Original Research Article

PRODUCTION, PERSERVATION AND SHELF-LIFE EVALUATION OF WINE FROM BANANA FRUIT (*Musa Acuminata*)

ABSTRACT

Aims: The investigation focused on production, preservation and shelf-life study of wine from banana fruit (*Musa acuminata*).
Study design: This work is based on completely randomized design with two replications and the average values calculated for mean comparison.
Place and Duration of Study: Food and Industrial Microbiology laboratory, Department of Microbiology, University of Port Harcourt, Nigeria, September, 2018 to March, 2019.
Methodology: Analyses performed using standard methods were microbiological, physicochemical and sensory evaluations. Sodium benzoate concentrations of 5 and 25 ppm were used for shelf life studies. Banana 'must' was analyzed at 4 day intervals for 12 days while produced wine was analyzed at 5 day intervals for 25 days during storage.
Results: Changes in total heterotrophic counts (THCs), total coliform counts (TCCs) and fungal counts (FCs) occurred during fermentation, resulting in maximum THCs of 5.02, TCCs of 3.60 and FCs of 8.87 $\log_{10}$ cfu ml^{-1} on days 4, 4 and 8 respectively. *Acetobacter* and *Saccharomyces* were pronounced in wine without preservative (control) throughout storage. Mean pH of 'must' was 5.8±0.30 while alcohol content was 0.28±0.03% on day 0 but as fermentation progressed, mean pH was reduced while mean alcohol content increased. Mean pH of wine preserved with 5 ppm varied slightly throughout storage but mean pH of control and 25 ppm preserved wine decreased from 3.7±0.20 on day 0 to 3.2±0.23 on day 25. Sensory attributes (overall acceptability) on day 12 was most preferred while during shelf-life studies, significant difference in overall acceptability of the different wines at $p<0.05$ occurred. Wine preserved with 5 ppm had the best organoleptic quality but 25 ppm preserved wine showed the most acceptable microbial quality.
Conclusion: Findings show that banana is a good substrate for wine production and 5 ppm sodium benzoate retained the qualities of the wine.

KEY WORDS: Banana fruit, *Musa acuminata*, production, preservation, shelf-life.

1. INTRODUCTION

Banana is an edible fruit produced by several kinds of large herbaceous flowering plants that belong to the genus *Musa.* In some countries, bananas used for cooking may be called plantains, in contrast to dessert bananas, which are eaten raw [1]. It is an important staple fruit in Nigeria. Banana serve as a good nutritional source of carbohydrates, mineral such as potassium and vitamin, including B1, B2, B12, C and E. [2]. The banana fruit can be eaten raw or cooked (deep fried, dehydrated, baked in its skin or steamed), processed in flour, jam, juice or fermented for the production of beverages such as beer (e.g. Mbege brewed by Chagga people in Kilimanjaro region of Tanzania), vinegar and wine [1].

Banana has a short shelf-life under the prevailing temperature and humidity conditions in tropical countries, including Nigeria. This results in wastage of fruits as a result of poor handling and inadequate storage facilities [3]. Adequate microbiological knowledge and handling practices of these produce would therefore help minimize wastes due to deterioration and unacceptability [4]. Fermenting banana juice into wine or converting it into other derivatives is considered an alternative means of utilizing surplus banana, since the consumption of these products provides a rich source of vitamins and ensures harnessing of the fruits into useful by-products [5, 6]

Therefore the present work was undertaken to produce wine from banana fruit and to determine the effects of two different concentration of sodium benzoate (5ppm and 25ppm) on the micro-biological, physicochemical and shelf-life studies, including sensory evaluations.

2. MATERIAL AND METHODS

2.1 Sample Collection

Mature ripe banana fruits (*Musa acuminata*) (2.5 kg) were purchased from Rumuomasi Market in Obio Akpor Local Government Area, Rivers State and were transported to the Food and Industrial Microbiology Laboratory, Department of Microbiology, University of Port Harcourt, under aseptic conditions.

2. 2 Isolation of *Saccharomyces cerevisiae* from banana fruit

Small portions of the banana fruit were first washed sterile water and peeled. The portions were extracted by blending the pulp with aliquots of water (100 ml) and filtering into a sterile bottle using a sterile muslin cloth. The bottle was then corked and left to stand for 5 days [7]. The fermented juice was then prepared for the yeast isolation by aseptically making a homogenate of it (suspending 10 ml of the juice in 90 ml sterile saline), and diluted serially in physiological saline (0.85%) to the 10^{-6} dilution. Aliquots (0.1 ml of appropriate dilutions) were spread plated aseptically on freshly prepared yeast extract glucose peptone agar (YEGPA) plates in duplicate. The plates were then incubated at 28^{o}C for 3-5 days. After incubation, yeast colonies with morphological properties (creamy, round, smooth and glistening) typical of *Saccharomyces cerevisiae* were purified on fresh YEGPA plates and stored in potato dextrose agar slants until further analysis.

2.3 Characterization of *Saccharomyces cerevisiae*

The tentative *Saccharomyces cerevisiae* isolates were characterized on the basis of their microscopic, biochemical and molecular properties. Prior to characterization, stock cultures of the isolates were subcultured onto YEGPA plates to resuscitate them.

