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DETERMINATION OF THE LEVELS OF NITRITE IN HOMEMADE BREWS, SPIRITS AND RAW MATERILS USED USING UV – VISIBLE SPECTROSCOPY IN NAIROBI COUNTY

Jeremiah O. Masime*, Ruth Wanjau, Charles Onindo

Kenyatta University, Chemistry Department, School of Pure and Applied Sciences

ABSTRACT

Homemade brews and the raw materials used can be a major source of nitrate and nitrite in human diet. Because of the potential health hazards result in high intake of nitrate and nitrite, determination of these ions content in Homemade brews and the raw materials used. Sources of nitrites include vegetables, fruit, and processed meats The aim of this research was to determine the concentration of nitrite in Homemade brews and the raw materials sampled from eleven stations in Nairobi County - Kenya. So, a total of one hundred and thirty two (132) home-made alcoholic beverages, forty eight (48) water and eighteen (18) raw materials samples were analyzed for nitrite. These were analyzed using, a SHADZU (Model No. UV-2550) UV-Visible spectrophotometer. The measurement was based on ISO Method (N0.6635). The detection limit and analitation limit of nitrite determination were found to be 0.93 µg/ml and 2.82 µg/ml respectively. The recommended maximum contamination levels set by Kenya Bureau of Standards/World Health Organization for nitrate in alcohols is nitrite 0.003 mg/l Data has been analyzed with p value and ANOVA. According to the results, the average of nitrite content in all of the samples was more than standard limits. Homemade brews and the raw materials used had significantly higher nitrite differences ($p<0.05$).

1.0 INTRODUCTION

Sources of nitrites include vegetables, fruit, and processed meats. Nitrite is used predominately as a food preservative, especially in cured meats. It has been used as a food preservative and anti-butulinonl agent for decades [1]. This ion has been used to prevent the growth of the spore-forming bacterium *clostridium botulinum*, whose toxin causes botulism, leading to paralysis and potential death. This been a subject of controversy since the 1970's, when some of its reaction products (i.e. nitrosamines) were associated with cancer [1]. A significant amount of nitrite in the body is produced endogenously internally, rather than introduced from dietary sources [1]. Nitrites are produced endogenously through the oxidation of nitric oxide and through a reduction of nitrate by commensal bacteria in the mouth and gastrointestinal tract. The body generates nitrite through normal nitrogen metabolism in which nitric oxide is produced, then converted to nitrite or nitrate in order to be excreted. Nitrite is formed when Nitrosomonas species of bacteria oxides ammonia produced by decomposing organic matter. Nitrite has been used as a food preservative and anti-butulinonl agent for decades [1]. It has been used to prevent the growth of the spore-forming bacterium *clostridium botulinum*, whose toxin causes botulism, leading to paralysis and potential death. It has been a subject of controversy since the 1970's, when some of its reaction products (i.e. nitrosamines) were associated with cancer [1]. Nitrite can form a variety of N-nitroso compounds by reacting with proteins in the stomach. Some of these compounds have been found to cause cancer in animals. However, according to the USEPA, the data is inadequate to determine whether exposure to nitrate and nitrite in drinking water can result in human cancer. Nitrite is mainly produced as a result of oxygen depletion, anaerobic biological conditions dominate and reduction sets in [2]. The variability of nitrates, nitrites, and nitrosamines in food items may also be a source of error. The scarcity of reported nitrate and nitrite values for alcoholic beverages makes it difficult to create relevant estimates. Potentially, the nitrate/nitrite content of water used to produce

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non-distilled beverages could be used to estimate these compounds; however, this information was not available [3].

Nitrate is reduced to nitrite, then to nitrogen gas. The conversion of nitrate to nitrite occurs readily under alkaline conditions. Nitrite is absorbed in the intestine into the blood stream. At normal levels, nitric oxide is a life supporting biological messenger that helps heal wounds and burns, promotes blood clotting, controls blood pressure, enhances brain function, and boosts immunity to kill tumor cells and intracellular parasites [1]. Moreover, when nitrite is acidified in the stomach it stimulates antimicrobial activity. It protects the human stomach against other food borne pathogens [1]. At the end, the objectives of this research were to;

1) determine the amount and the variability of the nitrite content in homemade brews and the raw materials used in Nairobi County by UV-Visible Spectrometry,

2) evaluate the relative safety of these homemade brews and the raw materials based on the maximum levels of European Commission Regulation (EC) No. 194/97.

