

Comparative Study of Physicochemical and Bacteriological Characteristics of Banana Wines Produced by Conventional and Modern Techniques in Southern province of Rwanda

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Abstract This study aimed to evaluate the physicochemical and bacteriological characteristics of different types of banana wine (Urwagwa) produced in Southern province of Rwanda. Both conventional (not bottled i.e from Bulinga (B) and Gahogo (GA)) and modern produced banana (bottled i.e Igisubizo from Ruli (I) and Ganzinyota from Ruvumera (G)) wines were analyzed and compared. Results showed that the alcohol content (v/v) of I, G, B and GA was 25.9% , 26.1%, 12.0% and 40.1 % , respectively while their total soluble contents were 15.2%, 12.2%, 21.7% and 21.8%, respectively. Titratable acidity (g/L) varied from 5.29 to 6.95 while pH varied from 4.45 to 3.30. The volatile acidity was found to be 0.49, 0.040, 0.147 and 0.19 g of acetic acid for wines I, G, B and GA, respectively. The turbidity was significantly higher in conventional wines than in modern ones. *Sulfur dioxide content*, heavy metals and minerals respected the standard norms however, potassium content (mg/L) was higher in wines B (1207) and GA (1107,21) and Pb was not recognized in all samples. Furthermore, wines GA accounted higher *Escherichia coli* (3×10^7 cfu/ml) and *Staphylococci aureus* (1.5×10^7 cfu/ml). In conclusion, these results indicated that the safety of un-bottled wine was far lower compared to that of bottled ones.

Keywords: banana wine, physicochemical analysis, safety, processing techniques

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1. Introduction

Indigenous food fermentation is one of the oldest food biotechnological processes which is dependent on the biological activity of microorganisms [1]. Fermented beverages constitute a major part of the diet of traditional African rural and peri-urban homes, including Rwanda [2].

Bananas (*Musa sapientum*) are important staple starchy foods in African countries especially in Rwanda. This plant can be used as direct food (green and no bitter banana), as fruits (sweet and ripe banana) or to produce wine [3]. Banana wine commonly known as "urwagwa" is one of the oldest and major alcoholic beverage produced in most East African countries. It is mostly consumed on three quarters of Rwandan territory, where banana growing is a major agricultural activity [4]. It is produced from banana juice extracted from a variety of local bananas including harsh tasting Igikashi and the milder tasting Kamara, and blended with sorghum flour to

improve the color and flavor of the final product. However, banana wine from undiluted juice is regarded as very special and has a higher price than that from diluted banana juice, because of its strongest [2].

The processing of banana wine "Urwagwa" is still traditional in most parts of Rwanda due to the lack of appropriate instruments and this poor processing leads to different health issues [5]. A wine fault or defect is an unpleasant characteristic of a wine often resulting from poor winemaking practices or storage conditions, and leading to wine spoilage [6]. There are many causes for the perception in wine faults: (i) poor hygiene at the winery, (ii) excessive and/or insufficient exposure of the wine to oxygen and to sulphur, (iii) overextended maceration of the wine either pre- or post-fermentation, (iv) faulty fining, (v) filtering and stabilization of the wine, (vi) the use of dirty oak barrels, over-extended barrel aging and the use of poor quality corks. Outside of the winery, other factors within the control of the retailer or end user of the wine can contribute to the perception of flaws in the wine [7].

Furthermore, depending on the method and materials used during processing and storage, banana wine presented different contamination risks including chemical and microbial contamination [8]. Therefore, it is highly recommended to monitor and control the processing of banana wines in order to insure the health of consumers. The aim of this study was to evaluate the physico-chemical and bacteriological properties of banana wines produced in Southern province of Rwanda.

2. Materials and Methods

2.1. Conventional Processing of Banana Wine

Fifty kilograms of ripen banana were washed in tap water and pilled with hands. Five liters of water were added to the pilled banana and macerated with hand to produce banana juice. After the maceration process, the juice was filtered through five layers cheese cloths. Then, the filtered juice was not further diluted. Two kilograms of sorghum flour were added to the filtered banana juice to induce the fermentation and no enzymatic fermenters were added. Sorghum flour helped to improve the color and flavor of the final product [9,10]. The mixture was stored at 25°C for 5 days, and the banana wine was filtered and ready for consumption. This process was given and executed by producers from Gahogo and Bulinga. Banana wines (Igisubizo and Ganza) were purchased from local supermarkets. The collected samples were stored at 4°C prior to further analysis. The control sample was prepared according to Rwanda Bureau of Standard (RBS).