2.4 Microscopic characterization

This was carried out to determine the properties of the isolates under the microscope. The properties observed were the cell shape, presence of spores and spore characteristics (if any). The cells were stained using a simple methylene blue dye staining technique before microscopic examination. This involved emulsification of a small portion of the yeast culture on clean, grease-free slides with a loopful of water. The smear was allowed to air-dry and then heat fixed. The heat-fixed smear was stained with crystal violet and allowed to stand for 1 min. The smear was then gently rinsed with water and allowed to dry after which, the slide was examined under oil immersion objective.

2.5 Biochemical characterization

This was carried out to determine the biochemical properties of the isolates. This included starch hydrolysis, urea hydrolysis, nitrate reduction and carbon (glucose, sucrose, maltose, galactose, lactose and fructose) utilization test. [8]

2.6 Molecular characterization

The genetic property of the yeast isolates, which is the measure of their nitrogenous base pairing pattern, was determined using the molecular approach. The steps involved during this characterization method include deoxyribonucleic acid (DNA) extraction, polymerase chain reaction (PCR) amplification, agarose gel preparation, agarose gel electrophoresis, 18 subunit ribosomal ribonucleic acid (18S rRNA primer), sequence analysis and construction of phylogenic tree [9, 10, 11]

Banana Fruits (2 kg)

Washing and Peeling

Juice Extraction

Filtration (Muslin cloth)

Must

Fortification (0.4 g Sodium metabisulphite, 100 g granulated sucrose, 29.4 g ammonium sulphate, 4.2 g potassium dihydrogen phosphate).

Pasteurization (60^{o}C for 15 min.).

Pitching (5 ml 3.0 x 10^{8} cfuml^{-1} *S. cerevisiae*)
Fermentation (28^{o}C for 12 days)

Wine Extraction (Whatman filter paper)

Addition of Preservative (5 ppm and 25 ppm Sodium benzoate)

Bottling and Pasteurization (63^{o}C for 30 min.)

Shelf-Life Study (25 days)

Figure 1: Wine production process from banana fruit and shelf-life study

2.7 Microbiological Analysis

The microbiological quality of the banana 'must' at different intervals during wine production, and that of the produced wine, at different intervals during shelf-life study were carried out using Plate Count Agar (PCA) for total heterotrophic counts, Mac Conkey Agar (MCA) for total coliform counts and potato dextrose Agar (PDA) for total fungal counts.

2.8 Physicochemical Analysis

This analysis was carried to determine the physicochemical quality of the banana 'must' at different intervals (days 0, 2, 4, 6, 8, 10 and 12) during wine production. The produced wine was subjected to shelf-life studies involving evaluation of its physicochemical properties at intervals of 5 days for 25 days. The parameters that were tested included: pH, reducing sugar content (%), alcohol content (%), titratable acidity (ppm) and specific gravity. These parameters were determined according to the method described by AOAC [2].

2.9 Sensory Evaluation

This was carried out to determine the sensory attributes (colour, taste, aroma and intensity) and general acceptability of the banana 'must' during production and the produced wine. In carrying out this evaluation, a panel of 10 persons was given the wine samples to rate using the 9-point hedonic sensory scale [12]) after tasting. Their ratings were recorded as "like extremely" (9), "like very much" (8), "like moderately" (7), "like slightly" (6), "neither like nor dislike" (5), "dislike slightly" (4), "dislike moderately" (3), "dislike very much" (2), "dislike extremely (1) [12].

3. RESULTS AND DISCUSSION

3.1 Isolated *Saccharomyces cerevisiae* from Banana Fruit

The cultural, microscopic and biochemical characteristics of the yeast isolates obtained from the banana sample is presented in Table 1. *Hanseniaspora* species were isolated alongside the *Saccharomyces cerevisiae*. They were all positive for starch and urea hydrolysis, negative for nitrate reduction, but showed variations in the fermentation of sugars.

Table 1:-Cultural, microscopic and biochemical characteristics of yeast isolated from banana fruit.

Isolate code	Macroscopy	Microscopy	Isolation medium	Starch hydrolysis	Urea hydrolysis	Nitrate reduction	Carbon source							Fungal species
							Glucose	Sucrose	Maltose	Galactose	Lactose	Fructose	Dextrose	
Y_1	Creamy, round, flat, smooth and glistening yeast like colony	Budding spherical yeast cells with no pseudohyphae	YEGPA	+	+	-	+	+	+	+	-	+	+	*Saccharomyces cerevisiae*
Y_2	Creamy, smooth and glossy yeast-like colony with a slightly raised center	Budding and ovoidal cells with one or two ascospores formed per ascus	YEGPA	+	+	-	+	-	-	-	-	+	+	*Hanseniaspora* sp.
Y_3	Round whitish and shiny yeast-like colony	Budding yeast cells with apiculate zygote and	YEGPA											*Hanseniasp*

persistent asci	+	+	-	+	+	-	-	-	+	+	*ora uvarium*

YEGPA = Yeast extract glucose peptone agar. + = positive reaction; - = negative reaction.