Nitrite levels in most water supplies are very low. The Federal-Provincial-Territorial Committee on Drinking Water has established a guideline for nitrite of 3.2 milligrams per litre of drinking water [4]. The maximum contamination limit for nitrite is 0.003 mg/l [5].

1.2 Raw materials for home brewed alcoholic beverages and spirits in East Africa

If a starchy food grain is fermented, it produces enzymes, which start to break the starch down into sugar. This is how growing plants derive energy; and this is how people release sugar from grains so that they can make alcohol from them, by brewing the grains into beer. In the nineteenth century, brewing was the usual process of making alcoholic beverages in most parts of East Africa; mostly the grain used was finger millet (*elevisine)* but some people used sorghum. Busaa is prepared from cereals, chang'aa is a distilled brew consumed in most parts of Kenya [6]. They are made from a variety of grains - malted millet and malted maize being the most common. It has a pleasant sweet flavour and contains at least 50% alcohol [6]; miti is prepared from boiled roots and honey, while muratina is prepared from sugarcane or honey, which is fermented using sausage plant (K*igelia african*). The conditions and raw materials used to prepare these home made brews/spirits may introduce toxic materials into the alcohols and hence the need for continuous monitoring of the levels of nutrients in the alcohols to make sure that the population is not exposed to dangerous levels.

In the twentieth century, maize has become a common ingredient in the making of alcoholic brews [6]. There are many other raw materials as well, which include bananas, coconuts, palm fluid, honey, pineapples, paw paws and many other fruits. Some of the techniques used are by no means new. Other techniques like those for distilled brews are new. Brewing from grain takes several days. In most cases there is no attempt to control the yeast other than the constant reuse of the same containers for brewing. Once brewed, the beer lasts for only a day or two; as a 'live' brew, spoils quickly, and if not drunk within about forty-eight hours it will be spoiled. Nor can it be transported any great distance, for the continuing fermentation produces gases, which make it impossible to seal the beer in a container [6].

1.3 Health hazards of nitrites

In the blood nitrite combines with hemoglobin preventing carrying of oxygen resulting in a condition called methaemoglobinemia, which can result in death [8]. Most humans over one year of age have the ability to rapidly convert

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methemoglobin back to oxyhemoglobin; hence, the total amount of methemoglobin within red blood cells remain low in spite of relatively high levels of nitrate uptake [9].

The best-known effect of nitrite is its ability to react with haemoglobin (oxyHb) to form methaemoglobin (metHb) and nitrate:

$$NO_2^- + oxyHb(Fe^{2+}) \rightarrow metHb(Fe^{3+}) + NO_3^-$$

As a consequence of the formation of metHb the oxygen delivery to tissue is impaired.

For infants under six months of age, the digestive system has an underdeveloped capability to secrete gastric acid, thus the pH level in the digestive system may rise. At a higher pH, bacteria levels may rise, increasing the transformation of nitrate to nitrites [9]. In addition, the enzyme systems for reducing methemoglobin to oxyhaemoglobin are incompletely developed in infants under six months of age. Thus, methamoglobinemia can occur, resulting in asphyxia. Symptoms include shortness of breath and blues of the skin [9]. Older persons who have a gastrointestinal systems disorder producing high pH level which allows for increased bacteria growth may be at greater risk.

1.4 Ultraviolet and visible absorption spectroscopy (UV-Vis)

This was done according to specification in the Eurasian Journal of Analytical Chemistry 4(2): 204-214, 2009, Pgs 204-214 [10].