2.2. Determination of Total Alcohol Content

The alcohol contents of the banana wine were taken using the procedures adapted from Woo [11]. Briefly, 100 mL of the sample was run through a distiller until around 70 mL was collected. The collected sample was set to 100 mL with distilled water and the alcohol content (%) was measured using an alcohol hydrometer (Triple scale hydrometer, France). The alcohol-temperature correction table was used with the sample's alcohol content and temperature.

2.3. pH, Total Acidity, and Total Soluble Solid Content

The pH was measured by using a HI 9126W-01 pH Thermo (Thermo Electron Co., Beverly, MA, USA). After measuring the pH, 10 mL of the sample was combined with the indicator phenolphthalein and titrated with a 0.1 N NaOH solution. The amount of NaOH (mL) was then converted to tartaric acid. The total soluble solid content was determined using a digital Refractometer (Model Dr-103L, Bellingham Stanley Ltd., Sturbridge Wells, UK) and measured in Brix (°Bx).

2.4. Determination of Turbidity

The turbidity of wine was measured using a Bentonite Photometer (Hanna Instruments HI 83749, USA). The

turbidity was expressed in nephelometric turbidity units (NTU).

2.5. Determination of Free Sulfur Dioxide

Free SO₂ was determined according to Zoecklin [12]. Fifty milliliters of sample were placed into a 250 mL Erlenmeyer flask then 5 mL of starch, indicator solution and 5 mL of sulfuric acid solution were added. The mixture was titrated with 0.020 N iodine solutions. The endpoint is indicated by the first bluish color persisting for about 30 seconds. The content of free sulfur dioxide was calculated according to the following formula:

$$\text{Free SO}_2 = \frac{V_I \times N_I \times 32000}{V_s} \quad (1)$$

Where, Free SO₂= expressed in ppm

N_I=concentration of iodine used for titration

V_I = volume of standard Iodine used for titration, in mL

V_s= volume of sample.

2.6. Determination of Volatile Acidity

Volatile acidity (VA) is a measure of acetic acid in wine and is an indication of spoilage. Two hundred milliliters of each sample were placed in 500 mL distillation flask containing about 25 mL of distilled water and a few pieces of pumice stone. The obtained mixture was distilled for 35 min and collected in 200 mL volumetric flask till a volume of 50 mL was recuperated. The distillate was left to cool at room temperature and three drops of phenolphthalein were added. Directly after, the mixture was titrated against standard 0.1 N NaOH and result was expressed as acetic acid grams per liters through the following formula [13]:

$$\text{VA} = \frac{V_B \times N_B \times 0.060 \times 1,000}{V_s} \quad (2)$$

Where, VA= Volatile acidity expressed in g acetic acid/L

N_B= concentration of standard NaOH used for titration

V_B = volume of standard NaOH used for titration, in mL

V_s= volume of sample

2.7. Determination of minerals and Heavy Metals

Minerals were determined according to the methods described by Enidiok [14]. Sixty milliliters of sample were transferred into a conical flask of 150 mL and 5 mL of concentrated HNO₃ was added. The mixture was slowly heated to boiling. The boiling was maintained till a small volume of about 15 mL remained. The solution was filtered and minerals, Ca, K, Mg, Na, Fe, Co, Cr and Pb, were determined by Atomic Absorption Spectrometry (AAnalyst, 200, Perkin Elmer, USA). The obtained values were expressed in mg/L.