The gel electrophoresis result for the isolated yeasts species in the study is presented in Plate 1, while the phylogenetic characteristics of the evolutionary relationship between the fungal isolates and their accession numbers are presented in Figure 2 and Table 2 respectively. The amplified internal transcribed space (ITS) bands of two of the isolates (*Hanseniaspora* sp. and *Hanseniaspora uvarium*) had similar (Plate 1) number of nitrogenous base pairs (slightly above 500 bp control), while that of the third isolate (*Saccharomyces cerevisae*) has a lower number of nitrogenous base pairs than the 500 bp control. The phylogenic analysis (Figure 2) indicated that the fungal isolates were 100% evolutionarily related.

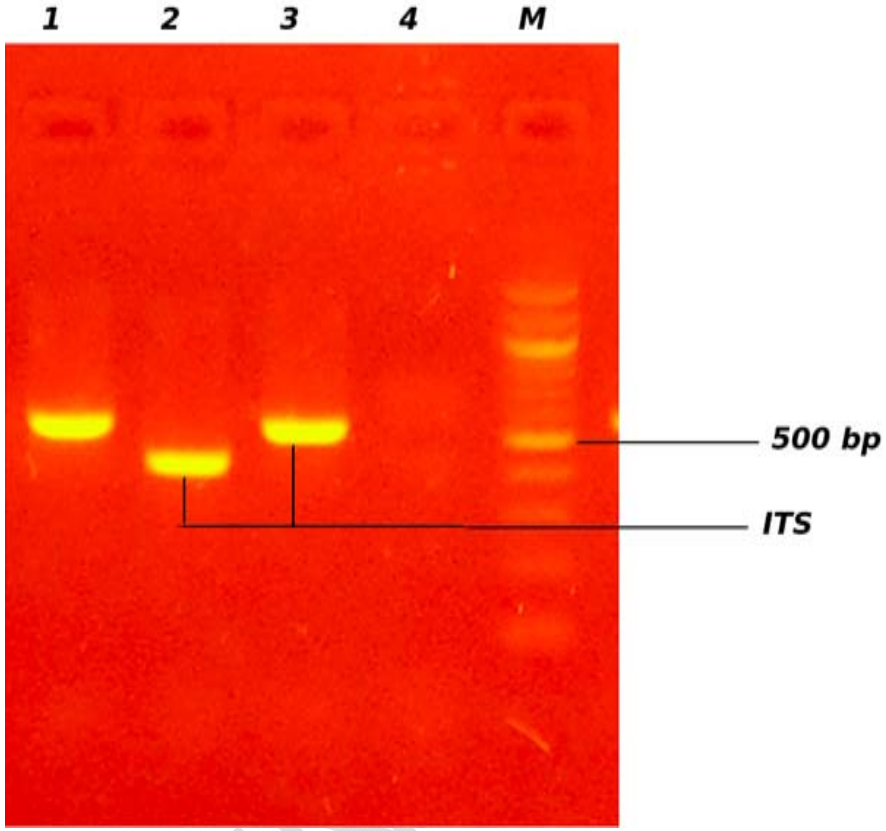

Plate 1:- Agarose gel electrophoresis of the amplified internal transcribed space (ITS) bands of the fungal isolates. Lanes 1, 2, 3 represent the ITS bands, lane 4 represents the negative control while lane M represents the 100 bp molecular ladder.

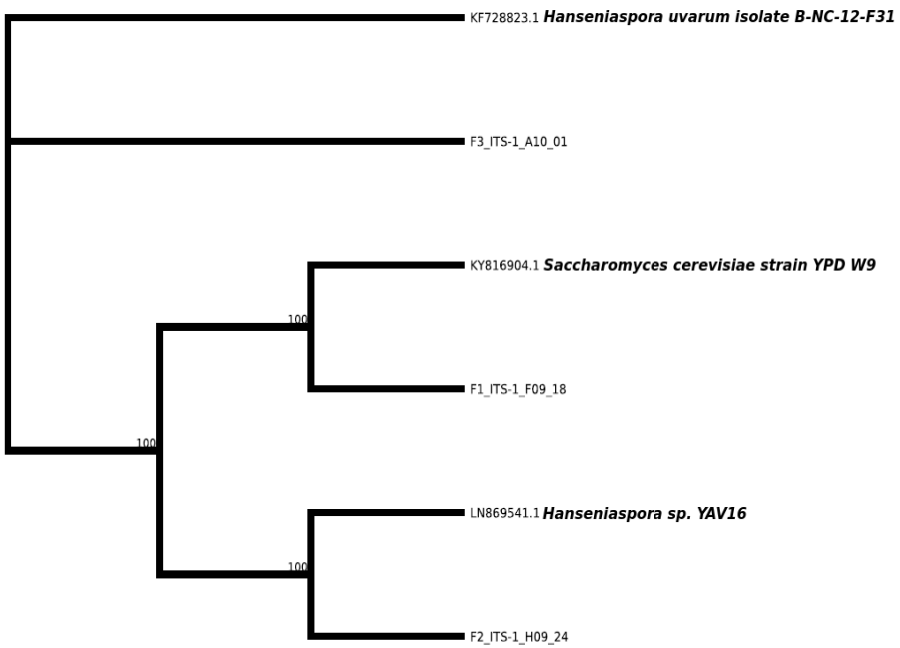

Figure 2:- Phylogenetic characteristics of evolutionary relationship between the fungal isolates.