2.0 METHODOLOGY

2.1 Samples and sampling

Sixteen (16) stations were targeted and ten samples of each brew and water were selected. A total of one hundred and thirty two (132) home-made alcoholic beverages, forty eight (48) water and eighteen (18) raw materials samples were analyzed for nitrate and nitrite. Six different raw materials were selected. Three samples of each were obtained from various places in the sixteen stations. These samples were randomly obtained from various parts of Nairobi and outskirts taking into account the requirements for the preparation of the brews. This information was obtained from the people who sold the brews. Sample of raw materials were obtained from market places nearest to the beverage sampling stations. A 100 ml samples were collected directly into specially cleaned, pretested, polypropylene bottles using sample handling techniques specially designed for collection of sample for the analysis of metals at trace levels. The samples were then either laboratory preserved by the addition of 5 ml of pretested 10 % HNO_3 per litre of sample, depending on the time between sample collection and arrival at the laboratory.

1.5 Sample size and study site

A total of 16 sampling stations were chosen on the basis of the home made alcohol brewing and drinking activities on those areas together with general financial status of people living in those areas. Most of the occupants are known to be living below the poverty level. The sampling stations were majorly the slum areas around Nairobi for example; Gikomba, Githurai, Kibera, Kawangware, Kangemi, Mathare, Runda and Uthiru. This sampling was carried out between December 2003 and August 2004. Map showing some of the sampling stations is given on figure 3.1.

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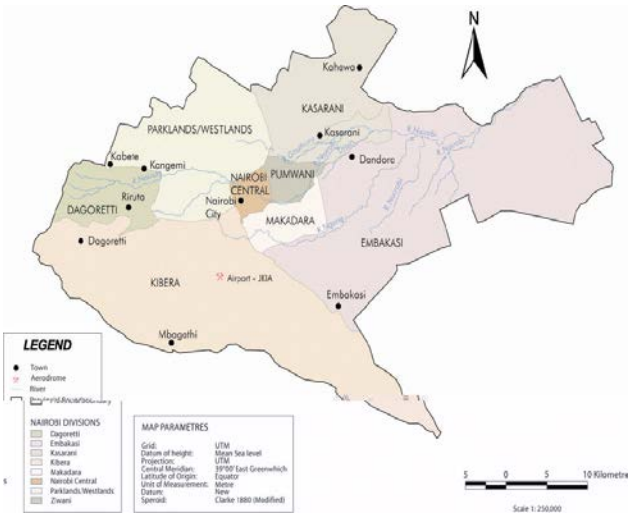

Figure 2.1: The Map of Nairobi County

Three samples of the various brews and spirits to be analyzed were collected from each of the 16 stations that is, Kibera (S_1), Kariobangi (S_2), Kawangware (S_3), Gikomba (S_4), Githurai (S_5), Uthiru (S_6), Kangemi (S_7), Mathare (S_8), Kiambu (S_9) and Runda (S_{13}) in Nairobi (Figure 2.1).

2.2 Nitrate and nitrite standards

All chemicals for nitrite and nitrate analysis were analytical reagent grade. Doubly distilled water was used in the preparation of all solutions in the experiments. Working standard solutions were prepared by appropriate dilution. Sulfanilic acid (0.5 g in 100ml water) and methyl anthranilate (0.5 ml in 100 ml of alcohol) were used. The following reagents were prepared by dissolving appropriate amountsin water 2 M of HCl and 2 M NaOH (Narayana *et al.,* 2009).

Nitrite stock solution (1000 µg/l) was prepared by dissolving 0.1500g sodium nitrite in water and diluting to 100 ml. Nitrate stock solution (1000 µg/l) was prepared by dissolving 0.7220 g potassium nitrate in water and diluting to 100 ml.

2.4 Blank solutions

Two blank solutions each 250 ml were made and approximately 100 ml of 1M hydrochloric acid added to each of the 250 ml volumetric flasks, followed by 20 ml of sulphuric acid (AnalaR) [11].

2.5 Equipment blanks

Before any sampling equipment was used at a given site, the laboratory or cleaning facility was required to generate equipment blanks to demonstrate that the sampling equipment was free from contamination. Two types of equipment blanks were required: bottle blanks and sampler check blanks.

