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Original Research Article

The Physicochemical and Microbiological Characteristics of Fermented *Hibiscus sabdariffa* Calyxes Using Probiotics Starter Cultures

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ABSTRACT

Zobo produced from aqueous calyxes extract of Roselle in Nigeria is a non-alcoholic local beverage widely consumed by people across different socio-economic classes. However, rapid deterioration in quality and spoilage remains a major concern for the large-scale production of zobo. Thus, this research aimed to assess the sensory evaluation and physicochemical attributes of zobo fermented with probiotic cultures of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* isolated from Hebron yoghurt and smooth cayenne pineapple in a bid to extend its shelf-life. During fermentation of the drink by *Lactobacillus fermentum*, the pH, titratable acidity, vitamin C and total dissolved solids were within the range of 3.47 – 2.76, 0.236-0.252%, 2.44 - 5.22 mg/100 g, 9.97 – 11.95 mg/L while the equivalent values for wine fermented by *Saccharomyces cerevisiae* were 3.48-2.8, 0.2 - 0.252%, 6.33 – 8.56 mg/100 g and 9.71 – 13.32 mg/L, respectively. The alcohol content of the drinks increased steadily during the fermentation process. In the end, 5. 71% and 5.61% alcohol content was recorded for the drink fermented by *L. fermentum* and *S. cerevisiae*, respectively. The results of the sensory evaluation on a 7-point hedonic scale show that the wine produced from fermentation by *Saccharomyces cerevisiae* was moderately accepted at a value of 3 while that of *Lactobacillus fermentum* had a value of 3.10 with less acceptance from the panel. The mean heterotrophic microbial load of *Saccharomyces cerevisiae* being $3 \pm 0.2 \times 10^3$ CFU/ml and *Lactobacillus fermentum* $5 \pm 0.2 \times 10^3$ CFU/mL indicated that the formulated drink may be safe for consumption.

Keywords: *Hibiscus sabdariffa*, Calyxes, *Saccharomyces cerevisiae*, Zobo, Vitamin C, Probiotics.

Introduction

Hibiscus sabdariffa is an underappreciated ornamental plant found in the subtropical and tropical regions of West, East, and Southeast Asia. The calyx of *Hibiscus sabdariffa* has a beautiful anthocyanin pigment with strong antioxidant properties.[25] Various nutritional and phytochemical bioactive constituents such as vitamins, phenolics and flavonoids are embedded in its flower which can be used to combat cancer, hypertension, and inflammatory diseases. These compounds can trigger peristalsis in the intestine, induce milk production and milden blood viscosity.[21, 28] *Hibiscus sabdariffa* is used as a purple colourant and flavouring agent in sauce, jam, jelly, wine, confectionery, juice, syrup, pudding, cake, ice cream, tea, marmalade, and chocolate to improve flavour, aroma, and overall acceptance.[38,45] Other uses of plant components (particularly calyxes) include the production of jam, non-alcoholic beverages, and tea.[8,30]

Zobo drink is a non-alcoholic beverage locally prepared by housewives or those on low income as a means of getting extra income to help meet ends. It is commonly sold in restaurants, parks, markets, and school premises, especially during the dry season when the temperature is fairly hot.

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It is a casual drink best served chilled as refreshment during marriage and child naming ceremonies as well as in festival periods in Nigeria.[4] Being a local beverage, the drink is prepared in different ways known to each culture and this can range from boiling to soaking of the sepals of the Hibiscus in distilled water and sweetened by either sugar, pineapples, or any sweetener of choice. The drink can be flavoured with ginger and lemon before refrigeration and consumption. Zobo has shown potential as a source of vitamin C, proteins, and carbohydrates.[29,14] Wine has previously been made from the juice of the zobo flower (Hibiscus sabdariffa) using yeast isolated from palm wine. The fermentation of zobo flower juice yielded the highest alcohol production of 2.6% (v/v).[39] Zobo drinks though commonly consumed in Nigeria across geopolitical and religious groups has not been successfully produced at a commercial scale. This is associated with its short shelf life attributable to microbial activity. This short shelf life of the drink associated with degradation of the nutrient component erodes the health benefits of the rich vitamin C and antioxidant property of Zobo.

