



A Comparative Study on Microbiological and Physico-chemical Properties of Three Local Alcoholic Beverages Produced and Consumed in Mombasa County, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EWM, JBM, JMK, MMA and HM designed the study and wrote the protocol. Authors MLC and JBM wrote the first draft of the manuscript, managed the literature searches, analyses of the study, performed the structural equation modelling and discuss the conclusion. All authors read and approved the final manuscript.

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ABSTRACT

Physico-chemical properties and microbial contamination of three local brews (*Mnazi*, *Mchuchula* and *M'bangara*) consumed in Mombasa County were assessed. The bacteria concentration enumeration was determined by incubation of each sample for two days at 35°C to 37°C on plates containing colony forming units (CFUs) on Aerobic plate count (APC) agar and Lactic acid bacteria (LAB) on Rogosa agar plates. Growth on plates were positive for heterotrophic and LAB bacteria

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with high levels recorded in 10^{-2} aliquots of each sample. *M'bangara* recorded higher levels of LAB (201.0 ± 24.79), with *Mnazi* and *Mchuchula* having slightly low levels of LAB of 182.0 ± 26.96 and 129.0 ± 5.20 respectively despite some of the plates not being used for enumeration since they had above 250 CFUs. Yeast detection and enumeration was also determined. Low numbers of yeast cells were recorded in *M'bangara* (30.0 ± 5.72), with *Mchuchula* recording the highest number of yeast cells (66.30 ± 3.07). Physico-chemical properties determined included colour, acidity, pH and alcohol in terms of volume i.e Alcohol by Volume (ABV). *M'bangara* recorded the highest titratable acidity (0.9 ± 0.04), and lowest pH (2.7 ± 0.07) whereas the highest ABV levels were recorded in *M'bangara* (4.6 ± 0.12). Confirmatory results for total coliforms, faecal coliforms and *E. coli* indicate the absence of contamination from sewage material. There were significant variation ($p > 0.05$) in terms of physico-chemical properties and microbial contamination in the three analyzed local brews. In this study, local brews consumed in Mombasa County contain non-microbial and microbial contaminants which may be related to effects of consumption of these brews.

Keywords: Local brews; physico-chemical properties; microbial contaminants.

1. INTRODUCTION

Alcoholic beverages are among the oldest and most commonly abused substances in the world. Although they are psychoactive substances, the society has allowed their uses by the public either socially or medically [1]. They may exist in many forms; commercial and non-commercial which can either be licit and illicit [2]. They can also be categorized into various types depending on the content of ethanol in it. For example Beer 5% v/v, Wines 12%v/v, Spirit 40%v/v. Chang'aa and other traditional brews contain varying amounts of ethanol, often as high as 90% v/v. In chemical terminology, alcohols are a large group of organic compounds derived from hydrocarbons and containing one or more hydroxyl group.

Non-commercial alcohols are estimated to account for a significant portion of all alcohol consumed worldwide [3] increasing to 90% in East Africa [4]. The main local brews produced and consumed in Mombasa County include coconut palm wine (*Mnazi*), fermented sugarcane juice (*M'bangara*) and *Mchuchula*. *Mnazi* was legalized by an act of parliament and its production occupy a prime position in the cultural, social and economic lives of coastal people of Kenya. *Mnazi* provides food and livelihood security and employment opportunities to major segment of the coastal rural population in Kenya [5] contributing 60% of the coconut subsector value [6]. There is a well-established *Mnazi* production cluster in Kilifi, Mombasa, and Kwale counties, but less *Mnazi* business in Lamu, Tana River and parts of Kwale counties where Islamic religion dominates [6]. Notably, even in these districts where *Mnazi* production is

low, *Mnazi* still contribute the highest return within the coconut sub-sector productivity [6].

M'bangara is prepared from fermented sugarcane juice while the origin of *Mchuchula* is not clear but it's thought to be an adulterated form of *Mnazi*. Although, commonly produced by the local population, *M'bangara* and *Mchuchula* are illicit brews since they have not been legalized by any Act of parliament. However, they not only provide a source of recreational and social activity, but also as an informal source of income and employment to the venders, and producers. The production, sale and consumption of local brews is not reflected in government statistics but represents an important part of local economies [7]. In rural Africa, many women engage in the production and sale of local brews as their main economic activity to support their families [8]. Moreover, since these products are untaxed and can use low cost ingredients and production methods, they tend to be cheaper than their commercial counterparts [7,8].