2.6 Bottle blanks

After undergoing appropriate cleaning procedures, bottles were subjected to conditions of use to verify the effectiveness of the cleaning procedure. A representative set of sample bottles was filled with reagent water acidified to pH < 2 and allowed to stand for a minimum of 24 hours. After standing, the water was analyzed for any signs of contamination. If any bottle showed slight contamination, the problem identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles re-cleaned.

2.7 Instruments and apparatus

All the weighing were done using a research analytical balance (Sartorious research, R 200D, model-40110044, Analos, Belgium). Other apparatus included the following; graduated pipettes (10 and 5 ml), micropipettes (200 ml) and tips, test tubes (13 x 100 ml), small square of parafilm, volumetric flasks (50 and 100 ml) and computer.

2.8 UV-visible spectroscopy instrument

Nitrite were analyzed using, a SHADZU (Model No. UV-2550) UV-Visible spectrophotometer with 1 cm matching quartz cell

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were used for the absorbance measurements. A WTW pH 330 pH meter was used [10].

2.9 Cleaning of apparatus

Cleaning of apparatus was adopted from Mendham *et al*, (2002), and AOAC (2000) [12]. Research apparatus as recommended by Association of Official Analytical Chemists (AOAC) were used. Sampler check blanks were generated in the laboratory or of the equipment cleaning contractor's facility by processing reagent water through the sampling devices using the same procedure sampling i.e. bottles were cleaned with liquid detergent and thoroughly rinsed with reagent water.

The apparatus were cleansed using detergents, water, rinsed with distilled de - ionized water and dried overnight in the oven at 100 °C.

3.0 Sample collection and pretreatment

The brew sample bottle (acid-washed, 125 ml polyethene bottle) were rinsed 3 times before sampling. Filled to approximately 2/3 full, tighten cap and freeze cruise, cast Niskin bottle number were recorded on the bottle and data sheet. All the brew sample bottles were first rinsed with the alcohol for alcohol samples before the brew samples were collected. The samples were then filtered, the residue discarded and the filtrates from home made brews were decolorized using activated charcoal and re-filtered until the colour disappeared.

3.1 Raw materials

In the sample pretreatment, modified procedures for washing and drying proposed by Santos *et al.* [13] and Kawashima & Soares [14], respectively, were used. First, each raw material samples were rinsed with distilled water to remove dirt and other debris. Then the raw material samples were brushed with polypropylene bristles and washed with deionized water. The raw materials were then grated with a polypropylene grater into porcelain containers. Then the containers with the raw material samples were dried in a laboratory oven at 65 ± 5 ºC for 24 h or until reaching constant weight. Immediately afterwards, the samples were stocked in polypropylene beakers and covered with a PVC film. Finally, they were stored in a desiccators awaiting digestion [15].

3.2 Digestion of raw materials

One (1) gm of the raw materials was weighed and digested using 6 ml of concentrated HNO_3, 0.5 ml of concentrated Hydrochloric acid and 1 ml of H_2O_2 were added to each one in Teflon vessel in order to dissolve the organic matter. These were placed in the microwave and digested for 30 minutes. A multiwave 300 microwave oven (Perkin-Elmer, Shelton, CT USA) was used for the microwave-assisted digestion [16]. A predidested spike of nitrite was added to some of the samples to measure analyte recovery through the digestion process. The vessels were sealed and placed into the rotor for the microwave digestion. After digestion process, the digested products were transferred to polypropylene 50 ml auto sampler vials (Perkin Elmer part number B0193234) and laboratory ASTM type 1 water was added to a final total weight of 25 gms of the container and its content [16]. The resulting solution was transferred into a 15 ml centrifuge tube and made to the mark with deionized water.

Table 3.2: Microwave digestion program [16]

Step	Power	Ramp (min)	Hold (min)	Fan speed
1	750	10	10	1
2	1200	10	10	1
3	0 (cool Down)	0	15	3

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To ensure a safe digestion, the multi wave 3000's IR sensor measures the temperature of each vessel. If a vessel nears its maximum operating temperature of 260 °C then the microwave oven automatically decreases the applied power. Also, the pressure sensor sends data to the multi wave oven controller during the digestion. The multi wave oven will automatically reduce power if the maximum pressure of 60 bars was applied [16].