In 2016, Nigeria had spent about N9 billion on the importation of champagne (sparkling wine) alone. High duty on imported wines stimulated interest in the promotion of our local drinks (kunu and zobo) in the Presidential villa.[40] However, in 2010, the importation of wine and spirits from the U.S had grown from $120,000 in 2008 to $2.7million. In 2019, Michael Ani and Segun Adams published in the Business Day the decline in the importation of wine by 24%.[6] However, production of zobo drink in commercial volume is hindered by the rapid deterioration of the drink with shelf life (storage) of approximately twenty-four (24) hours following production if not refrigerated, which is attributed to the presence of spoilage microbes. Thus the objective of this research is to extend the viability of the drink by fermentation into red wine using probiotic organisms.

Probiotics are live bacteria and yeasts that have been shown to have a variety of health advantages. They're often termed as "good" or "friendly" bacteria and are commonly added to yoghurts or taken as dietary supplements.[32] Examples of common probiotics are strains of lactic acid bacteria; Lactobacillus and fungi; *Saccharomyces cerevisiae* also known as 'brewer's yeast.

Lactobacillus fermentum is present in probiotic foods such as yeast and kimchi, and it contains two potent superoxides, such as dismutase and glutathione, which are antioxidants. These antioxidants assist digestion and boost immunity by neutralising digestive toxins in the stomach.[19] *Saccharomyces cerevisiae* is a commonly used model organism because it is a quickly reproducing eukaryote that has helped scientists better understand molecular, cellular, and metabolic processes, as well as the pathophysiology and potential treatments for many human diseases. In good to ideal conditions, S. cerevisiae reproduces at rates comparable to bacterial cells as single-celled organisms.[17]

In light of this, this study focused on assessing the sensory evaluation and physicochemical attributes of zobo fermented with probiotic cultures of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* isolated from Hebron yoghurt and smooth cayenne pineapple in a bid to extend its shelf-life.

Materials and Methods

Collection of sample

Calyxes of *Hibiscus sabdariffa* for wine production were obtained from suppliers in Ota, Ogun State and verified at the biology laboratory of Covenant University, Ota in October 2018. Similarly, samples of yoghurt, corn gruel and queen pineapple were acquired for the isolation of *Lactobacillus fermentum* and *Saccharomyces cerevisiae*. The Hibiscus calyxes were employed in the fermentation process for wine production, with sugar added as both a carbon source and sweetener.

Microbiological analysis

Isolation of probiotic cultures

The probiotic cultures were isolated and characterized using De Man Rogosa and Sharpe Agar (MRS) and Potato Dextrose Agar (PDA) prepared according to manufacturer's guidelines. Aliquots (0.1ml) of the dilution factors 10^{-3}, 10^{-5}, and 10^{-9} were plated aseptically on MRS agar and PDA. PDA plates at room temperatures and MRS agar plates placed in an anaerobic jar with 5% CO_2; were both incubated for 3 days. Isolates were examined for shape, size, pigmentation, and then sub-cultured until pure colonies were obtained.

Microbiological culture

During fermentation, aliquots were plated on PDA and MRS agar at intervals of 0, 12 and 24 hours to determine the variations of organism growth used in fermentation**.**

Characterization of the isolates

The cultural, morphological, biochemical, and physiological characteristics of isolated species were employed to identify the species of the organisms. The tests performed included Gram staining,[11] oxidase,[44] catalase,[42] indole,[26] urease and citrate test,[16,47] endospore,[23] as well as the ability of the organisms to ferment sugar[43] were employed to determine the identity of the organism.

Preparation for calyx extract fermentation

Manual sorting was employed in dirt removal from hibiscus calyxes before 100g was weighed in and masked in three different foil paper and sterilized using the autoclave at 121°C for 15 minutes. Weighed and sterilized calyx (100g) were introduced into 1000ml of sterile distilled water in three different reaction flasks and allowed to stand, to hasten extraction. Thereafter, using a sterile muslin cloth, the settled extract was filtered before the introduction of 100g of refined sugar to each portion to stimulate fermentation. The suspension was sterilized using the autoclave at 121°C for 15 minutes to eliminate any microorganism that must have been introduced during preparation.

