

## Quality Assessment of Banana Juice and Beer in Rwanda

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**Abstract:** Banana juice and beer samples were collected at different points along the processing and marketing chain from four localities in Rwanda and analyzed for microbiological and physico-chemical quality by adapting a Hazard Analysis and Critical Control Points (HACCP) methodology. The results showed high total bacterial counts of 9.02-9.86 log<sub>10</sub> cfu mL<sup>-1</sup> with yeast and moulds as well as lactic acid bacteria being the predominant microbes. Coliform counts were high in artisanal processed banana beer, 7.65-8.11 log<sub>10</sub> cfu mL<sup>-1</sup> but were low or undetected in semi-industrial processed beer samples. The presence of coliforms in the artisanal processed banana beer indicated post-fermentation contamination. High total bacteria and coliform counts were associated with samples from artisanal processors, those drawn from plastic non-food grade containers and diluted with water indicating these as potential critical points for microbial ingress.

**Key words:** Banana beer, juice, microbiological, physico-chemical, coliforms, bacterial counts

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### INTRODUCTION

Food safety has become an increasingly important public health and market development issue in developing countries. Besides its important role in determining access to export markets, food suppliers in developing countries are faced with the challenge of improving food safety for their growing urban middle class as the large burden of disease associated with poor food safety becomes widely appreciated. Evidence of food safety concerns in developing countries can be seen from the vast literature on quality assessments of different food types from a public health perspective from the 1990s (Mosupye and von Holy, 1999; Bryan *et al.*, 1997). This study contributes to the literature by investigating the microbiological and physico-chemical quality of banana juice and beer in Rwanda by adapting a HACCP methodology. Banana juice and beer are important beverages in the Great Lakes region.

Bananas occupy 35% of cultivated land area in Rwanda and play a key role contributing to rural populations' household food security and revenue (Mpawenimana, 2005). The three main banana types grown include the cooking type, mainly the East African Highland banana which is largely produced for home consumption with surplus sold to the market dessert types mainly comprising of AAA and AB types and beer banana cultivars (AB, ABB) which is a common source of household income as it is transformed into juice and beer

which are then sold to consumers or used for social and ceremonial events (Gaidashova *et al.*, 2005). The beer banana cultivars account for 60% of banana production in Rwanda though about 4,000 tonnes per annum is still imported from the Democratic Republic of Congo (Bingen and Laurent, 2000; Akabor *et al.*, 2003).

Production of banana beverages, predominantly juice and beer in Rwanda has been estimated at 700 million litres per annum and a per capita consumption of about 1.2 L per day (Gensi *et al.*, 2000). The banana beverage processing and marketing is dominated by the informal sector, comprising smallholders operating individually or collectively using artisanal methods (Informal sector here refers to the non-registered and unregulated actors). Most of them carry out these activities as a survival strategy for income generation. A few formal processors exist at association/cooperative and small scale industrial levels utilizing semi-mechanized and mechanized equipment.

**Banana juice and beer processing procedure:** A survey was carried out in 2008 covering a random sample of 610 processors and market chain actors in Rwanda, Burundi and South Kivu Province of the DRC to obtain specific and relevant information on banana juice and beer processing procedures. Specific information was collected with regard to the type of raw materials and ingredients used and fermentation equipment, fermentation time and types of traders involved in marketing.

The process begins by harvesting mature bananas and ripening for about 3 days. The ripened bananas are then peeled manually. This is then followed by extraction of banana juice. For the artisanal processors, this involves utilization of the grass, *Imperata cylindrica* commonly known as inshinge to squeeze out the banana juice using hands or feet. Prior to filtration, the juice is diluted with water which is in most cases untreated, under local settings. The juice is then filtered using the *I. cylindrica* to obtain a clear product. The filtered juice is then fermented for about 24-48 h using sorghum flour to obtain banana beer which is then filtered and stored in plastic drums. The raw materials are not sterilized by boiling and therefore provide an excellent substrate for microbial growth (Karamura *et al.*, 1998).