3.3 Digestion of brews

No digestion is performed on unfiltered samples prior to analytical determinations. Portions of 20 ml of the neutralized filtered brew samples were evaporated to dryness in separate beakers. The residues were each cooled and extracted with 1 ml phenol disulphonic acid {prepared from 25 g of phenol crystals (BDH Chemicals Ltd, Poole, UK), 150 ml of concentrated H_2SO_4 (Fischer Chemicals, UK), 75ml of fuming H_2SO_4 (Fischer Chemicals, UK)} and each mixture heated for 2 hours on water bath. All samples (homemade brews, water, raw materials) and blanks (n=3) were digested and diluted using the same procedure.

3.4 Sample analysis

Samples were analyzed using UV-Visible spectroscopy**.** The maximum holding time for NO_2-N was 48 hours. The concentration of the nutrients in solution was determined by measuring the absorbance. Samples were analyzed for nitrite at 493 nm, and then applying the Beer-Lambert law the concentrations of the solutions were obtained.

3.5 Sample analysis for nitrite in UV-visible spectroscopy

Aliquots of stock solution containing 0.2-8.0 µg /l of nitrite were transferred into series of 10 ml calibrated flask. To each flask, 1 ml of 0.5 % sulfanilic acid and 1 ml of 2 mol/l hydrochloric acid solution were added and the solutions were shaken thoroughly for 5 minutes to allow the diazotization reaction to go to completion. Then, 1 ml of 0.5 % methyle anthranilate and 2 ml of 2 M sodium hydroxide solution were added to form an azo dye and the concents were diluted to 10 ml using water. After dilution to 10 ml with water, absorbance of the red colored dye was measured at 493 nm against the corresponding reagent blank and the caliberation curve constructed [10].

3.6 Data analysis

The quantitative relationship between absorbance and concentration was first done by using a standard curve (calibration curve). In this case our standards were known concentrations of phosphate as indicated in appendix 10. The concentration of each solution was calculated based on the regression equation for data analysis. P-values and ANOVA tests were used in data analysis.

4.0 Concentrations of nitrites in various homemade brews, spirits and tap water

The levels of the nitrites in various types of home made brews and spirits from various places were determined using the UV-Visible spectroscopy and the results obtained for various samples are presented in Table 4.1 and Figure 4.1.

From these, the highest levels of nitrite were obtained in Muratina from Mathare which had the concentration of 11.50 ± 0.67 mg/l. The lowest nitrite levels were obtained in Miti from kibera with a concentration of 0.14± 0.04 mg/l. The average levels of the nitrites were relatively high in some brews but low in spirits and water which had non detectable levels of the nutrient. Busaa from Kibera, Kariobangi, Kawangware, Gikomba, Githurai, Uthiru, Mathare and Kangemi had high levels of nitrite ranging from 1.01 ± 0.02 mg/l for Kibera to 11.10 ± 0.95 mg/l for Mathare. Nitrite levels in Chang'aa ranged from 0.58 ± 0.11 mg/l in the brew from Kibera to non detectable levels. Chang'aa from Kibera and Kariobangi had

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detectable levels of nitrites ranging from 0.34 ± 0.04 mg/l mg/l to 0.58 ± 0.11. The rest of the chang'aa samples had non detectable levels. The nitrite levels in Miti ranged from 0.14 ± 0.04 mg/l for Miti from Kibera to 1.78 ± 0.44 mg/l in Mathare. The nitrite levels in Muratina ranged from 1.18 ± 0.02 mg/l for Muratina from Kariobangi and Kawangware to 11.50 ± 0.67 mg/l for the brew from Mathare. Kumi kumi and Kangara had only one value analyzed since they were obtained from one site each, due to unavailability of sample at that time, hence they were not significant. Busaa from Mathare had the highest concentration of nitrite at 11.01 ± 0.95 mg/l and the lowest level of 1.04 ± 0.52 mg/l in Gikomba. Kangara had the highest mean concentration of nitrite ions of 6.85 ± 0.40 mg/l, followed by muratina at the level of 6.18 ± 5.35 mg/l and Chang´aa had the lowest mean concentration of 0.12 ± 0.22 mg/l. The concentration of nitrites in busaa, muratina and kangara were generally above the maximum allowable limit of 0.003 mg/l [5]. Hence they pose health danger to the consumer. All brews except, chang'aa and kumi kumi had levels higher than the maximum contamination limits. The higher concentration in these brews could be attributed to pollution from another source. The source of the high nitrite concentrations could be attributed the use of nitrogenous fertilizers used for growing raw materials or organic decomposition during the fermentation process, use of contaminated river water and some additives in some cases.