The three formulations were labelled A, B, and C. Formulation A was covered without microbial inoculation, whereas Formulations B and C were inoculated with 1 ml aliquot of the fermenting cultures of *Saccharomyces cerevisiae* and *Lactobacillus fermentum*, respectively, before corking.

These were incubated at 25^0C for 24 hours. Samples were taken intermittently at 12-hour intervals to assess the physicochemical properties. The drinks were generated were resolved and clarified with gelatin.

Physicochemical analysis

Bijoux bottles were appropriately labelled and used to collect and hold the fermentations at intervals 0, 12 and 24 hours for the three different formulations to prevent disruption of the main fermentation formulation.

Vitamin C

The vitamin C contents of the drink formulations were determined by iodine titration[22] using 1% starch indicator, iodine and vitamin c standard solutions. 25ml of formulation samples A, B and C was added to a 125ml Erlenmeyer flask and the iodine solution was added till the endpoint was reached which is when you get the blue colour that lasts longer than 20 seconds. This process was carried out at a 12-hour interval.

pH

Apparatus and reagents needed for assessing the pH of the formulation include a pH meter with an electrode and a Standard buffer solution of different graded level of pH 4.0 (Acidic), 7.0 (Neutral) and 9.0 (basic). The pH meter was calibrated by inserting the electrode into the graded level of standard solution by using the manual. Triplicate readings of the meter were observed, and the mean of the readings was recorded.

Assessment of total titratable acidity

The total titratable acidity (TTA) of the samples was assayed using the titrimetric method.[24] Fifteen (15) millilitres of each formulation were obtained, and deionized sterile water (100mls) was added. This was titrated against 0.1 equivalent weight NaOH solution, while phenolphthalein drops were introduced to act as an indicator, till there was a change to pink colouration.

$$\% \text{ Total Titratable Acidity} = \frac{A \times N}{W} \quad \ldots\ldots\text{equation 1}$$

where A is vol. of NaOH used; N is Equivalent weight (Normality) of NaOH; and W is Mass of formulations used.

Determination of total dissolved solids (TDS)

Total dissolved solids of the samples were assessed following the gravimetric determination procedure:[12] 50 cm^3 of the samples were weighed, filtered before it was turned into a clean, dry and pre-weighed evaporating dish and placed in the oven at the temperature of 105 ^{o}C till the sample was dried completely. It was placed inside a desiccator and weighed until the constant weight was achieved and noted.

$$\text{Total Dissolved Solids Calculation} = \frac{(w_3 - w_1)mg \times 1000}{Mass\ (mg) of\ water\ sample} \quad \ldots\ldots\text{equation 2}$$

Alcohol content

The formula for the alcohol content calculation was therefore computed using the specific gravity method.[13]

Alcohol content by vol. (%) = 131.25 × (Initial Gravity - Final Gravity)

Statistical analysis

The Pearson correlation coefficient 2-tailed (a = 0.01) was performed to evaluate the significance of differences between pH, acidity,

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vitamin c, total dissolved solids and alcohol content, using Microsoft Excel Version 2020 (where $P < 0.05$ and $P < 0.01$ shows significance).

Organoleptic assessment

Organoleptic assessment for all formulations was carried out according to the methods of Maragatham.[27] A panel of five judges familiar with sensorial attributes of wine were selected. The judges consisted of three men and two women aged between 23 and 30. The panel members elected according to their availability and interest, assessed the drinks using the 7-hedonic scale with value 1 as "like extremely" and value 7 as "dislike extremely".

Results and Discussion

Characterization of isolates

The colonial morphology and biochemical characteristics of the probiotics isolated from their respective food source revealed the presence of *L. fermentum* and *S. cerevisiae* (Table 1).