2.8. Bacteriological Analysis

2.8.1. Culture Media and Other Materials

For this test, specific culture media were used. Salmonella and Shigella were tested on Salmonella and

Shigella agar (SSA) [Biolab, Hungary], *Staphylococcus aureus* tested on Mannitol Salt Agar (MSA) [Biolab, PHEUR-USP], *Escherichia coli* was tested on Peptone water [OXOID, England] in which agar [HiMedia Laboratories Pvt, HIMEDIA RM026-500G, USA] was added while Total coliform was tested on Lactose Broth culture media [HiMedia Laboratories Pvt, HIMEDIA, M 1003-500G, USA] mixed with agar. All specific media, physiological saline (solution 0.09% NaCl), petri dishes, test tubes and all sterilizable required materials were sterilized at 121°C for 15 min.

2.8.2. Assessment of the Presence of Bacteria

The bacteriological analysis was done in cleaned and sterilized fume hood to avoid contamination during experiment. Around 20 mL of hot culture media were poured in sterilized Petri dishes for cooling. After solidification, one milliliter of a given sample was spread on surface of the solid media and left to dry. The number of micro-organisms in different sampled wines was determined by serial dilutions for traditional made wines while those made through improved technology were analyzed without any dilution. The traditionally made wines were 10^6 times diluted prior to facilitate counting process. Thus, all plates were incubated under aerobic conditions at 37°C for 24-46 hours. The mean number of colonies counted was expressed as log of colony forming units (\log_{10} cfu/ ml)

2.9. Statistical Analysis

All statistical analyses were performed using SPSS version 19 (IBM, Cary, NC, USA). Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared by using turkey test. Significance was defined at $P \leq 0.05$ level.

3. Results and Discussion

3.1. Alcohol Content of Different Banana Wines

The alcohol contents of the analyzed banana wines are shown in Figure 1. Banana wine from Gahogo (GA) showed higher alcohol content compared to the other wine samples, the alcohol content of different banana wines was ranged as follow: Gahogo (40.1%) > Ganza inyota (26.1%) > Igisubizo (25.9%) > Bulinga (12.0%). The higher alcohol content of banana wine might be due to: (i) the production procedure, (ii) species of banana used, (iii) the yeasts present, and (iv) the dilution ratio [15, 16]. Alcohol content is one of the factors that affect the quality of Banana wine and can also be used to show the degree of fermentation throughout the fermentation process [11]. During fermentation, starch is converted into glucose which is after transformed by yeast to give alcohol and CO₂. The weather is also contributing to the alcohol content of wines, since the fermentation process is conducted at ambient or room temperature without means to stabilize/control the fermentation temperature [2].

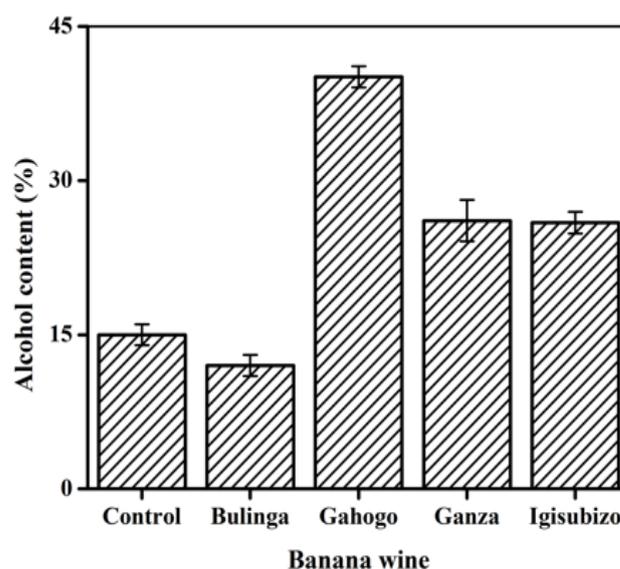


Figure 1. Alcohol content of banana wine from different sites in Muhanga district

3.2. pH, Total Acidity, Total Soluble Solid Contents and Turbidity of Different Banana Wines

The pH, total acidity, total soluble solid contents and turbidity of all banana wines are shown in Figure 2. Ganza inyota (G) wine showed the lowest pH (3.30) compared to other wines (Figure 2 (a)). pH values of Igisubizo (I), Bulinga (B) and Gahogo (GA) banana wines were not significantly different and were ranged as 4.45, 4.54 and 4.29 respectively. These results were in agreement with previous studies of Kim *et al.* [17] and Seo *et al.* [18] who reported that the pH of the Korean Traditional Rice Wine, Makgeolli, and Supplemented with Banana was ranged between 3.4 and 4.5, respectively.