F1: *Saccharomyces cerevisaie* YPD W9; **F2:** *Hanseniaspora* sp. YAV 16
F3: *Hanseniaspora uvarum* B-NC-12-F31

3.2 Colonial, physiological and biochemical characterization of bacterial isolates from banana 'must'

The colonial, physiological and biochemical characteristics of bacteria isolated from the ‘must’ during wine production are presented in Table 2.

Bacteria species	Occurrence at different days	Shape	Colour	Isolation medium	Size (mm)	Elevation	Texture	Morphological (Gram stain reaction)			Spore formation	Motility	Catalase	Citrate	Indole	MR	VP	Oxidase	Starch Hydrolysis	TSI			Sugar fermentation		
								Stain	**Shape**	**Arrangement**										**H_2S**	**Acid**	**Gas**	**Glucose**	**Lactose**	**Sucrose**
Aerococcus	+(1)	Circular	Milky	PCA	2.5	Raised	Soft	+	Cocci	Tetrad	-	-	-	+	-	-	-	+	+	-	+	-	+(-)	+(-)	+(-)
Hafnia	+(4)	Circular	Cream	MCA	1.5	Raised	Soft	-	Rods	Singly	-	+	+	-	-	+	+	-	+	-	+	+	+(+)	+(+)	+(+)
Lactobacillus	+(4)	Circular	Cream	PCA	2	Raised	Soft	+	Rods	Short chains	-	-	-	-	-	-	-	-	+	-	+	-	+(-)	+(-)	+(-)

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Acetobacter	+(4)	Circular	Pink	PCA	2	Raised	Soft	-	Rods	Singly	-	+	+	+	-	-	-	-	+	-	+	+	+(+)	+(+)	+ (+)
Citrobacter	+(8)	Circular	Cream	MCA	2	Raised	Soft	-	Rods	Pairs	-	+	+	+	-	+	-	-	+	-	+	+	+(+)	+(+)	+ (+)
Staphylococcus	+(2)	Circular	Golden yellow	MSA	2	Raised	Soft	+	Cocci	Cluster	-	-	+	-	-	-	-	-	+	-	+	+	+(+)	+(-)	+ (-)

Table 2:- Colonial, morphological (Gram stain reaction) and biochemical characteristics of the bacterial isolates obtained from banana 'must' during wine production.

PCA = Plate count agar; MCA = MacConkey agar; MSA = Mannitol salt agar; MR = Methyl red; VP = Voges Proskauer; TSI = Triple sugar iron agar; H_2S = Hydrogen sulphide.
+ = positive reaction; - = negative reaction; +(-)= acid and no gas production and +(+)=acid and gas produced.

3.3 Physicochemical properties of 'must' during wine production

The physicochemical properties of the banana 'must' at different days during wine production are presented in Table 3. The pH decreased from 5.8±0.30 on day 0 to 3.7±0.03 on day 14; reducing sugar decreased from 15.25±3.01% on day 0 to 1.31±0.07% on day 14; alcohol content increased from 0.28±0.03% on day 0 to 7.63±1.77% on day 10 but further reduced to 6.88±0.83% on day 14; acidity increased from 14.9±1.09 ppm on day 0 to 44.2±3.65 ppm on day 14 and specific gravity reduced from 0.76±0.02 on day 0 to 0.34±0.22 on day 10 and increased to 0.38±0.54 on day 14.

Table 3: Physicochemical properties of the banana 'must' on different days during wine production.

Days	pH	Reducing sugar (%)	Alcohol content (%)	Acidity (ppm)	Specific gravity
0	5.8±0.30	15.25±3.01	0.28±0.03	14.9±1.09	0.76±0.02
2	5.1±0.23	10.4±1.01	2.45±0.11	33.56±3.32	0.59±0.05
4	4.6±0.67	6.24±1.75	4.57±0.38	33.56±1.11	0.59±0.28
6	4.1±0.39	4.88±0.92	6.86±0.66	38.27±2.67	0.46±0.69
8	3.9±0.11	3.24±0.21	7.57±1.39	41.61±4.44	0.39±0.11

10	3.9±0.01	2.51±0.06	7.63±1.77	41.44±3.39	0.34±0.22
12	3.7±0.03	1.33±0.04	7.01±0.67	43.7±0.87	0.35±0.56
14	3.7±0.04	1.31±0.07	6.88±0.83	44.2±3.65	0.38±0.54

Each value represents the average ± standard deviation of duplicate values.

The Pearson's correlation coefficient (*r*) between microbial counts and physicochemical properties of banana 'must' during wine production are presented in Table 4.

Table 4: Pearson's correlation coefficient (*r*) between microbial counts and physicochemical properties of Banana 'must' during wine production.

Parameters	THC	TCC	TFC	pH	Reducing sugar	Alcohol content	Acidity	Specific gravity
THC	1							
TCC	0.950081158	1						
TFC	0.095916595	0.309241087	1					
pH	-0.025404983	-0.170532379	-0.955935444	1				
Reducing sugar	-0.086977437	-0.203621666	-0.929654484	0.99417838	1			

Alcohol content	0.118995239	0.291306416	0.986219314	-0.987911759	-0.97707002	1		
Acidity	0.100813369	0.234924087	0.951619142	-0.996864063	-0.99781079	0.988991869	1	
Specific gravity	-0.171053689	-0.392240704	-0.99503165	0.927726879	0.901158484	-0.97265207	-0.92785	1

THCs = Total heterotrophic bacteria counts; **TCCs =** Total coliform counts; **TFCs =** Total fungi counts.