Gram-positive organisms were identified by purple colouration after staining due to the ability of the organism to conserve the crystal violet stain within its cell wall, used in the Gram staining method of identification. The indole negative organisms were identified by the formation of a yellow-coloured ring in the alcohol layer (colour of Kovac's reagent). Catalase negative organism was identified as catalase-negative due to the absence of gas bubbles when mixed with hydrogen peroxide while catalase-positive organism was identified as catalase-positive due to the production of gas bubbles when tested. The citrate negative organisms that do not utilize citrate as a source of carbon hence causing no growth on the media were identified by the non-change of the Koser citrates' medium from green to blue. The utilization of sugar by both organisms resulted in a colour change of media from red into yellow which happened as a result of acid production by the organisms and the production of gas in the Durham tubes. The oxidase negative organisms were identified by the absence of a purple colouration which indicates the presence of an oxidase enzyme in a positive reaction. Urease negative organisms were not able to change the colour of the media from yellow to orange to pink due to the absence of the enzyme urease to break down urea present in the media. Observation of the organisms for spores under the microscope revealed the absence of spore formation for both organisms. The presumptive biochemical identification of the organisms identified the two organisms to be *Lactobacillus fermentum* together with *Saccharomyces cerevisiae* and these outcomes were compiled in Table 1.

Physicochemical analysis

Physicochemical parameters including pH, total dissolved solids, specific gravity, titratable acidity, vitamin C and alcohol contents were analysed during the experimental phase. For physicochemical parameters, pH, Vitamin C and Total Dissolved Solids reduced with an increase in fermentation time while percentage acidity increased as shown in Table 2. For the pH, values ranged from 3.50 to 2.79 for sample A, 3.48 to 2.80 for sample B and 3.47 to 2.76 for sample C. For Vitamin C, sample A gave 10.54 to 4.20 mg/100 ml, while samples B and C ranged between 10.5 to 6.33 mg/100 ml and 10.53 to 2.44 mg/100 ml respectively. For titratable acidity sample A gave 0.195 to 0.225%, while sample B and C ranged between 0.2 to 0.225% and 0.236 to 0.252% respectively. Alcoholic content was not detectable in sample A, while sample B and C had 5.65% and 5.71% respectively (Table 2). The average pH was lowered over 24 hours from 3.5 to 2.79. Titratable acidity measuring a product's acid level[1] increased in Formulation A through 24-hour time (0.195-0.225%). This agrees with the high acid content often observed in roselle drink which is a naturally acidic fruit endowed in organic substances with low pH: succinic acid, oxalate, malate and tartaric acid.[50] Formulations B and C demonstrated a gradual increase in acidity. For formulation B, the pH value decreased from 3.48 to 2.80 and the percentage acidity increased from 0.2 to 0.225 while for formulation C, the pH decreased from 3.47 to 2.76 while the titratable acidity increased from 0.236 to 0.252.

Table 1: Morphological and biochemical properties of the probiotic isolates

	L. fermentum	***S. cerevisiae***
Colony morphology	Rough, round	Raised, Oval
Cell shape	Rod	oval
Gram staining	+	Nil
Catalase test	-	+
Oxidase	-	-
Urease test	-	-
Citrate utilization	-	-
Sucrose utilization	+	+
Lactose fermentation	+	+
Glucose fermentation	+	+
Gas production	+	+
Spore staining	-	-
Indole test	-	-

Table 2: Physiochemical properties of the three different drink formulations

Parameters **Formulation**	**Fermentation Time (hr)**	**pH**	**Titratable Acidity (%)**	**Vitamin C (mg/100 ml)**	**Total Dissolved Solids (mg/L)**	**Alcohol Content**
	0	3.50	0.195	10.54	14.31	Not Detected
A	12	3.39	0.210	6.54	11.31	Not Detected
	24	2.79	0.225	4.20	5.16	Not Detected
B	0	3.48	0.200	10.50	13.31	Not Detected
	12	3.37	0.215	6.34	12.31	2.7
	24	2.80	0.225	6.33	9.71	5.65
C	0	3.47	0.236	10.53	11.95	Not Detected
	12	3.30	0.239	2.60	10.52	2.9
	24	2.76	0.252	2.44	9.97	5.71