As shown in Figure 2 (b), the total acidity of Bulinga and Gahogo wines was higher compared to Ganza inyota and Igisubizo wines. The total acidity of banana wine from different sampled sites were ranged as follow: Bulinga and Gahogo (6.96) > Ganza inyota (5.39) > Igisubizo (5.29). Value of organic acid content is an important parameter to ameliorate flavor, stability, and shelf life of the wine [19]. The increase in acidity could possibly be due to the activities of lactic acid bacteria breaking down sugars to produce lactic acid among other secondary products, resulting in the sweet-sour taste which makes banana beer popular among the Rwandan people. The decrease of acidity in wines explain the loss of its quality since it is often followed by formation of different types of acids which include succinic, lactic and acetic acids [2].

As indicated in Figure 2 (c), (B) and (GA) wines showed higher total soluble content than G and I wines. The total soluble contents of the I, G, B and GA wines were 15.2%, 12.2%, 21.7% and 21.8%, respectively. The total soluble solids decrease along with an increase in alcohol content which is due to the breakdown of sugars through the fermentation process. The variation of the pH, total acidity, and total soluble contents might also be dependent on the origin of samples [20].

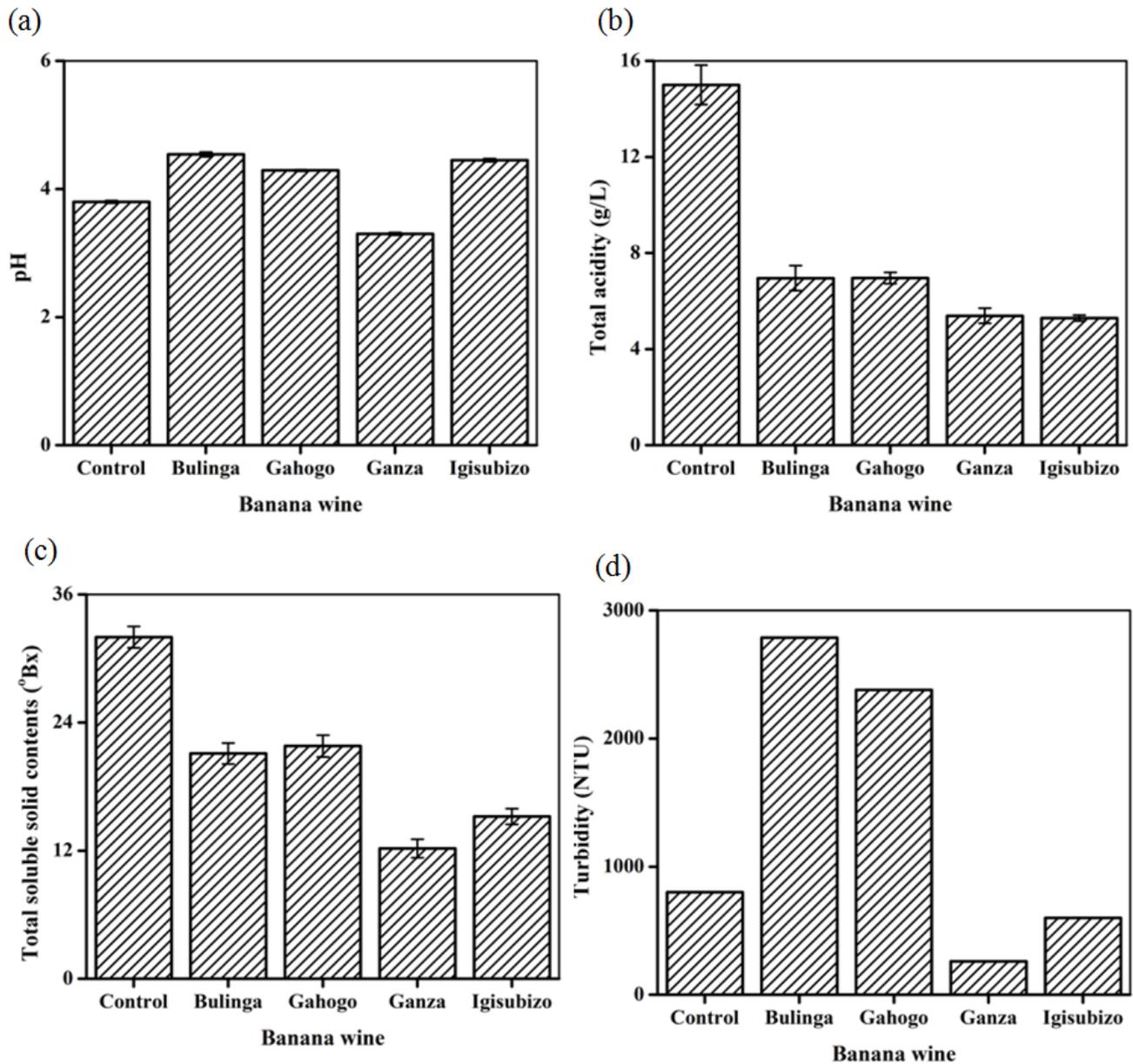


Figure 2. pH (a), Total acidity (b), Total soluble solid (c) and turbidity (d) of Banana wines

Turbidity is “an expression of the optical property that causes light to be scattered and absorbed rather than simply transmitted in straight lines through the sample. The wine turbidity is one of the quality parameters for consumer acceptability of food and beverage products. The lower turbidity of wine may affect its consumer’s acceptability. As indicated in Figure 2 (d), both B and GA wines showed the highest turbidity compared to other samples. The turbidity of I and G wines was within the range reported by Byarugaba-Bazirake [21]. The higher turbidity of wine might be due to poor processing of banana juice prior to fermentation and/or poor filtration of the final product which is assigned mainly to the polysaccharides, pectin and starch [22].

3.3. Volatile and Fixed Acidity Content of Different Banana Wines