Local brew contain a wide range of microbial flora that carry out natural fermentation of sugar-containing products converting them to ethanol under anaerobic conditions [9]. Although yeast is the major micro-organisms in many fermentation processes, involvement of other microbial flora has been reported [10,11,12,13,14,15,16,17] which may play a role in contamination and health related effects of the brews. The presence and survival of microbial flora supported by physicochemical properties, alongside other factors determines the quality of the brew. Furthermore the unhygienic conditions, innovative ways and novel ingredients used to make them more potent and maximize profits

overlook their health and safety related issues [18].

Mnazi is made from natural fermentation by tapping the juice from the young growing part of the coconut palm that bear the fruit. After removal from the fermentation container, it is left for at least 24 hours for it to mature. *Mchuchula* is thought to be an adulterated form of *Mnazi* and thus a lot of information is concerning its production is not available. *M'bangara* is produced via controlled fermentation of sugar cane juice through addition of yeast which is also left for at least 24 hours to mature before its consumption.

Despite the hygiene and safety issues, production and consumption of local brews is seemingly on the increase among low income earners [7,19]. Reported effects of the local brews on consumers range from stomach aches, loss of eyesight, and in extreme cases, even loss of lives [20,21,22], adverse health effects and health care costs, lost earnings and decreased productivity [23] to irresponsible behaviors and poor health [24,15]. Further studies conducted had no interest on whether the individual species of microorganisms found may cause health hazards. Since locally produced alcoholic beverages are not spared from contamination from either the environment or any other source, testing should be prioritized to identify the microbial contaminants that exist, together with those responsible for fermentation. The current study aims at illuminating on the types of microorganisms present and the physicochemical properties of the brews. This has the bearing of providing information for advising the producers and consumers on hygienic production, storage and consumption of local brews. Armed with the relevant information, producers can then be advised further on how to reduce the level of non-microbial and microbial contamination. Moreover, the Government can also use the information to recommend the sale or ban of certain brews for the health of its citizens.

2. MATERIALS AND METHODS

2.1 Sample Collection

Major producing and selling points of each brew were identified in Jomvu (*Mnazi*) Chagamwe (*M'bangara*) and Mvita (*Mchuchula*) constituencies where three points were randomly selected in each point and 100 mL of the brew

samples collected using 100 mL sterile bottles. Control sample for *Mnazi* was aseptically collected from the tappers whereas those of *Mchuchula* and *M'bangara* were prepared in the laboratory under controlled conditions. Three sample of each brew at the sampling points were collected. Samples collected were transported to Technical University of Mombasa (TUM), Pure and Applied Sciences Department and stored in the Microbiology laboratory under refrigeration at 4°C until sample processing.

2.2 Microbial Analysis

Microbial analysis in the samples was performed as described by Larry and James [25] according to Bacteriology Analytical Manual. The level of microorganism in the brew samples was analyzed by Aerobic Plate Count (APC) where Colony Forming Units, (CFUs) were determined. Coliforms, fecal coliforms and *E. coli* were detected by presumptive confirmed and complete tests using the 3 test tube Most Probable Number (MPN) technique. The MPN-presumptive test for *coliforms*, *fecal coliforms* and *E. coli* estimated using MPN per 100 mL was expressed as Mean \pm SEM of the estimates from each test tube containing samples. Biochemical tests; Indole production, Voges–proskauer (VP) reactive compounds, Methyl red reactive compounds and Citrate tests were used to characterize the colonies obtained as described by Peter et al. [26]. The samples were analyzed for detection of Lactic Acid Bacteria (L.A.B) using Rogosa agar where sample dilutions were plated and incubated at 37°C for two days [27]. Yeast detection and enumeration followed the same criteria as in LAB and Aerobic Plate Count (APC). The total yeast cells in each sample was enumerated by use of Sabroud dextrose agar. Aliquots of sampled brew were plated in petri dishes containing sabroud dextrose agar and incubated for 48 hours at 21°C. Yeast cell viability was determined by use of methyl blue dye and the cells were enumerated after observed under the microscope and the percentage recorded [27].

2.3 Physicochemical Analysis

The samples were analyzed for physico-chemical properties which included color by parallel comparison by the eye on a white tile, pH using a pH meter, acidity by titration using phenolphthalein indicator and Alcohol content (ABV) using a pycnometer as described by Singaravadivel et al. [28].

2.4 Data Analysis

Three independent replicates were used per analysis and the results were expressed as mean values \pm standard error of mean. Analysis of variance (ANOVA) followed by Duncans' test ($P \leq 0.05$) was used for comparison and separation of mean microbial load between brews. All statistical analysis was carried out by GenStat discovery 14th Edition.

3. RESULTS

3.1 Microbial Analysis

As indicated in Table 1, positive results were obtained in all test tubes containing the 10 mL of the brews (Fig. 1).