Key: A = Calyx extract + 100g sugar
B = Calyx extract + 100g sugar + *Saccharomyces cerevisiae*
C = Calyx extract + 100g sugar + *Lactobacillus fermentum*

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These observations are similar to an experiment carried out on roselle wine in which the pH gradually reduced and titratable acidity increased at the end of ageing by Alobo and Offonry.[3] The rise in titratable acidity and concomitant fall in pH in formulations B and C can be linked to the fermentative effect of the yeast and lactic acid bacteria in converting the sugars to produce organic acids.[36] Acidity, which determines wine quality and affords an environment that inhibits potential spoilage microbes but promotes the growth of desirable microorganisms, is essential to wine fermentation.[9]

For the three different formulations, the alcohol content at the beginning of the fermentation was not detected, A (without any starter culture pre-inoculated), B (with *Saccharomyces cerevisiae* as starter culture) and C (with *Lactobacillus fermentum* as starter culture). However, as time progressed, it slowly increased to 5.65 and 5.71% for formulation B and C respectively while Formulation A did not change because it was not inoculated with a starter culture. These observations agreed with Archibong and Okafor.[9,37] Microorganisms undergo metabolic activities during fermentation, leading to metabolites release including organic substances with low pH. This could be linked with the alcohol content increase observed across the different formulations. The low alcohol content is similar to work done by Yokotsuka where they observed that *Hibiscus sabdariffa* calyces carry minute amounts of alpha-ketoglutaric acid, pyruvic acid and ethanol which normally react with SO_2 to result in bisulphate complexes in fermentation, leading to the low alcohol content after fermentation goes to end.[51] Total soluble solids measure the sugars domiciled in the fermented must as well as wine. Total dissolved solids showed marked reduction across the different setups with formulation A having a reduction of 14.31-12.00 mg/L, formulation B (13.31-9.71 mg/L) and formulation C (11.95-9.97 mg/L). The observed reduction can be linked to microbial activities that metabolize the fermentable sugars into acids. In comparison to formulation A, the total dissolvable solid in formulations B and C was lower. This could be because formulations B and C were pre-inoculated with starter cultures. This is in line with Efiuvwevwere and Eka's report, as well as Nwafor and Ikenebomeh.[20,33]

All the formulations had a significant reduction in vitamin C concentration with formulation A having a reduction of 10.54-4.20mg/100ml, formulation B (10.50-6.33mg/100ml) and formulation C (10.53-2.44mg/100ml). Heat, light, and oxygen exposure have all been shown to improve vitamin C loss. The observed reduction could be attributed to oxidation, which occurs in fruit juices during storage and is particularly reliant on the amount of oxygen in the headroom or dissolve in the samples.[41,48]

Microbial load of formulations A, B and C

The total viable count for formulation A was not detected, formulation B had a viable count of 2, 3 and 5 CFU/ml and formulation C had a viable count of 3, 5 and 7 CFU/ml at 0, 12 and 24 hours respectively (Table 3). Formulation A had a viable count of zero due to the absence of microorganisms, formulation B had an increasing viable count of 2, 3 and 5 $\times 10^4$ CFU/ml and formulation C, 3, 5 and 7 $\times 10^4$ CFU/ml and this could be as a result of hygienic conditions in place during the production of the drinks.

The drink made from the extract of *Hibiscus sabdariffa* (Roselle drink) using *Saccharomyces cerevisiae* isolated was successfully developed in this study. Since the wine produced is a fruit fermented and undistilled product, it is expected that most of the nutrients originally found in the Roselle drink,-base is also present in the wine produced.[52] During the winemaking process, the result showed that no culturable microorganism was present in the fermenting at 0 hours whereas 12 hours and 24 hours, only yeast cells were detected which decreased from mean yeast count of 2.77 ×– 2.25 × 10^4 CFU/ml. The absence of culturable microorganisms in the 'must' could be attributed to the pasteurization of the 'must'. In a related study that involved the production of watermelon wine, Zainab and her colleagues reported that bacteria and coliforms were not detected in the fermenting Roselle drink except yeast cells which increased from 0 - 5.0 × 10^7 CFU/ml within the fermentation period.[52] In this study, undetected bacteria in the fermenting extract of *H. sabdariffa* throughout the fermentation period is an indication that the finished product is safe for human consumption.

