Microbiological Analysis of Traditional Banana Wine Prepared by Local Population with Feet and Hands in North and East (*Case Study Ngoma and Musanze*)

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Abstract—**This research has the objective of analyzing and compare the microbial content of TBW manufactured in rural areas. To achieve our objectives, 3 samples from Ngoma and 3 from Musanze sectors have been collected and analyzed using Nutrient broth, Sabouraud, and Eosin methylene blue agar media. The results have shown that TBW made from Musanze and Ngoma contains many microbes from different species *E Coli, Streptococcus, Staphylococcus, Lactobacillus, Enterobacter*, *Clostridium*, and *Bacillus* have been identified. The results showed that the TBW lacks hygienic conditions which were worse in TBW prepared by feet in Ngoma. The TBW made in Ngoma contained more fecal coliforms 330/ml, total germs $302.3x10^7$/ml, yeasts, and molds $784x10^6$/ml compared to TBW made from Musanze containing 50/ml fecal coliforms 325/ml yeasts and molds and $523x10^6$/ml total germs. The total coliforms were the only category of microorganisms to be more numerous in Musanze TBW $117x10^4$/ml compared to $140x10^3$/ml of Ngoma. TBW from Musanze had high pH and alcoholic degree compared to Ngoma. TBW made from Ngoma had a shorter shelf life and bad smelling. Both Tbw did not respect the norms fixed by RBS, so the producers must improve the hygienic conditions and respect the norms of RBS.**

Index Terms—**traditional banana wine, microbes, flavour, alcoholic degree, culture medium, pH, colour**

I. Introduction

In Rwanda, as elsewhere in the world there is production of various alcoholic beverages prepared by local population which can be authorized or not by Rwanda bureau of standards. The extraction of that juice from banana and fermentation to produce banana wine using traditional methods is an important post-harvest activity in the family banana farming in eastern Africa. The production and sale traditional banana wine has greater importance to local population because it generates incomes to the family household [1]. The most popular drinks are banana wine and liquors. These drinks are different from each other by the raw material used in their production and the method of manufacture [2].

Traditional banana wine is a beverage obtained by fermenting banana juice using or not sorghum flour as yeast source. These beverages produced are not controlled and hygiene might not be sufficient depending on mode of production. That can change the properties of banana wine because of presence of unwanted microorganisms. Those microorganisms appear using unsterilized material, improper water and producers lacking hygiene in the production of that traditional banana wine. Hygiene and sanitation play a role for the producers as it can affect the flavor and the banana wine shelf life which might result in fluctuation of income but also the consumers of banana wine can become a target for infection diseases transmission. In Rwanda, banana wine is made in different ways varying from one region to another. This leads to production of different kinds of wine in terms of quality.

II. Methodology

A. Sample Collection

Three samples of traditional banana wine were collected in 3 different families from north Musanze District, in different sectors such as (Rwaza, Muko, Muhoza) and 3 samples of traditional banana wines were collected Ngoma District to be analyzed in laboratory of K.I.E for total coliforms, fecal coliform, total germs, yeast and molds, pH and alcoholic degree. Those samples were putted in separate clean bottles, brought to laboratory and kept in refrigerator less than 24 hours before their analysis. For the microbial content analysis, the 3 samples collected from Musanze were pulled together in order to form one mix of samples, and the 3 samples collected from Ngoma were also pulled together in order to form one mix of samples.

B. Materials Sterilization

All materials used for microbial contents analysis were washed with water, dried and sterilized in autoclave at

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121°C for 15 minutes apart for plastic Petri dishes and pipettes which were sterilized using ethanol 70%. All techniques were done aseptically around the Bunsen burner. All of working tables were cleaned and disinfected with ethanol 70% so as to prevent cross contamination that could occur during operations.

C. Culture Medium

A culture medium is solid preparations that have been used to grow and store microorganisms in order to be used in identifying and isolating bacteria and fungi strains.

1) Different culture medium used in experiment

Eosin methylene blue agar (EMB) was used for isolation and differentiation of total and fecal coliforms.