3.4 Sensory attributes of 'must' during wine production

The result obtained for the sensory rating of the banana 'must' during wine production is presented in Table 5. The overall acceptability of the 'must' had the highest rating on day 12 of fermentation. Also, there was a significant difference in the overall acceptability of the banana 'must' on the different days of fermentation at *P*=.05.

Table 5: Sensory characteristics of the banana 'must' at different days during wine production.

Sensory attributes	Day 0	Day 4	Day 8	Day 12
Colour	2.40±0.03[a]	4.50±0.31[b]	6.20±0.11[c]	7.20±0.44[d]
Taste	6.10±0.12[a]	2.90±0.02[b]	5.90±0.23[c]	7.70±0.02[d]
Aroma	2.40±0.04[a]	3.60±0.52[b]	6.30±0.23[c]	8.00±0.22[d]
Intensity	1.60±0.12[a]	3.40±0.23[b]	5.50±0.54[c]	7.20±0.31[d]
Overall Acceptability	2.00±1.82[a]	3.42±0.70[b]	6.02±0.33[c]	7.54±0.41[d]

Each value represents the mean ± standard deviation of duplicate determinations. Values in rows with different superscripts for the different days during wine production are significantly different at P=.05.

3.5 Microbial counts during shelf-life studies

The total heterotrophic bacteria, total coliform and total fungal counts during shelf-life studies are presented in Figure 3. For the control wine, fungal were the most predominant (7.69 $\log_{10}$ cfu ml^{-1}), followed by heterotrophic bacteria (2.78 $\log_{10}$ cfu ml^{-1}) and lastly, the coliforms (1.41 $\log_{10}$ cfu ml^{-1}) on day 0.

Wine preserved with 5 ppm sodium benzoate had fungal as the most predominant (7.25 $\log_{10}$ cfu ml^{-1}) microbial group, followed by heterotrophic bacteria (2.05 $\log_{10}$ cfu ml^{-1}) on day 0 and lastly, coliforms (0.48 $\log_{10}$ cfu ml^{-1})on day 0.

For the wine preserved with 25 ppm sodium benzoate, fungal were the most predominant (3.95 $\log_{10}$ cfu ml^{-1}), followed by heterotrophic bacteria (0.85 $\log_{10}$ cfu ml^{-1}). Coliforms were not detected throughout the shelf-life study.