Elevated levels of acetic acid can be detrimental to wine quality by imparting vinegary aroma to the wine, a

sensory aspect characterizing the wine as minimally flawed to downright fault [23]. The measurement of a wine’s volatile acidity is thought to be a measurement of its acetic acid. The acidity in wine is made up of two types: the non-volatile “fixed” and the steam distillable acids, or “volatile.” Volatile acidity magnifies the taste of fixed acids but can be masked by high levels of sugar and alcohol. Volatile acidity is used routinely as an indicator of wine spoilage [15]. As shown in Table 1, the volatile acidity in all samples was lower than the legal limits of USA [24]. I wine (0.490 g/L) showed the highest concentration of volatile acidity, while G wine (0.040 g/L) the lowest concentration of volatile acidity. The increase acetic acid concentration might be due to fermentation problems (e.g. nutrient deficiency) that occurred during the primary fermentation [13]. Spontaneous alcoholic fermentations from wild yeasts present on the fruit skins, or reliance upon natural fermentations from the winery’s “native” yeast (residing within the winery and on equipment) may produce enough acetic acid to lead to a

sluggish/stuck fermentation. In addition, the higher temperature of wine storage and higher wine pH might favor the development and metabolism of acetic acid bacteria species (*Acetobacter*, *Gluconobacter* and *Gluconacetobacter*) [25]. The presence of Lactic acid bacteria (LAB) within a winery may also account for acetic acid within the wine. It was suggested to the winemaker that they should limit wine's access to oxygen, and keep the levels of free SO₂ at the pH-adjusted ppm to provide protection against acetic acid bacteria growth [26].

Table 1. Volatile Acidity and Fixed Acidity Content of Different Banana Wines

Samples	Volatile acidity (g acetic acid/L)	Fixed acidity (g Tartaric acid/L)
Control	3.50±0.02 ^e	10.00±1.11 ^d
Bulinga	0.18±0.01 ^b	6.78±0.85 ^c
Gahogo	0.19±0.02 ^c	6.77±0.46 ^c
Ganza	0.04±0.00 ^a	5.35±0.35 ^b
Igisubizo	0.49±0.04 ^d	4.80±0.21 ^a

Results are expressed as mean values±standard deviations. Means with same superscripts in a column are not significantly different (p < 0.05) as assessed by Duncan's multiple range test.