Nutrient Broth (NB) was used for isolation and differentiation of total germs.

Sabouraud dextrose agar (SAB) was used for isolation and differentiation of fungi, yeast and lactic acid bacteria.

2) Culture medium preparation

NB preparation 3.9 grams was weight using the electronic balance and was added with 3grs of agar and suspended in 300 ml of distilled water, heated until completely dissolved and autoclaved at 121°C for 15 minutes and poured carefully into Petri dishes.

SAB preparation 19.5 grams were weight using electronic balance of the medium and suspended in 300 ml of distilled water, heated until completely dissolved and autoclaved at 121°C for 15 minutes, and poured into Petri dishes.

EMB preparation 7.2 grams were weight using electronic balance of the medium and suspended in 200 ml of distilled water, heated until completely dissolved and autoclaved at 121°C for 15 minutes and poured into Petri dishes.

D. Serial Dilution

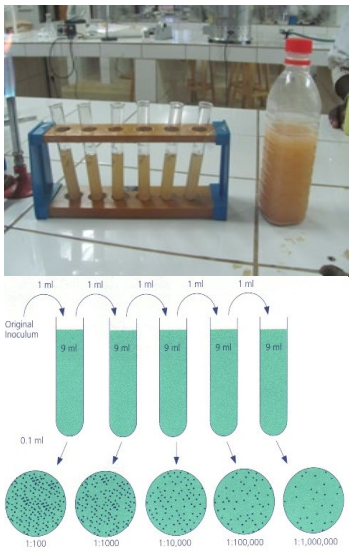

Figure 1. Serial dilution of traditional banana wine in K.I.E laboratory.

Dilutions were very important in order to get a number of colonies that could be countable. Seven test tubes were filled with 9 ml of sterilized traditional banana wine as shown below on Fig. 1. By using a micropipette, 1ml of the original sample was taken and put in 9 ml (dilution10^{-1}). After mixing, 1ml was taken from that tube and then put in the next tube (dilution10^{-2}). The same process was repeated up to the tube number seven.

E. Inoculation

The inoculation has been done by putting 100 µl of the diluted suspension samples into Petri dishes by using micropipette. Then the samples were spreaded on the agar media by using a spreader.

F. Incubation

The Petri dishes were putted into incubator for 24 hours at 37°C and 44°C to enable total coliforms and fecal coliforms to grow on EMB. For Petri dishes of NB and SAB, they were putted for 72 hours at 28-30°C to enable yeasts and fungi to grow.

G. Description of Colonies

1) Description

In order to differentiate the microorganisms, the description of colonies is needed. The colonies are described using different characteristics which are:

Size: diameter in millimeter, *Form:* Punctiform, circular, filamentous, irregular, rhizoid, *Elevation:* flat, raised convex, pulvinate, umbonate, umbilicate, *Margin:* entire, undulate, lobate, *Color:* white, yellow, black, buff, orange, pink, *Density:* opaque, translucent, transparent, *Consistency*: viscid, membranous, brittle, butyrins.

H. Microscopic Observations

A small portion was taken from each type of colony and was putted on slide containing a drop of methylene blue and well mixed. The slide was covered by cover slip and microorganisms were observed at magnification of x1000. A drop of immersion oil was added on the cover slip to clarify the image. Characteristics taken into consideration in microscopic observation for bacteria were: - the form (cocci, rods, and spirillum), arrangement (cluster, pairs, tetrade, and chain), size (big, small), mobility, presence of spores (terminal, subterminal, central).

For mold the characteristics taken into consideration were: the presence of septa or not in hyphae and the mode of asexual reproduction (fission, arthrospores, chlamydospores, sporangiospores, conidiospores or blastospores).

For yeasts, the characteristics taken into consideration were: shape (spherical, oval, cylindrical), size (big or small)

I. Physicochemical Parameters

1) Alcoholic degree

The vinometer used to measure the alcoholic degree of the banana wine samples during six days, was hold vertically with the funnel upwards. Each sample of traditional banana wine was poured one by one into funnel without air bubble allowed in capillary traditional banana wine column. In looking that the vinometer end of capillary the vinometer was turn upside –down. Then percentage was read directly from the graduation where the upper side of traditional banana wine stays.