The MPN value of *Mnazi* was the lowest followed by *Mchuchula* while *M'bangara* had the highest MPN value (Table 1). The high MPN values might be due to poor handling during preparation of the *M'bangara* and *Mchuchula*. Natural fermentation in *Mnazi* might be the cause of lower MPN values in *Mnazi* as compared to and *M'bangara*.

The APC revealed the availability of microbes in the brews. Upon dilution, aliquots growth was observed with 10^{-2} having a dense growth on the plates indicating higher CFUs/mL (Table 2).

After dilution and further incubation, some of the plates indicated growth of LAB (Table 3), with high levels of CFUs/mL in *Mchuchula* and *M'bangara* respectively.



Fig. 1. Test tubes showing (a) positive and (b) negative MPN-presumptive test for coliforms, fecal coliforms and *E. coli*

Table 1. Most Probably Number (MPN) index for the different brews and their control

MPN index per 100 mL \pm SEM					
Sample brews			Control brews		
<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>
264.35 \pm 87.07	526.09 \pm 59.88	886.04 \pm 174.19	165.0 \pm 31.82	185.06 \pm 10.61	135.03 \pm 21.21

Table 2. Colony forming units (CFUs) on plate count agar on different brews under different serial dilutions

Dilution	Sample brews			Control brews		
	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>
10^{-2}	>250	>250	>250	273.05 \pm 5.77	251.5 \pm 4.91	269.5 \pm 1.44
10^{-3}	200.0 \pm 21.36	>250	>250	131.5 \pm 2.02	123.0 \pm 3.46	130.5 \pm 4.33
10^{-4}	158.0 \pm 17.03	95.0 \pm 24.54	109.0 \pm 23.04	77.0 \pm 2.31	59.5 \pm 2.02	44.5 \pm 4.91

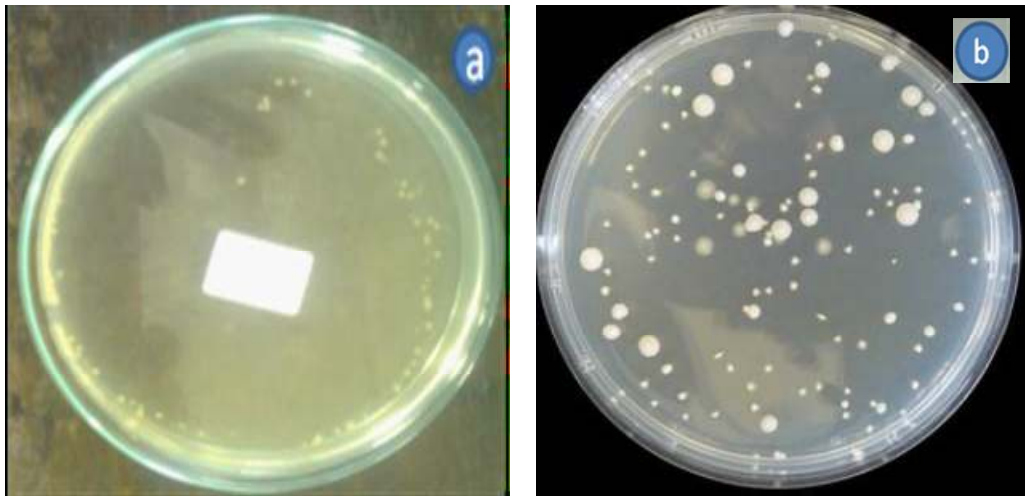


Fig. 2. (a) A plate with enumerated microbes (b) A plate with lactic Acid Bacteria colonies

Table 3. Lactic acid bacteria colony forming units (CFUs) on plate count agar on different brews under different serial dilutions

Dilution	Sample brews			Control brews		
	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>
10^{-2}	N/A	N/A	N/A	49.5±1.44	48.5±2.02	49.5±3.75
10^{-3}	N/A	129.0±5.20	201.0±24.79	20.0±1.73	29.5±3.75	26.5±1.44
10^{-4}	182.0±26.96	117.0±4.48	117.0±12.25	14.0±2.31	15.0±0.58	15.5±1.44