Fixed acidity is taken as difference between total acidity and volatile acidity. It is expressed in grams of tartaric acid per liter of wine [27]. Tartaric acid is the preferred acidulating agent in low-acid wines; it is relatively more resistant to microbial breakdown and can thus be added before the onset of alcoholic fermentation without the risk of off-flavors [28]. The fixed acidity was ranged between 4.802 g/L and 6.779 g/L. The concentration in fixed acidity is in agreement with the results reported by Enidiok and Attah [14].

3.4. Sulfur Dioxide Content of Different Banana Wines

Sulfur dioxide (SO₂) is a natural by-product of winemaking as a small quantity is produced during the alcoholic fermentation by yeasts. It is also added to wine as a preservative and antioxidant in juices to prevent unwanted microbial infection and oxidation. Arubi and Offonry [29] reported that the low pH and sulphite in wine are desirable, as they inhibit the activity of undesirable microorganisms that may be present in wine. The free SO₂ content was ranged as follow: GA wine (176.64 ppm) > B wine (148.48 ppm) > I wine (143.36 ppm) > G wine (104.32 ppm) (Figure 3). The free SO₂ in banana wine might be produced by yeast in wine during fermentation [30].

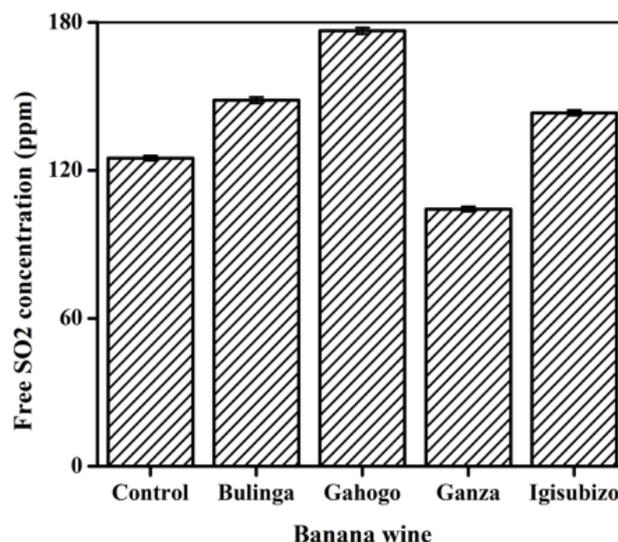


Figure 3. Free SO₂ concentration in samples I, G, B and GA

3.5. Heavy Metals and Minerals Content of Different Banana Wines

The minerals such as Calcium (Ca²⁺), potassium (K⁺), magnesium (Mg²⁺), sodium (Na⁺) and iron (Fe²⁺), were measured in different banana wine samples. As shown in Table 2, Ca content (mg/L) was between 6.51 to 74.67 and was higher in I wine and lower in B wine. K content was ranged between 118.90 to 1207.57, with lowest concentration in G wine and highest concentration in B. K concentration in B and G wines was far higher than the control. This might be attributed to the origin of banana used during wine processing. Na and Mg contents (mg/L) were ranged between 24.27 to 24.29 and 4.11 to 4.37, respectively. Na and Mg concentrations were not significantly different among the samples, and their concentrations were lower compared to the control. Fe content was ranged between 2.70 and 14.88 (mg/L). I showed the highest Fe concentration compared to the other wines. The content of minerals in all wine samples was ranged as follow: K > Ca > Na > Fe > Mg.

Lead was not detected in all samples while copper and chrome were significantly detected in lower concentrations compared to the control (Table 2). GA wine showed higher content in copper and chrome, accounting 0.22 (mg/L) and 0.80 (mg/L), respectively. All samples meet the National standard in heavy metal content. The contamination of wines by heavy metals might arise during manufacture or juice dilution [31].