2) pH

The pH was measured during 6 days using pH meter. The electrodes were washed using distilled water and

were putted into each sample of traditional banana wines. Then, the pH was readable on pH scale.

III. Results and Discussion

A. Results

Descriptive and qualitative analysis of microorganisms grown in SAB, NB, EMB

TABLE I. Number of Microorganisms between Traditional Banana Wines Made from Musanze and Ngoma

Samples	Musanze tbw	Ngoma tbw
Total coliforms grown on EMB	1.17×10^{6}	1.40×10^{5}
Fecal coliforms grown on EMB	50	330
Total germs grown on NB	5.23×10^{8}	3.02×10^{9}
Yeast and Molds grown on SAB	3.25×10^{8}	7.84×10^{8}

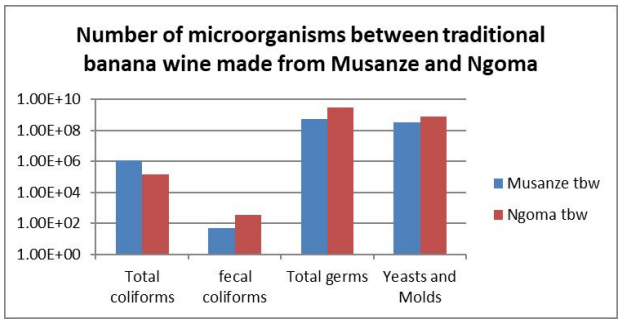

Figure 2. Number of microorganisms between traditional banana wines made from Musanze and Ngoma.

On the above Table I and Fig. 2 the traditional banana wine made in Ngoma contained more fecal coliforms 330/ml, total germs 302.3×10^{7}/ml, yeasts and molds 784×10^{6}/ml compared to traditional banana wine made from Musanze containing 50/ml fecal coliforms 325/ml yeasts and molds and 523×10^{6}/ml total germs. The total coliforms were the only category of microorganisms to be more numerous in Musanze traditional banana wine 117×10^{4}/ml compared to 140×10^{3}/ml of Ngoma.

On the below Table II, above Fig. 3, and Fig. 4 the average pH of traditional banana wine brewed in Musanze at day 1 (4.84) is higher compared to pH of Ngoma (4.48) and alcoholic degree in Musanze traditional banana wine (9.03) is higher than alcoholic degree of Ngoma (7.63). From day 1 to day 3, the pH in both traditional banana wine decrease to increase again at day 6. From day 1 to day 3 the alcohol in both traditional banana wines increase then at day 6 decrease. Also the gas in Musanze traditional banana wine was tolerable compared to Ngoma which bad smelled.

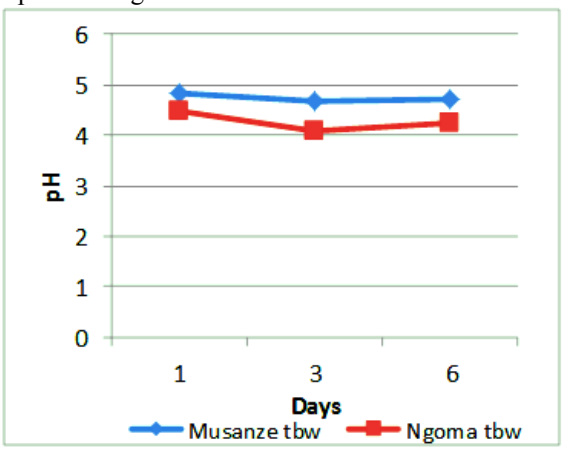

Figure 3. pH in tbw made from Musanze vs Ngoma.