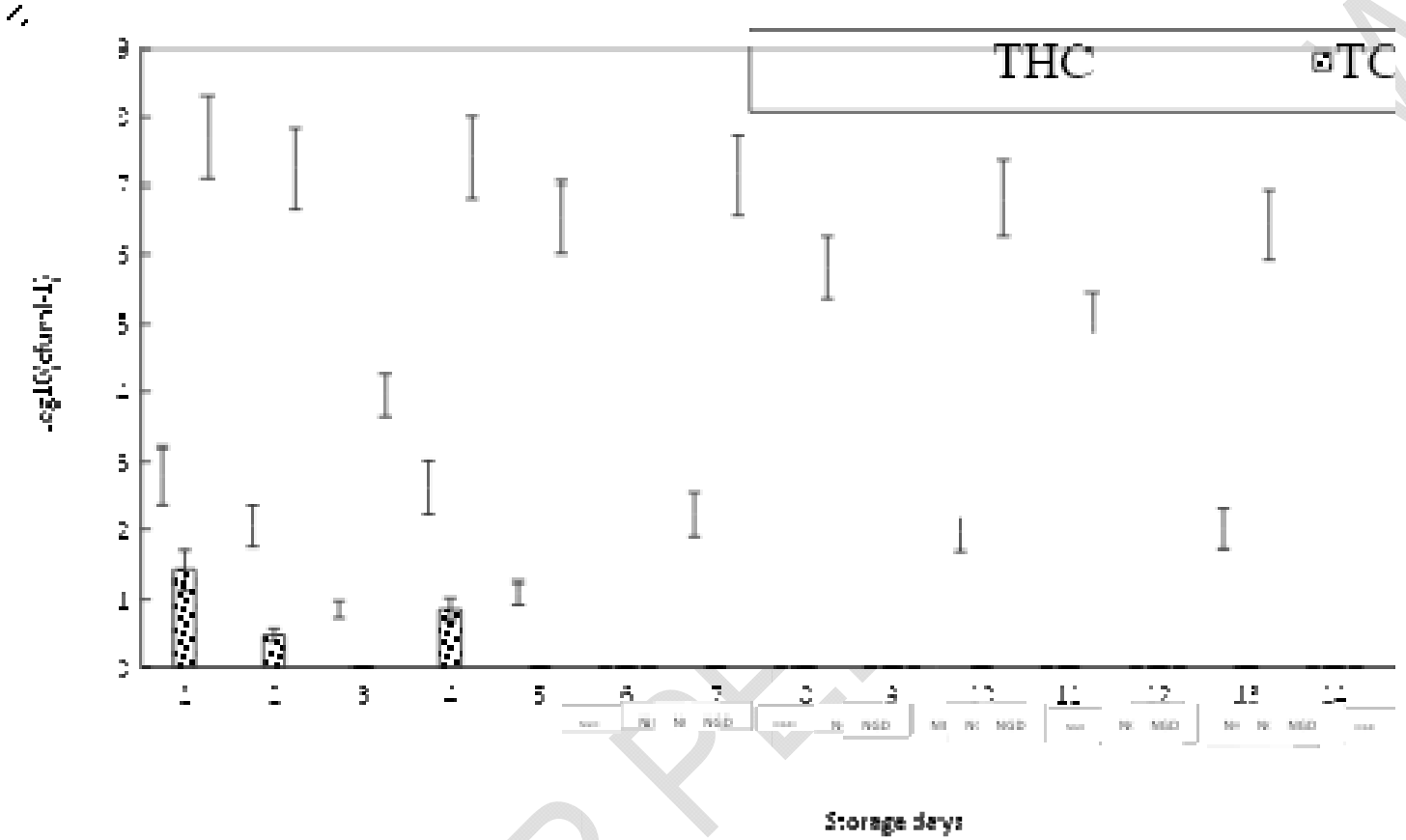

Figure 3: Microbial counts of different microbial groups of the different banana wine preserved with 0, 5 and 25 ppm sodium benzoate at different shelf-life days. **THC =** Total heterotrophic count; **TCC =** Total coliform count; **TFC =** Total fungi count; **NGD** = No growth detected.

Error bars represent mean ± standard deviation of duplicate determinations

The physicochemical properties of the banana wine during the shelf-life studies are presented in tables 6, 7 and 8.

Table 6:- Physicochemical properties of the control banana wine on different days during shelf-life studies

Days	pH	Reducing sugar (%)	Alcohol content (%)	Acidity (ppm)	Specific gravity
0	3.7±0.20	1.31±0.30	6.88±0.22	44.2±1.51	0.43±0.02
5	3.4±0.13	0.39±0.05	7.29±0.59	45.1±0.61	0.47±0.02
10	3.3±0.10	0.25±0.02	7.11±0.34	45.4±0.23	0.49±0.05
15	3.3±0.00	0.19±0.01	7.03±0.44	45.8±1.01	0.53±0.07
20	3.2±0.24	0.18±0.03	6.94±0.67	45.9±0.44	0.55±0.01
25	3.2±0.23	0.15±0.01	6.92±0.83	46.2±0.76	0.59±0.02

Each value represents the average ± standard deviation of duplicate determinations.

Table 7:- Physicochemical properties of the banana wine with 5 ppm sodium benzoate on different days during shelf-life studies

Days	pH	Reducing sugar (%)	Alcohol content (%)	Acidity (ppm)	Specific gravity
0	3.7±0.04	1.31±0.48	6.88±1.32	44.2±0.15	0.43±0.09
5	3.7±0.02	1.19±0.33	7.10±0.76	44.6±0.33	0.39±0.04
10	3.7±0.04	1.13±0.05	7.33±0.22	44.7±0.81	0.36±0.02
15	3.7±0.01	0.87±0.01	7.35±0.44	44.7±0.77	0.33±0.02
20	3.7±0.03	0.85±0.04	7.35±0.12	44.7±0.63	0.33±0.02
25	3.7±0.02	0.85±0.02	7.35±0.63	44.8±0.38	0.32±0.05

Each value represents the average ± standard deviation of duplicate determinations

Table 8:- Physicochemical properties of the banana wine with 25 ppm sodium benzoate on different days during shelf-life studies

Days	pH	Reducing sugar (%)	Alcohol content (%)	Acidity (ppm)	Specific gravity
0	3.9±0.03	1.31±0.04	6.88±0.23	42.7±1.45	0.61±0.17
5	3.8±0.00	0.93±0.07	7.63±0.04	43.4±0.33	0.48±0.02
10	3.8±0.01	0.93±0.02	7.63±0.01	43.4±0.02	0.48±0.03
15	3.8±0.04	0.93±0.03	7.63±0.01	43.4±0.05	0.48±0.05
20	3.7±0.03	0.92±0.03	7.63±0.02	43.4±0.09	0.48±0.01
25	3.7±0.01	0.92±0.01	7.63±0.01	43.4±0.04	0.48±0.03

Each value represents the average ± standard deviation of duplicate values.

3.6 Sensory Evaluation of banana wine during shelf-life studies

The results obtained from the sensory evaluation of the banana wine during shelf-life studies are presented in Table 9. There was significant difference at *P*=.05 in the overall acceptability of the different wines during the shelf-life study.