Table 2. Heavy Metals and Minerals Content of Banana Wine from Different Sites (mg/L)

Banana wine	Pb	Cu	Cr	Fe	Ca	K	Mg	Na
Control	0.10±0.00	2.00±0.00 ^c	5.00±0.00 ^e	7.00±0.02 ^c	100.00±1.02 ^e	633.00±1.22 ^c	32.00±0.00 ^c	63.00±0.12 ^b
Bulinga	ND	0.21±0.01 ^a	0.68±0.01 ^c	3.03±0.01 ^a	6.51±0.05 ^a	1207.57±2.01 ^e	4.37±0.05 ^b	24.29±0.02 ^a
Gahogo	ND	0.22±0.00 ^a	0.80±0.03 ^d	5.82±0.03 ^b	47.57±1.00 ^b	1107.21±0.99 ^d	4.37±0.03 ^b	24.29±0.00 ^a
Ganza	ND	0.13±0.01 ^b	0.15±0.02 ^b	2.70±0.05 ^a	56.65±1.06 ^c	118.90±0.35 ^a	4.15±0.00 ^a	24.29±0.02 ^a
Igisubizo	ND	0.14±0.01 ^b	0.04±0.00 ^a	14.88±0.09 ^d	74.67±0.98 ^d	295.15±0.56 ^b	4.11±0.01 ^a	24.27±0.04 ^a

Results are expressed as mean values±standard deviations. Means with same superscripts in a column are not significantly different (p < 0.05) as assessed by Duncan's multiple range test. ND: not detectable.

Table 3. Bacteriological Parameters of Different Banana Wines

Samples	E. coli	S. aureus	Salmonella & Shigella	Total coliform
Control	< 100	< 100	< 100	ND
Bulinga (10 ⁶)	3	4	ND	ND
Gahogo (10 ⁶)	30	15	ND	ND
Ganza	Uncountable	9	ND	uncountable
Igisubizo	15	4.5	ND	ND

ND: Not Detectable.

3.6. Bacteriological Parameters of Different Banana Wines

Salmonella & Shigella were not detected in all samples. According to Kotloff *et al.* [32], infection with Shigella develops diarrhea, fever and stomach cramps starting a day after exposition to bacteria while the contamination of people by Salmonella may be caused by infected persons, animals and direct contact of those with fluids. Salmonella also has an important role in producing pathogens that cause food poisoning [33]. The absence of these bacteria from the analyzed wines was taken as good news for the safety of consumers. Total coliform was uncountable in G wine but it was not detected in other wines. Ganza inyota sample showed also uncountable number *E. Coli* while other sample showed lower content of this bacteria compared to the control (National Standard). The presence of *E. coli* indicates a contamination of fecal origin. The presence of *Staphylococcus aureus* or its toxins in processed foods or processing equipment is generally an indication of poor sanitation. GA showed higher content in *S. aureus* than other samples. The content of *S. aureus* in all samples was lower than the control (National standards). Seo *et al.* [18] reported that the total microorganisms increased throughout the fermentation process. Another reason of presence of microorganisms in banana wine (Urwagwa) might be the poor hygiene of the processing area, water, hygiene of the equipment and personnel. The contamination with different microorganisms may lead to the poor hygienic quality, short shelf-life, poor yield of ethanol and variations in organoleptic and hygienic quality [34,35].

4. Conclusion

The Physico-chemical and bacteriological properties of banana wine “urwagwa” from Southern province of Rwanda were evaluated. Results showed that the alcohol content of Gahogo banana wine was far higher than the control (National Standard) while Bulinga wine was lower than the control. The higher alcohol content might be attributed to the dilution ratio of the banana juice during processing. Results also showed acceptable pH, sulfur dioxide content, fixed acidity, total soluble solids, heavy metals and minerals content compared to the control. In addition, the total acidity of Gahogo and Bulinga wines was significantly higher compared to the control and other wines indicating that high activities of lactic acid bacteria breaking down sugars to produce lactic acid. Volatile acidity as an indicator of wine spoilage was found to be lower than the legal limits. Bulinga and Gahogo wines showed the highest turbidity compared to other samples probably due to poor processing of banana juice prior to

fermentation and poor filtration of the final product. Furthermore, Igisubizo wine showed the presence of some major microorganisms found were total coliform, *Escherichia coli* and *Staphylococci aureus*, but their content was less than the standard limit. There was no Salmonella and Shigella detected in all wines.

Statement of Interest

The authors have no conflict of interest.

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