No standards and control measures have been set up by the government regulatory authorities yet hygiene and sanitation of processing equipments and personnel are often very poor. Composition quality is usually highly variable and may have safety implications on human health and product shelf life. The small scale industrial processors use enzymes for juice extraction after which it is pasteurized. Sorghum flour is then added to the cooled juice for fermentation in stainless steel pans to obtain beer which is then packaged in drums and bottles. Generally, the fermentation process occurs spontaneously brought about by natural yeasts that are present in the banana and sorghum. Uncontrolled fermentation may result in quality unpredictability of the final product, for example excess acids, fusel oils and esters (Moshia *et al.*, 1996). A number of studies such as Birch and Lindley (1985) and Goodman and Gilman (2001) have shown that fusel oils and esters affect the human sensory motor system, liver and kidney while excess acids may result in stomach ulcers.

## MATERIALS AND METHODS

**Sample collection:** Fifty juice and beer samples were collected from a purposive random sample of formal and informal processors as well as market chain actors in Rwanda in 2010. The samples were collected from different points along the processing line and analyzed for microbiological and physico-chemical parameters. The coverage included Kigali town, Rwamagana, Gisenyi and Butare districts. The samples were collected in sterilized containers of 500 mL. The 20% of the samples were drawn from various market actors and outlets while the rest were from known processors at various defined processing stages: juice extraction, dilution and beer after fermentation. After collection, the samples were stored and transported in cooler boxes to the laboratory for

analysis. The 10 mL of the sample was diluted with 90 mL of sterile buffered peptone water and mixed well. This was used in dilution for total aerobe, Lactic Acid Bacteria (LAB) and yeast count while bacto-peptone diluent was used in dilution for coliform count. Serial dilutions were prepared and spread plate technique was used on appropriate selective media.

**Microbiological and physico-chemical analysis:** The methods and procedures used were as described by Harrigan and McCance (1976) and the FDA, bacteriological analytical manuals. The sample serially diluted in 0.1% of sterile peptone solution or bacteriological peptone. Dilution plates were for total plate count on Plate Count Agar (PCA), Yeast on Potato Dextrose Agar (PDA), Lactic acid on deMan Rogosa Sharpe agar (MSR agar) and coliform count on Violet Red Bile Agar (VRBA). All plates were incubated under aerobic conditions for 24-36 h. VRBA plates were incubated at 37°C while the rest were incubated at 25°C.

The pH meter was calibrated using buffers of pH 4 and pH 7 after which the pH of the samples was measured using a Fisher Model. Total soluble solids were determined using a refractometer and the reading was taken in °Brix (AOAC, 1995). Titratable acidity was also determined. A sample of 10 mL was titrated with 0.1 N NaOH using 1% phenolphthalein as an indicator. The titratable acidity was calculated as a proportion of lactic acid.

**HACCP process:** The Hazard Analysis Critical Control Points (HACCP) process, recommended by FAO/WHO (1998) is now a widely accepted methodology in risk analysis for processed foods. A number of studies such as Mwangi *et al.* (2000) have utilized HACCP to identify hazards and critical points along the food processing and marketing chains in developing countries. HACCP identifies the critical points in a process that may be hazardous, their risk factors and potential level of risk so that controls for remedial action can be implemented. Controls are specific actions that need to be undertaken to prevent health risks. The application of HACCP is however a major challenge in developing countries where food processing and marketing is mostly informal with minimal documentation of procedures. Identification of hazards, critical points and controls along the banana beverage processing-marketing continuum is an invaluable input towards setting up processing standards and minimizing on health risks that may be associated with consumption of such beverages.

Two strategies were employed to identify critical points that were associated with high total bacterial and

coliform counts in banana beverages. The first was through descriptive statistics and the second was stepwise regression models of the logarithm of total and coliform bacterial counts as dependent variables with all potential critical points as independent variables with  $p < 0.05$  for entry and retention using STATA Statistical Software.