Table 9: Sensory attributes of the banana wine at different days during shelf-life studies

Days	Sensory attributes	CSL	SLD1	SLD2
0	Colour	7.80±0.09[a]	7.50±0.45[a]	7.60±0.04a
	Taste	7.70±0.02[a]	7.20±0.06[a]	6.60±0.61[b]
	Aroma	8.00±0.11[a]	7.80±0.77[b]	7.10±0.06[b]
	Intensity	7.20±0.34[a]	7.20±0.12[a]	7.20±0.32[a]
	Overall Acceptability	6.16±0.42[a]	7.28±0.41[b]	6.94±0.55[a]
5	Colour	8.20±0.06[a]	7.70±0.07[b]	7.70±0.07[b]
	Taste	8.10±0.13[a]	7.10±0.61[b]	6.40±0.45[c]
	Aroma	8.30±0.69[a]	7.60±0.04[b]	6.80±0.33[c]
	Intensity	7.80±0.15[a]	7.30±0.09[a]	7.10±0.07[a]
	Overall Acceptability	7.98±0.33[a]	7.26±0.44[a]	6.90±0.57[b]
10	Colour	7.70±0.33[a]	7.70±0.11[a]	7.70±0.21[a]
	Taste	7.40±0.03[a]	7.40±0.62[a]	6.50±0.30[b]
	Aroma	7.70±0.09[a]	7.40±0.07[a]	7.00±0.85[a]
	Intensity	8.20±0.42[a]	7.50±0.55[b]	7.60±0.71[b]
	Overall Acceptability	7.74±0.29[a]	7.28±0.46[a]	7.04±0.60[a]

Table 9 Continued

Days	Sensory attributes	CSL	SLD1	SLD2
15	Colour	7.80±0.23[a]	7.60±0.45[a]	7.60±0.01[a]
	Taste	6.30±0.06[a]	7.40±0.01[b]	6.20±0.20[a]
	Aroma	7.50±0.46[a]	7.40±0.21[a]	7.10±0.09[a]
	Intensity	7.60±0.22[a]	7.50±0.91[a]	7.30±0.32[a]
	Overall Acceptability	7.26±0.59[a]	7.28±0.44[a]	6.90±0.62[b]
20	Colour	8.10±0.55[a]	7.70±0.78[b]	7.80±0.45[b]
	Taste	5.70±0.62[a]	7.10±0.34[b]	6.30±0.23[c]
	Aroma	7.60±0.04[a]	7.10±0.18[a]	6.90±0.31[b]
	Intensity	7.20±0.21[a]	7.30±0.04[a]	7.40±0.04[a]
	Overall Acceptability	7.12±0.90[a]	7.10±0.51[a]	6.96±0.64[b]
25	Colour	8.20±0.22[a]	7.70±0.31[b]	7.90±0.13[b]
	Taste	4.40±0.08[a]	7.20±0.18[b]	6.10±0.03[c]
	Aroma	7.20±0.61[a]	7.00±0.06[a]	7.10±0.75[a]
	Intensity	7.40±0.44[a]	7.20±0.82[a]	7.10±0.66[a]
	Overall Acceptability	6.60±1.44[a]	7.06±0.55[b]	6.96±0.67[c]

Each value represents the mean ± standard deviation. Values in rows with different superscripts for the different wine types during shelf-life studies are significantly different at P=.05.
CSL = Wine without preservative; **SLD1 =** Wine preserved with 5 ppm sodium benzoate; **SLD2** = Wine preserved with 25 ppm sodium benzoate.

Tropical fruits as substrates for the production of wines have been a subject of research for some years [13, 14, 15]. Several factors influence the microbiological, physicochemical and organoleptic qualities of wine.

The results of this research indicate the marked increase in the microbial load of the different microbial groups during the wine production stage with maximum occurrence in total fungal count of 8.87 $\log_{10}$ cfu ml^{-1}. According to Columbie *et al.* [16], the growth of microorganisms in any given food ecosystem is greatly enhanced by the presence of readily available carbon and nitrogen sources. Therefore, this rapid

increase in the different microbial groups within the first few days of fermentation (during the wine production) could be attributed to the metabolism of the readily available carbon (sucrose, fructose and glucose) and nitrogen sources (nitrates and amino acids) in the banana must. This was clearly illustrated by the correlation coefficient (*r*) (Table 4) of the populations of the different microbial groups and reducing sugar content. Most yeasts including *Saccharomyces cerevisiae*, ferment a variety of sugars to produce ethanol [7, 17]. The yeasts metabolized the carbon sources in the 'must' most effectively into biomass and alcohol as indicated by the strong inverse correlation coefficient value between the reducing-sugar and fungal biomass (r = -0.93) and reducing-sugar and alcohol-content (r = -0.98) (Table 4). Although there was an increase in microbial loads as production progressed up to day 4, for both total heterotrophic counts (THCs) and total coliform counts (TCs), and on day 8 for total fungal counts (TFCs), their population reduced on day 8 for both THCs and TCCs and on day 12 for TFCs with the reduction in sugar content and increase in alcohol content and acidity. Additionally, from day 8, there was a total elimination of *Staphylococcus aureus*, and this indicated that the fermentation medium had become unfavourable for this organism as a result of increase in alcohol and titratable acidity content [18].

The increase in wine acidity during production was as a result of the apparent increase in the metabolism of some acid-producing bacterial groups (*Hafnia, Acetobacter, Citrobacter,* and *Lactobacillus*) which fermented the sugars or alcohols into various organic acids such as acetic acid, lactic acid and others [17]. This increase in titratable acidity of the wine during production contributed to the reduction in its pH as shown by the strong inverse correlation between pH and acidity (r = -0.997) (Table 4). However, during the wine production stage, organic acids produced by bacteria were due to the metabolism of sugars to alcohol as indicated by a moderate positive correlation coefficient value (r = 0.48) (Table 4) between alcohol content and acidity. The antimicrobial activity of organic acids lies in their ability to dissociate. As these acids permeate into the cytoplasm of microbes, they dissociate and make the pH of the cellular environment to drop due to the excessive release of H^+ within the microbial cell [5]. This eventually leads to death [19].