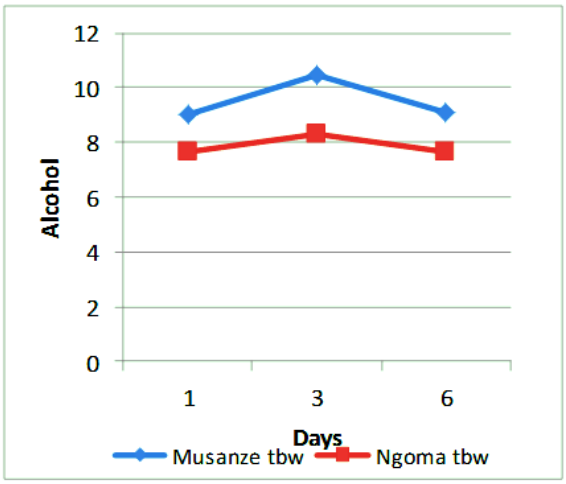

Figure 4. Alcohol in tbw made from Musanze vs Ngoma.

TABLE II. pH and Alcoholic Degree from Traditional Banana Wines of Musanze and Ngoma

samples		Day 1 for pH	Day 1 for alcoholic degree	Day 3 for pH	Day 3 for alcoholic degree	Day 6 for pH	Day 6 for alcoholic degree
Musanze	Average	4.82	9.03	4.68	10.5	4.70	9.1
	Color	Grey		Grey		Grey	
	Smelling	Odor tolerable		Odor tolerable		Oder non tolerable	
	Release of gases	✓		✓		-	
Ngoma	Average	4.48	7.63	4.1	8.3	4.23	7.66
	Color	Clear kaki		Kaki		Kaki	
	Smelling	Odor tolerable		Bad smelling		Bad smelling	
	Release of gases	✓		-		-	

✓ presence of gas - no gas

B. Discussion on Microbial Analysis

Both traditional banana wines made from Musanze and Ngoma contain varieties of microorganisms such as total coliforms, fecal coliforms among which we have identified probably *Klebsiella*, *Escherichia coli* and *Enterobacter*; Yeasts and Molds among them we have identified probably *Saccharomyces* and *Candida sp* and total germs among which probably *Lactobacillus, Staphyloccoccus, Colosridium, Bacillus* and *Streptococcus* have been identified. These varieties of microorganisms found are similar with the ones identified in traditional sorghum beer as it has been investigated by Lyumugabe *et al*. [3].

The results presented in Table I and Fig. 2 showed that in the traditional banana wine made from Ngoma, the number and variety of microorganisms is higher compared to traditional banana wine made from Musanze for all the microorganisms category found except for the total coliforms. The cause of that high number and variety of microorganisms can be that the people use their feet which are not well washed and river's water or lake water, while in Musanze tap water is preferably used. As they lack the sense of hygiene that can have led to high contamination of traditional banana wine made from Ngoma.

Among the total germs, potential pathogens, general indicator of potential contamination have been identified. Among them *Staphylococcus, Streptococcus, Clostridium* and Bacillus have been identified in both banana wines but the number and variety was higher in the one from Ngoma as shown on Fig. 5 and Fig. 6. *Staphylococcus* and *Streptococcus* are frequent commensals on nose, throat and on the skin of healthy of people that may lead to the contamination of food stuff or drink in producing enterotoxin which can poison the food. *Clostridium* and *Bacillus* are sporulating bacteria which are found mostly in the soil, *Clostridium* being also found in the intestinal tract of humans [4]. In food, they have also been found to be causal agent of food intoxication. Their presence indicates that the producers were probably carrier of *Staphylococcus* and *Streptococcus* and would have introduced these microorganisms in the banana wine due to unhygienic practices. Also, these findings reveal that during the production of banana wine, the producer's feet and hands would have been in contact with soil containing *Clostridium* and *Bacillus*. Also, when the bananas are put in the pit for their ripening, the soil which is add to cover the banana might have been contaminated with the bacteria which would have subsequently been introduced in the banana wine.

Figure 5. Total germs grown on NB Dilution10-5 banana wine Made in Musanze.

Figure 6. Total germs grown on NB Dilution10^{-5} banana wine Made in Ngoma.