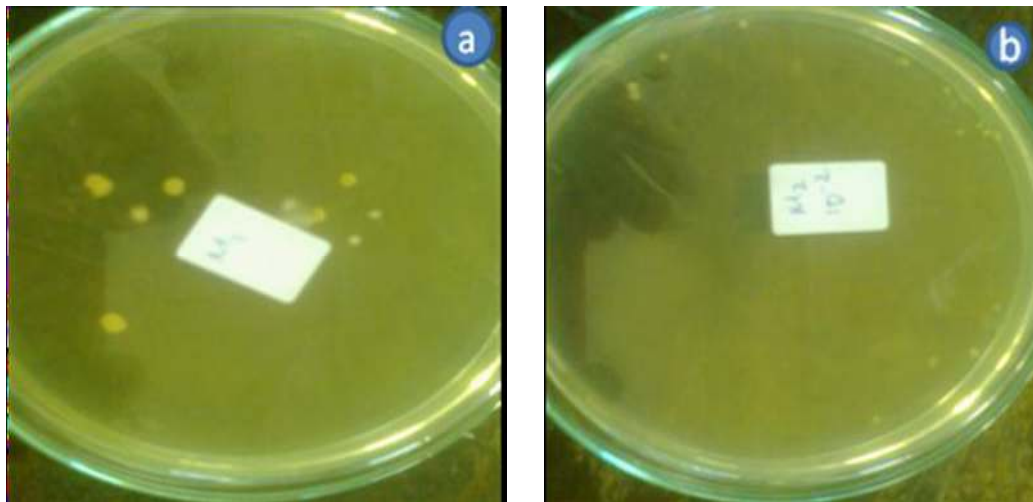


Fig. 3. Plates indicating positive growth of yeast cell after incubation of the samples on sabroud dextrose agar

The total yeast cells present in each sample was enumerated. Positive results on the samples are presented in Fig. 3.

The number of yeast cells (CFUs/mL) on the different brew samples are presented in Table 4. The percentage yeast cell viability which is an

indicator of the brews toxicity was calculated and the results are presented in Table 5. *Mchuchula* had the highest yeast cells compared to *Mnazi* and *M'bangara* in all the dilutions made. This is attributed to the fact that yeast is one of the ingredients in the manufacture of *M'bangara*.

Table 4. Yeast counts on plate count sabroud dextrose agar on different brews under different serial dilutions

Dilution	Sample brews			Control brews		
	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>
10 ⁻²	41.70±2.60	66.30±3.07	30.0±5.72	49.50±1.44	33.50±0.87	31.00±1.15
10 ⁻³	29.70±3.66	32.7±4.72	19.7±7.41	21.50±0.87	16.50±0.87	16.00±0.58
10 ⁻⁴	11.30±1.19	20.0±1.70	0.70±0.54	18.0±1.15	13.50±1.44	14.00±0.58

Table 5. Percentage viable of yeast cells on sabroud dextrose agar on different brews

Parameter	Sample brews			Control brews		
	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>
No. of Viable Cells	39.7±1.36	45.7±5.19	27.3±4.72	47.5±2.60	40.0±2.31	28.0±2.31
Total No. of Cells	75.0±5.25	71.3±8.09	77.0±2.16	79.5±2.02	69.5±3.75	57.5±0.87
% Viability	52.9±2.19	64.0±3.16	35.5±6.71	59.7±1.75	57.6±0.22	48.7±3.29

Table 6. Physico-chemical properties of different brews

Parameter	Sample brews			Control brews		
	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>	<i>Mnazi</i>	<i>Mchuchula</i>	<i>M'bangara</i>
Color	Milky White	Brown	Brown	Milky White	Brown	Brown
% Acidity	0.8±0.05	0.4±0.02	0.9±0.04	0.39±0.02	0.59±0.01	0.59±0.03
pH	3.4±0.05	3.4±0.03	2.7±0.07	4.1±0.09	4.5±0.06	4.2±0.09
Alcohol	3.7±1.67	2.2±0.09	4.6±0.12	5.0±0.09	3.4±0.06	5.2±0.09

Highest percentage viability of the yeast cell was recorded in *Mnazi* than *M'bangara* and *Mchuchula*. This indicate the less toxic effects of *Mnazi* as compares to the other brews.

3.2 Physico-chemical Properties

The results on the physico-chemical properties of the brews are summarized in Table 6.

4. DISCUSSION

The consumption of local alcoholic beverages among the Kenyan Coast is rampant. These local brews such as *Mnazi* has been associated with irresponsible behaviors and poor health especially among men and youths in the Kenyan coast [24,15]. Notable in this study the CFUs/mL among the three brews, *Mnazi* had the highest compared to the other two. This perhaps may be the contributing factor of the health related effects reviewed by several literature [24,15]. Moreover, most plates of *M'bangara* and *Mchuchula* had growth of colonies exceeding 250 CFUs, thus the highest counts. The APC bacteria included a wide range of bacteria, comprising of both primary and secondary pathogens which have been attributed to increase in the chances infections [29].