**Stepwise Regression Model:** Stepwise Regression is a method of model specification that selects variables based on the significance of their t-scores and their contribution to good model fitness also referred to as  $R^2$  (Greene, 2003). It starts from a multiple linear or nonlinear regression model and is used to study the relationship between a dependent variable,  $y$  and one or more independent variables,  $x_n$ . The random error term,  $\epsilon$  is included to capture omitted factors not included in the model:

$$y = f(x_1, x_2, \dots, x_n) + \epsilon$$

The objective of stepwise regression is to find a subset of the independent variables,  $x_n$  that best predict the dependent variable,  $y$ . Several approaches are used including forward selection, backward elimination or a combination of the two. Forward selection approach involves starting with no variables in the model, trying out the variables one by one and including them if they are statistically significant. Backward elimination approach involves starting with all candidate variables and testing them one by one for statistical significance, deleting any that are not significant (ibid.). There are other methods that are a combination of both forward selection and backward elimination approach testing at each stage for variables to be included or excluded.

**RESULTS AND DISCUSSION**

**Laboratory results:** Table 1 shows the microbial counts of banana juice and beer samples. High total bacterial counts  $>9.02 \log \text{ cfu mL}^{-1}$  were observed with the yeast and moulds as well as lactic acid bacteria being the predominant microbes. High coliform counts were also observed in both the banana juice and beer, though higher counts were associated with diluted juice samples and beer samples drawn from the market.

Results of the physico-chemical analysis of the samples are presented in Table 2. The observed pH of 4.2-4.6 shows both juice and beer samples to be relatively acidic. The juice samples, both concentrated and diluted had higher total soluble solids compared to the beer samples. Microbial counts in banana juice and beer varied by location from which the samples were drawn. Figure 1 and 2 show the microbial counts for banana juice and beer samples, respectively based on location. High total bacteria, yeast and coliform counts were associated with both juice and beer samples drawn from Gisenyi and Butare. Coliforms were not detected in both juice and beer samples drawn from the formal processors. The samples from the formal processors had the lowest total bacteria counts although they were associated with high lactic acid bacteria counts.

**Stepwise regression results:** The  $R^2$ -value of 54 and 41% shows relatively good model fitness for total bacterial and coliform counts respectively. The results showed significantly high total bacteria and coliform counts for samples from Gisenyi ( $p < 0.01$ ). Lower total bacteria counts were associated with the use of mechanized/semi-mechanized juice extraction method and samples obtained from the extraction tank or pasteurizers compared to those obtained from the plastic jerrycans ( $p < 0.05$ ). Lower coliform counts were also linked to samples obtained from the extraction tank or pasteurizers compared to those obtained from the plastic jerrycans. There was a strongly positive association between coliform counts and samples that were diluted with water ( $p < 0.1$ ).