It was reported that 2 × 10^4 CFU/ml was the mean count of bacteria in the fermenting 'must' during Roselle winemaking (wine made from the extract of *H. sabdariffa*) which they generally considered to be of no significance.[7] Also, reported in the study was 2 × 10^4 CFU/ml spore count in the fermenting extract. Meanwhile, during the production of Roselle wine, Zhang et al. (2018) reported an increase in viable count in the fermenting 'must' from 1.64 × 10^6 CFU/ml at 0 hours to 8.80 × 10^8 Cfu/ml at 12 hours. A recent study reported that juice from sweet orange (*Citrus sinensis*) demonstrated a remarkable inhibition against clinical bacteria species isolated from wounds namely *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* which they attributed to the presence of secondary metabolites.[10] The result from that study collaborated with the findings reported by Anitha which involved testing the inhibitory properties of sweet orange juice against bacteria wound isolates, namely *Klebsiella pneumonia, Proteus mirabilis, Acinetobacter baumannii,* and *S. aureus*.[7]

Table 3: Total Viable Count of the three formulations

Parameters Formulation	Fermentation Time (Hr)	Total Viable Count (Cfu/Ml)
A	0	Not Detected
	12	Not Detected
	24	Not Detected
B	0	2
	12	3
	24	5
C	0	3
	12	5
	24	7

Table 4: Statistical Analysis of physicochemical properties of drinks after clarification

Physicochemical Properties Statistics	Ph	Acidity (%)	Vitamin C (Mg/100ml)	Total Dissolved Solids (Mg/L)	Alcohol Content (%)
STDEV	0.09	0.01	1.92	2.74	3.28
Count	3.00	3.00	3.00	3.00	3.00
Standard error of Mean (SEM)	0.05	0.01	1.11	1.58	1.89
Degrees of Freedom	2.00	2.00	2.00	2.00	2.00
Hypothesized Mean	2.50	0.25	3.00	7.00	3.00
T-statistics	0.13	4.44	1.08	0.80	0.42
P-value	0.91	0.05	0.39	0.51	0.72

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Both reports suggested that the sweet orange juice used in this study to prepare red wine influenced the absence of nonculturable bacteria in the fermenting extract. The low pH of the fermenting extract also may have contributed to the non-detection of bacteria due to the inhibition of the growth of some pathogenic microorganisms.

At pH below 3.5, only a few microorganisms involved in the fermentation process survived whereas most microbes were eliminated.[15] Critically, the increasing concentration of alcohol in the fermenting 'must' most likely created an unconducive environment for microorganisms to thrive with the exception of yeast cells which decreased in population between 12 hours and 24 hours. Bacterial growth was inhibited by alcohol by plasmolyzing the cell wall of the bacteria. The possible reasons for the decrease in the population of yeast cells as reported in their study include high cell density, depletion of nutrients, suspected presence of toxic metabolic byproducts as well as rupturing of cell membrane of the yeast cells by increasing concentration of alcohol.[35]

Physicochemical analysis result of formulations after clarification

Similar values to results before clarification for pH, Vitamin C, total dissolvable solids (TSA) and alcohol content were obtained after clarification. However, there was a slight increase in titratable acidity in Formulation C (Figure 1). The results of the statistical analysis carried out on physicochemical analysis are displayed in Table 4.

Organoleptic Evaluation Results

From the result of the organoleptic evaluation, formulation B was adjudged best by the taste panel with a 3-point value, with formulations A and C scoring 3.30 and 3.10, respectively on a 7-point Hedonic scale (Figure 2).

Formulation B was the most acceptable wine to the panellists, as well as the most preferred in terms of product preference.