The high MPN in the presumptive test results recorded in all the three alcoholic beverages indicated concentration with total coliforms. However, the confirmatory MPN test was negative. Therefore the contamination of the brews may be from other sources as reported [30,31]. *M'bangara* and *Mchuchula* was highly contaminated with coliforms compared to *Mnazi*. This may be attributed by the fact water is used as a major raw material thus suspected to be the source. The *E. coli* as a fecal coliform indicator was not found to be present as no growth of colonies was observed on EC broth, indicating no fecal contamination on the sample.

The LAB are the most frequent encountered spoilage bacteria in fermenting products and render the products unpalatable due to production of lactic acid [32]. Despite the ability and advantages of LAB to act as probiotics, conferring healthy benefits to the user [33], they have been implicated and associated with infections such as bacteremia, endocarditis and dental carries [34]. The presence of LAB in all the brews; *Mnazi*, *M'bangara* and *Mchuchula* indicate that the users are at risk to suffer from these kinds of infections. Yeast cell count and viability were highest in *Mnazi* followed by *Mchuchula*, attributed to low levels of LAB leading to low production of acids making it

favourable for their growth. However, the yeast cell count and viability of *M'bangara* reduction was attributed to increase in LAB leading to increase in acidity hence unfavourable environment for growth. The result of this study therefore indicate that *Mnazi* is safer to the user as compared to *M'bangara*. Since *Mchuchula* is thought to be an adulterated form of *Mnazi*, it has an agreeable amount of safety which conforms with the results of this study.

Physicochemical properties of a brew determine and differentiate a brew from others. Among these properties is color. Color as characteristic differentiated the brews, *Mnazi* was milky white which contrasted with the standard sample which was nearly a colorless liquid. The milky white color of *Mnazi* is suspected to be due to increased microbial suspension [35,36] and adulteration by either the producer or the vendors. *Mchuchula* had a distinctive yellow brown color which differentiated it from *M'bangara* which was brown in color. The two slightly differentiated from their standards. The brown color in *Mchuchula* is suspected to be from the molasses added during processing. The pH of the samples varied, with *M'bangara* having the highest pH. Drop of pH from the standard can be due to increase in LAB which facilitated the production of lactic acid hence reduction in pH levels. The highest titratable acidity was recorded in *M'bangara*. This high values can be linked to high levels of LAB which ferment sugars into lactic acid. Further, the high ABV levels were due to presence of high levels of fermentable sugars and yeast cells.

Significant levels of alcohol were produced by the brews. However, *M'bangara* recorded the highest alcohol content. Although there was decrease in yeast cell count and viability, the LAB could be the source of the high levels of alcohol recorded in *M'bangara* due to mixed fermentation. However, all the standards had low alcohol production compared to samples which can be attributed to reduction in yeast cell count and viability. The LAB produced lactic acid lead to high levels titratable acidity thus low pH lowering the numbers of yeast cells.

Based on the results obtained from this study on microbial load and physico-chemical properties, the brews tested are contaminated with both non-microbial and microbial contaminants which may affect the health of the consumer. Contamination of these local brews is suspected to be due to unsterile handling during production, processing, collection, storage and serving containers [37].

From the studies it can be concluded that the presence of microbes pose a health risk to consumers especially to opportunistic infections increasing their vulnerability to such infections. Presence of LAB, considered a spoilage bacteria in fermentation, in the brews could offer health benefits as they can act as probiotics, although the in high levels recorded could a health risk to the consumer.

5. CONCLUSION

From the results of this study, contamination of local brews may be attributed to unsterile handling and processing before sale. Therefore collecting, storage and serving containers may be major sources of contamination. Contamination of this brews has direct effect on the consumers' health both positively and negatively. From the studies it can be concluded that brews served for sale in Mombasa County are contaminated. High levels of APC indicate presence of many bacterial species which pose a health risk to consumers. Although, the presence of LAB, considered a spoilage bacteria in fermentation, this could offer health benefits as they can act as probiotics. However, the high numbers recorded in the brews could surpass this ability thus the health related risk of these brews.

6. RECOMMENDATION

Although *Mnazi* was legalized by an act of the parliament by the Government of Kenya, only its consumption is monitored. Therefore there is need to monitor its production processes to ensure high quality products to the consumers. In this context therefore it will of great value to the public to analyze sample for the different parameters conducted in this study in national laboratories such as Government Chemistry to ascertain its safety before being released to the market. Further, research should be conducted to look at ways of preserving and packaging to increase its self life. More studies on the brews should be conducted to characterize specific microbes present in brews and their effect on consumers' health. Moreover, concentration of different types of alcohols of the brews such as methanol and the types and level at which contamination of the brews occurs should be ascertained.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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