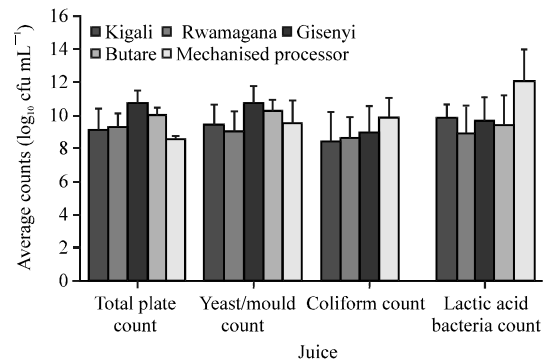


Fig. 1: Microbial counts in banana juice samples by location

Table 1: Microbial counts in banana juice and beer samples

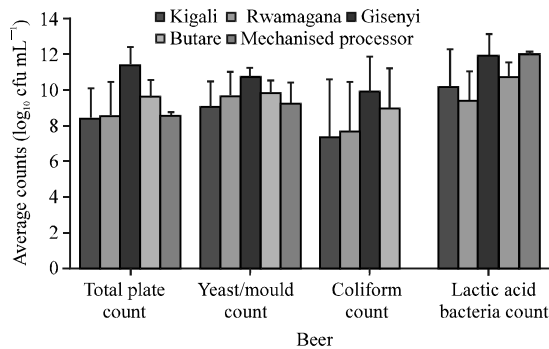
Type of banana beverage	Mean total plate count (log <sub>10</sub> cfu mL <sup>-1</sup> )	Mean yeast and moulds count (log <sub>10</sub> cfu mL <sup>-1</sup> )	Mean coliform count (log <sub>10</sub> cfu mL <sup>-1</sup> )	Mean Lactic acid bacterial count (log <sub>10</sub> cfu mL <sup>-1</sup> )
Concentrated juice	9.25±1.31 <sup>a</sup>	9.46±1.48	6.53±3.36	9.20±1.87
Diluted juice	9.86±1.08	10.06±1.07	9.49±1.40	10.08±1.30
Beer:Processors	9.86±1.70	9.97±1.23	7.65±4.26	10.91±1.69
Beer:Market	9.02±1.89	9.69±1.23	8.11±2.23	10.38±1.75

<sup>a</sup>After ± values show standard error of mean

**Table 2: Physico-chemical parameters of banana juice and beer samples**

Type of banana beverage	Titratable acidity (lactic acid %)	pH	Total soluble solids ( <sup>o</sup> Brix)
Concentrated juice	0.50±0.34 <sup>a</sup>	4.56±0.33	13.18±4.85
Diluted juice	0.49±0.21	4.58±0.49	10.81±5.91
Beer:processors	0.55±0.15	4.27±0.24	7.91±4.09
Beer:market	0.54±0.14	4.23±0.14	7.82±4.77

<sup>a</sup>After ± values show standard error of mean



**Fig. 2: Microbial counts in banana beer samples by location**

High total bacteria counts in banana juice and beer ranging from 9.0-9.9 log<sub>10</sub> cfu mL<sup>-1</sup> were observed showing potential contamination due to poor handling, type of packaging material used and processing procedures which could allow microbial ingress as found in other studies by Mahale *et al.* (2008). The microbes in the banana juice and beer comprise of yeast and moulds most of which are known to thrive in medium rich fermentable substrates such as sugars found in bananas leading to the production of acids during fermentation (Amusa and Odunbaku, 2009). This is possibly why there were high counts of Lactic Acid Bacteria (LAB), an average of 10.7 log<sub>10</sub> cfu mL<sup>-1</sup> in the banana beer and the resulting low final pH (Table 3).

Total soluble solids decreased in the banana beverage samples after fermentation with lower counts reported in banana beer samples compared to the unfermented juice. Gensi *et al.* (2000) found similar results and attributed the decrease in soluble solids in banana beer to increase in alcohol content during storage. Similar total microbial counts (9.3-9.5 log<sub>10</sub> cfu mL<sup>-1</sup>) have been obtained for kirario, an indigenous Kenyan fermented porridge and the Ethiopian fermented beverage, borde (Kunyanga *et al.*, 2009; Kebede *et al.*, 2002).

Stepwise regression results for banana juice samples showed lower total bacterial count for samples obtained from semi-mechanized compared to manual extraction method. Lower hygiene standards are usually associated with manual extraction methods utilizing hands and feet. These are potential critical points for microbial ingress especially if there is poor sanitization. Low total bacteria