As shown in the tables 4.2, 4.3 and 4.4 the probability of the between-treatments MS being ≥ 3.098 times the within-treatments MS. The mean was 4.117, the median 1.41 and the SD was 4.516. We can therefore conclude that statistically there were significant differences between the concentrations of nitrates in the brews means as determined by one-way ANOVA (F = 3.098, p = 0.019). All at considered at 95 % confidence interval. In all the homemade brews the value of $p < 0.05$, implying that there were significant differences in the levels of nitrite in the homemade alcoholic beverages.

From these values we can conclude that the levels were in some cases higher than the maximum contamination levels of 0.03 mg/l for water [5], Kenya Bureau of Standards did not have standards for nitrites in alcoholic beverages. Though in some cases, concentrations were found to be higher than the maximum limits of 2.0 mg/l set by AMPHORA for alcohol [17].

4.1 Concentrations of nitrite in various raw materials

The levels of nitrites in the raw materials used to prepare the home made brews were determined using UV-Visible spectroscopy and the results are presented in the Table 4.5 and Figure 4.5.

From here, honey had the highest concentration of nitrite ions of 193.00 ± 18.52 mg/kg, followed by sorghum at 180.00 ± 16.02 mg/kg and Jaggery at 145.00 ± 47.79 mg/kg. Millet seeds had the lowest concentration of nitrite at 116.00 ± 41.93 mg/kg. Maize, millet and sorghum used in the preparation of Busaa had relatively low levels of nitrite, 130 ± 7.69, 116 ± 41.93 and 180 ± 16.02 mg/kg respectively. Honey which is used in the preparation in miti and muratina had a mean level of 193 ± 18.52 mg/l. All these means were higher than those observed in the brews, indicating that the raw materials could be their source. The levels of nitrites in the raw materials were found to be higher than the maximum allowable limits set by the WHO of 2 mg/kg [8]. The high concentration must be due to the use of nitrogenous fertilizers together with the reactions that take place during the fermentation process. Figure 4.4 illustrates that the levels of nitrite in the raw materials were generally high. The high concentration must be due to the use

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of nitrogenous fertilizers. This implies that the raw material whose nitrite concentrations were generally high contributed positively towards elevating the concentrations of the nutrient in the brews/spirits.

The mean levels of nitrites in various raw materials used were to determine whether there was any significant difference between the levels of nitrites in the various raw materials using the ANOVA test.

As shown in the table 4.6, 4.7 and 4.8 the probability of the between-treatments MS being ≥ 3.609 times the within-treatments MS. The mean was 148.2, the median 137.5 and the SD was 31.42. We can therefore conclude that statistically there were significant differences between the concentrations of nitrates in the raw materials used means as determined by one-way ANOVA ($F = 3.609$, $p = 0.032$). All were considered at 95 % confidence interval. In all the raw materials the value of $p < 0.05$, implying that there were significant differences in the levels of nitrite in the raw materials used.

SUMMARY AND CONCLUSION

105 out of 132 samples analyzed had levels above the maximum contamination levels of 0.03 mg/l level for water. These levels exceeded the maximum levels recommended by the World Health Organization [18]. Contaminant levels measured in the home made alcoholic beverages more likely implied high levels of nitrite from water and the raw materials used in the brewing processes.

