rd Ed. Minneapolis. Burgess Publishing Company, p. 241.

Barnett, J. A., Payne, R. W. and Yarrow, D. (2000). Yeasts characteristics and identification. 3rd Ed. Cambridge. Cambridge University Press, p. 1139.

Bianco, L. J., Richardson, G. H., Sherbon, J. W., Burke, J. A., Leaman, D., Rota, W. and Liden, R. (1978). Standard methods for the examination of dairy products. Washnigton DC. Springerlink Publishers, p. 401.

Chowdhury, P. and Ray, R. C. (2005). Fermentation of Jamum (*Syzgium cumini L.*) fruits to form red wine. ASEAN Food J. 14 (1): 15 – 23.

Cullimore, D. R. (2000). Practical Atlas for Bacterial Identification. Florida. CRC Press, p. 209.

Harley, J. P. and Prescott, L. M. (2002). Laboratory Exercises in Microbiology. 5th Ed. New York. Mac Graw Hill, p. 449 .

Imade, E. E. (2012). Microbiological and physico-chemical analysis of microbial profile and enzyme involved in the bioconversion of soursop (*Annona muricata)* must to wine. Ms.c Thesis, Dept. of Microbiology, University of Benin. Benin City.

Iredale, J. P. (2003). Cirrhosis: new research provides a basis for rational and targeted treatments. British Med. J. 327: 143 – 147**.**

Lutchmedial, M., Ramlal, R., Badrie, N. and Chang-Yen, I. (2004). Nutritional and sensory quality of stirred soursop (*Annona muricata L.*) yoghurt. Internat. J. Food Sci. Nutri. 55: 407–414.

McCarthy, M. (1994). Measurement of Titratable Acidity and pH. Nutrition information research centre, South Australian Research and Development Institution p. 1-19.

Morton, J. F. (1987). Fruits of warm climates. Miami. Coral Gables Publishers, p. 780.

Okigbo, R. N. (2009). Mycoflora and production of wine from fruits of soursop (*Annona Muricata* L.) Internat. J. Wine Res. 1: 1 – 9.

Osuide, G. E. (1999). Post-harvest losses implication for food quality and safety, In: Elemo G. N, (Ed.) Post harvest loss reduction. Proceedings of the 23rd Annual Conference of Nigeria Institute of Food Science and Technology, Abuja, Nigeria. P. 270–272.

Roberts, D. and Greenwood, M. (2003). Practical Food Microbiology, 3rd Ed. Oxford. Blackwell Publishing, p. 296.

Sharma, P. (2009). Manual of Microbiology, tools and techniques. New Delhi. Ane books. Pvt. Ltd., p 405.

Umme, A., Salmah, Y. and Asbi, B. A. (1997). Microbial changes in natural soursop puree during storage. Food Chem. 63: 315–322.

Van der Walt, J. P. (1970). Genus 16, *Saccharomyces,* pp. 732-745. In: Lodder J. (ed). The yeasts, a taxonomic study: North-Holland Publishing Company, Amsterdam.