**Table 3: Regression models for log<sub>10</sub> of total bacterial and coliform counts for banana juice samples**

Variables	Coefficient	SE	p> t
<b>Regression Model for log<sub>10</sub> total bacteria counts (PCA)</b>			
Container type (extraction tank/pasteuriser vs. jerrycan)	-0.823	0.330	0.023
Extraction method (semi-mechanised vs. manual)	-0.979	0.404	0.027
Total soluble solids	0.082	0.036	0.035
Kigali	-0.372	0.382	0.343
Gisenyi	1.430	0.439	0.005
Intercept	5.079	0.499	0.000
Adjusted R <sup>2</sup>	0.542		
<b>Regression Model for log<sub>10</sub> coliform counts (VRB)</b>			
Extraction method (semi-mechanised vs. manual)	-1.752	1.222	0.172
Container type (extraction tank/pasteuriser vs. jerrycan)	-5.754	2.191	0.019
Kigali	2.953	2.712	0.345
Gisenyi	3.852	1.834	0.049
Diluted of juice with water (1 = yes, 0 = no)	4.351	2.442	0.093
Intercept	6.050	1.586	0.000
Adjusted R <sup>2</sup>	0.412		

SE: Standard Error

counts were also associated with samples drawn from the extraction tank or pasteurizers (Fig. 3 and 4) compared to those from plastic jerrycans. Plastic jerrycans enhance continuous fermentation and multiplication of microbes due to its heat retention properties. The association between bacterial counts and plastic jerrycans in banana beverage processing can be partly attributed to poor hygiene and the general lack of a cold chain after fermentation.

Absence of coliforms in both banana juice and beer samples drawn from the formal processors can be attributed to higher hygiene standards in the industrial/semi-industrial set up and the effect of inhibition of growth of coliforms due to production of acids and fall of pH during fermentation. High coliform counts observed in banana juice from the artisanal processors indicate some high degree of contamination probably from raw material and equipment contamination and improper handling. The presence of the coliforms in banana beer even after fermentation may be attributed to poor post-fermentation handling through storage equipment and prevalence of unhygienic conditions.

From the stepwise regression results, the positive linkage between coliform counts and water dilution of banana juice suggest water source as a critical point for contamination. In most cases, the water used is mainly from unprotected springs, also used for other household chores. The presence of coliform bacteria in the banana beverages is a source of concern because most coliforms are known to be causative agents of food borne gastroenteritis and bacterial diarrhea diseases (Onuorah *et al.*, 1987).

The quality of banana beverages varied by location with higher total bacteria and coliform counts reported



Fig. 3: Extraction tank for an artisanal processor



Fig. 4: Pasteuriser for a semi-industrial processor

where a high number of individual processors bulked the final product for sale such as in Gisenyi and Butare sites. The government of Rwanda has encouraged formation of processing associations and cooperatives even though still using artisanal processing methods. In Butare and Gisenyi, the existing associations were banana beer selling associations but the processing was done at individual level then bulked for sale. In Kigali, the scenario was different with some associations having members specializing in certain functions such as provision of the raw materials (bananas), processing and marketing. In Rwamagana, members of the association were employed by an entrepreneur to carry out processing at a larger scale.

### CONCLUSION

This study has investigated the microbiological and physico-chemical quality of banana juice and beer, both important beverages in the Great Lakes region by adapting a HACCP methodology. Results show better quality of banana beverages from formal processors

relative to the artisanal processors. Generally, banana beverage manufacturing is characterized by absence of documented procedures and analytical control. However, the formal processors have set their own unwritten self-regulated quality control measures in order to target higher value markets. Given that banana beverage processing is still dominated by artisanal processors who largely serve low-income consumers, government and private sector investments in standardized specifications, routine quality control and HACCP plans should be encouraged as this would result in higher value products leading to improved welfare for both processors and consumers.

Potential critical points for microbial access have been identified as water quality used for dilution, type of container used for storage and manual juice extraction methods under poor hygiene. Some practices of artisanal processors such as use of plastic containers, water quality and poor sanitization procedures could be improved through extension and training and promotion of good practices by public and private sector players. The use of commercial enzymes for juice extraction as carried out by the formal processors can also reduce potential contamination from hands and feet and other materials that increase microbial load.

Although, the government has encouraged formation of banana beverage processing associations and cooperatives which are actually producing better quality products than individual processors, a lot of effort is still needed in strengthening their capacity from artisanal to semi-industrial processing as well as reinforcing their market linkages in order to improve their welfare.

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