Also, *Lactobacillus* probably identified have probably contributed to decrease pH as they perform homolactic, heterolactic and malolactic fermentation producing lactic acid as well as *Clostridium sp* which can perform propionic fermentation producing acid butyric. In these fermentations, as different other products are also released, such as acetone and butanol, these might have contributed to the banana wine aroma and to spoilage of wines [5], [6].

Among the yeasts identified, it is known that *Saccharomyces* is used for alcoholic fermentation to produce alcohol and might have been among the ones which have increased the alcohol content of our banana wines. However, C*andida sp* which have also been identified might have caused the spoilage observed after day 3 and as some are known pathogens, they might expose the consumers to candidiasis [7].

Also number of coliforms indicated serious lack of hygiene during the preparation of traditional banana wine either using feet or hands and these results are higher to the ones found in traditional sorghum beer [8] but similar to result found by (Twagirimana, 2010). Those coliforms might come from the endogenous microflora found into raw material used such as (banana, sorghum, and banana leaves), in the water, the soil for incubation and material used. The fecal coliforms found show that the environment, the water used in preparation, or hands and feet of people making banana wine were contaminated by feces. Also, they might have contributed to degradation and bad smelling noticed in traditional banana wines as they might have produced different acids such as formic, succinic, acetic and lactic responsible for odors and taste [9]. The samples of traditional banana wine present different colors from clear kaki to grey and smelling were from tolerable to bad odor as the days after the banana wine production were increasing. Also, the banana wine of Musanze produced a lot of gas during six days compared to Ngoma. The pH and alcohol degree results in traditional banana wine made from Musanze were also surprising. It was expected that in Ngoma, as the number of microorganisms is high due on one hand to the hot climate, and on the other hand to the material used, that the alcohol content would have been higher and the pH lower. However, in Musanze, during the banana wine preparation, the quantity of sorghum flour add is higher than in Ngoma, adding more nutrients. This might have allowed the growth of microorganisms, among, them the yeasts, for a longer period of time producing more alcohol as seen 3 days after the collection. Also, we can presume that the yeasts found in Musanze were maybe

higher alcohol performers and the big number of other microorganisms found in Ngoma might have decrease the pH, cause spoilage, with less alcohol production. Also the traditional banana made in Rwanda has high pH and alcohol compared to traditional banana wine made in Tanzania [10].

All those banana wine have microbiological pathogens that can cause diseases and the requirement for traditional banana wine have not been set by RBS, so the producers must know the standards of traditional banana wine in order to avoid those diseases.

IV. Conclusion and Recomandation

A. Conclusion

Microbial Pathogenicity of traditional banana wine has greater effect on human consumption resulted from lack of hygiene, skills and equipment. The results we have seen show health risk associated to traditional banana wine due its indicator was much below the limit set by RBS. The result showed that there are many sources of contamination of traditional banana wine they include air, water, soil, humans. According to all those traditional banana wines all processing in production are not controlled that leads to the contamination of microorganisms and cause some infectious diseases. The consumption of the traditional banana wine must have predicted to the local producer to obey and follow microbial tolerance limits defined by RBS related to banana wine, number of microbes can be minimized through proper handling through all process.

B. Recommendations

The recommendation that can be given to the producers, consumers and other people in common of traditional banana wine for better improvement of mode production because it constitutes the source of revenue and it is consumable.

- Improvement of hygiene in process of fermentation in order to avoid unwanted microorganisms such as bacteria.
- Methods of using feet in production of traditional banana wine must be well improved in district of NGOMA in order to get the good quality of banana wine.
- Quality control measures should be explained to all traditional producers.
- There must use pasteurization in order to kill the unwanted microorganisms and sterilize the materials their use Local producer should be aware of standards set by RBS.

Conflict of Interest

The authors declare no conflict of interest

Author Contributions

Author Bunani Parfait conducted this research from the beginning until to the end. Author Karine Bernard supervised this research project. Author Isabane Remy Serge wrote the paper and corrected grammar mistakes. Author Ishimwe Patrick analyzed data and plotted different graphs in this research.

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