Following the increase of ethanol and organic acids and the reduction of sugar content during the fermentation process of the banana must, the fermentation medium became adverse due to high alcohol content of 7.63±1.77% on day 10 and high acidity of 44.2±3.65 ppm on day 14 for the different microbial populations present in the 'must'. These resulted in a drastic reduction in their population and change in microbial community dynamics. Thus, in some cases, the total elimination of some of these microbial

populations such as *Aerococcus* on day 4, *Hafnia* and *Staphylococcus* on 8 day as fermentation progressed. This indicates the wine's characteristics in controlling some pathogenic and spoilage microorganisms like *Staphylococcus*, *Hafnia* and *Citrobacter* [18, 20].

During production, there was a significant decrease (P=.05) in the overall acceptability of the wine. The most preferred acceptability however, was obtained on the day 12 of production given that all the sensory attributes of the wine had the highest mean ratings by the panel. The overall acceptability of the wine was most preferred by the panel on that day probably as a result of decreased reducing sugar content and increased alcohol content. The changes in the physicochemical properties as reported by Binning and Possman [21] of wine produced from apple using a consortium of indigenous yeast species present in the fruit compare favourably with the physicochemical properties of the banana wine produced in this study.

The shelf-life extension of the produced wine was achieved using two different concentrations (5 ppm and 25 ppm) of sodium benzoate and the effects of these concentrations on the sensory properties of the wine were also evident. Sodium benzoate is a 'Generally Regarded as Safe' (GRAS) chemical preservative which in very low concentration inhibits the activity of microbes (fungi and bacteria) in many food products [22] and may impart a slight tang in their taste at low pH which is as a result of undissociated benzoic acid [19]. The two concentrations extended the shelf-life of the produced wine but the use of 5 ppm concentration of the preservative was preferred. The introduction of sodium benzoate (5 ppm and 25 ppm) impacted greatly on the microbial quality of the wine and the sensory attributes. Wine preserved with 25 ppm sodium benzoate inhibited all the microbial groups by day 5 while that preserved with 5 ppm inhibited all microbial groups by day 20. The two concentrations of the preservative also had similar activities on coliforms and fungi. The 25 ppm sodium benzoate concentration was more effective in inhibiting all microbial groups thus extending shelf-life more effectively than the 5 ppm concentration. This is because at this concentration, the microbial populations are exposed to the H^+ released as a result of the dissociation of the acid at a faster rate. Although the 25 ppm concentration performed better at shelf-life extension than 5 ppm concentration, wine preserved with the 5 ppm concentration of sodium benzoate was preferred on the basis of sensory properties. This is as a result of the slight tang taste given to the wine at 25 ppm concentration. This observation on the use of preservatives is in agreement with the work reported by Laplace *et al.* [23] on apple wine. They observed that not only was the wine preserved, the taste and nutritional content were affected.

The change in pH in the wine preserved with 25 ppm sodium benzoate however, did not affect the activity of the preservative because sodium benzoate exhibits poor antimicrobial activity when pH is above 5.5 and is ineffective in neutral pH [24].

In the wine without the preservative at the early stages of shelf-life monitoring, microbial population progressed at a much reduced rate due to the depleted sugar concentration. As a result of this, the environment of the wine gradually became more selective to the microbial groups (Figure 2) due to the continuous increase of alcohol and titirable acidity with the sharp decrease in pH, which resulted from further fermentation of the sugars. A continuous drop in alcohol content and increase in acidity of the wine from day 15 indicated the metabolism of alcohol by the microbes into organic acids. The correlation coefficient value (r= -0.56) between these two parameters also demonstrated their moderate inverse relationship. This change in metabolism was due to the action of the acid-producing bacteria species – *Acetobacter,* given that it was the only bacteria species isolated during the period. During this period, due to the depletion of reducing sugars, this organic acid producing organism changed its metabolism such that it utilized the alcohols produced during fermentation as carbon sources to yield organic acids [18, 20]. *Saccharomyces cerevisiae* occurred throughout the shelf-life study but its population also kept declining due to carbon depletion and other unfavourable conditions [20]. The wine without sodium benzoate treatment (control) tasted a little bit sour/tarty at the end of shelf-life studies as a result of increase organic acid, hence, its significant difference at P=.05 in overall acceptability with the wines with 5 ppm and 25 ppm sodium benzoate (Table 9).

4. CONCLUSION

Banana fruit 'must' is generally a good substrate for the production of wine that is nutritionally rich and this process can be very helpful in curbing the loss of the fruit. Particular attention should be paid to quality during the wine production process to minimize the contaminations that may arise. Due to the high nutritional composition of the 'must', microbial contaminants tend to proliferate at high rates and may persist through the entire fermentation process causing some undesirable effects in the resulting wine. The use of sodium benzoate as the study indicates is good for preserving banana wine giving that the properties of the wine (microbiological, physicochemical and sensory) maintained good standards when treated with 5 ppm and 25 ppm

of the preservative. However, the study demonstrated that the lower concentration of the preservative (5 ppm) retained the qualities of the wine.

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