In terms of the sensory features studied among the test wines, Formulation B was the most acceptable wine to the panellists, as well as the most preferred in terms of product preference. Sensory analysis of the wine produced indicated that the mean sensory score for colour is interpreted as 'like slightly'. The taste and overall acceptability of the red wine produced are interpreted as 'like moderately' were higher than the values assigned to the same sensory parameters for both imported wine and the wine produced in Nigeria. Although the interpretation of the sensory score for the flavour of the red wine produced (Like slightly) is the same as Baron de Vall (imported wine), the mean sensory score was slightly higher. The overall sensory analysis of wine produced in comparison with Baron de Vall and Concorde wine indicated that the wine made from sweet orange juice and extract from *H. sabdariffa* using palm wine yeast (*S. cerevisiae*) is preferable to the two brands of wines evaluated in this study. An extract from *H. sabdariffa* for wine production possibly influenced higher sensory scores for the alcoholic beverage compared with Concorde wine and Baron de vall wine. This report is in agreement with a similar study.[2] The preference of the wine produced over the two brands of wine already commercialized could also be attributed to many volatile and non-volatile compounds released into the product which gives it a typical taste and odour. The concentration of ethanol in the alcoholic beverage will determine the extent to which the olfactory system will perceive the volatile compounds released.[18]

The relationship between titratable acidity and pH demonstrated in this study is such that as the pH of the fermenting must decreases, the titratable acidity increases. This occurrence could be attributed to the accumulation of organic acids during the fermentation process. Different researchers have also reported a similar trend in studies involving fruit winemaking.[35] Considering the relationship between reducing sugar, alcohol content and specific gravity, this study reveals that as the reducing sugar decreases due to increased utilization of sugar by the yeast cells, the concentration of alcohol released increases which result in decreases in the specific gravity.

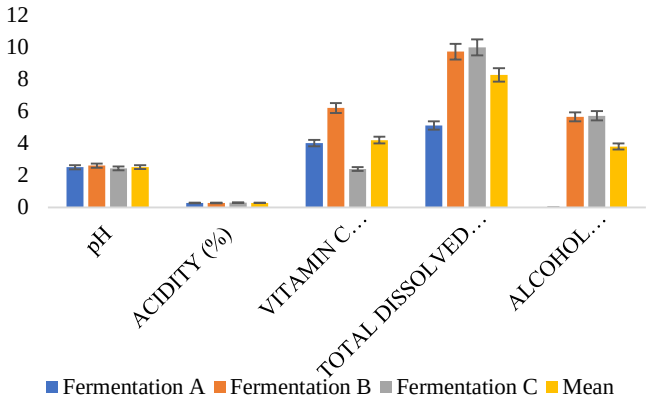

Figure 1: Physiochemical properties of drinks after clarification

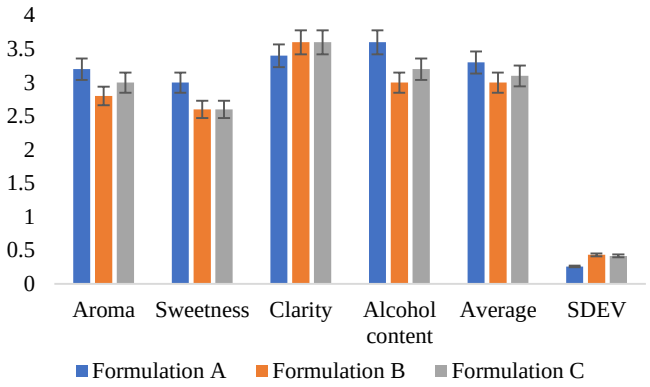

Figure 2: Organoleptic evaluation of wine

Conclusion

The results reveal that *Saccharomyces cerevisiae* and *Lactobacillus fermentum* were able to ferment *H. sabdariffa* red calyxes extract to make a drink that is safe to consume. The physicochemical qualities of the drink made from *H. sabdariffa* calyces were acceptable, with a reduction in pH, an increase in total titratable acidity, a decrease in carbohydrate value, and a decrease in total soluble solids. Furthermore, the results of this study present *Saccharomyces cerevisiae* as a better probiotic to be employed in the manufacture of wine from aqueous extract of *Hibiscus sabdariffa*.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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