Table 4.1 The levels of Nitrite in homemade brews

BREW / PLACE	BUSAA [n = 24]	CHANG'AA [n = 33]	MITI [n = 24]	MURATINA [n = 33]
KIBERA	1.01 ± 0.02	0.58 ±0. 11	0.14 ±0.038	1.18 ± 0.06
KARIOBANGI	10.10 ± 0.19	0.34 ± 0.04	0.18 ±0.04	1.18 ± 0.02
KAWANGWARE	1.05 ± 0.07	ND	1.60 ± 0.17	1.18 ±0.02
GIKOMBA	1.04 ± 0.52	ND	0.17 ± 0.04	1.20 ± 1.14
GITHURAI	3.83 ± 1.48	ND	1.49 ± 0.33	11.40 ± 0.65
UTHIRU	10.40 ± 0.63	ND	1.39 ± 0.37	10.30 ± 0.73
KANGEMI	1.08 ± 0.92	ND	1.43 ± 0.27	11.50 ± 0.57
MATHARE	11.10 ± 0.95	ND	1.78 ± 0.44	11.50 ± 0.67
RUNDA	NA	NA	NA	NA
MEAN	4.95± 4.725	0.12 ± 0.22	1.02 ± 0.72	6.18 ± 5.35

BREW / PLACE	KUMI KUMI [n = 3]	KANGARA [n = 3]
KIBERA	0.39 ±0.50	NA
KARIOBANGI	NA	NA
KAWANGWARE	NA	NA
GIKOMBA	NA	NA
GITHURAI	NA	NA
UTHIRU	NA	NA
KANGEMI	NA	NA
MATHARE	NA	NA
RUNDA	NA	6.85 ± 0.40
MEAN	0.39 ±0.50	6.85 ± 0.40

NA = Not analyzed **ND = Not detected**

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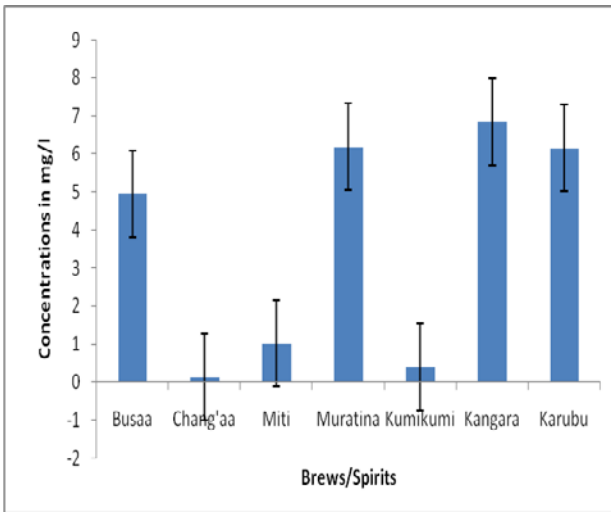

Figure 4.1: Overall mean concentrations of nitrites in various homemade brews/spirits with standard error bars

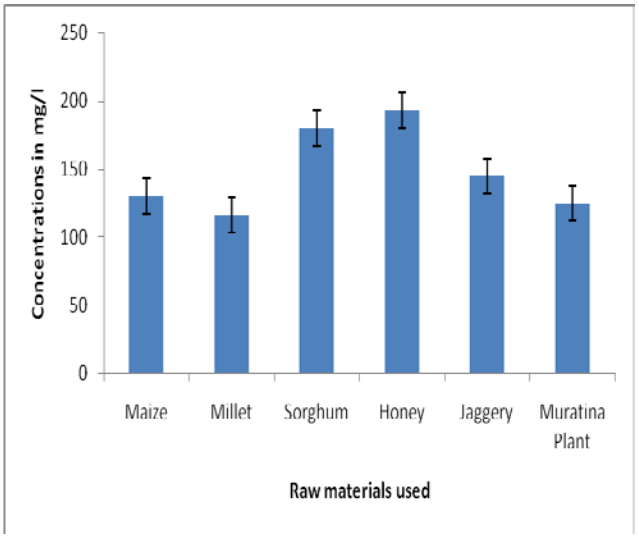

Figure 4.5: Mean concentrations of nitrites in raw materials in mg/kg with standard error bars

Table 4.2 ANOVA values for nitrite in homemade brews

	SS	df	MS	F	p
Between	239.369	6	39.895	3.098	0.019
Within	360.604	28	12.879		
Total	559.973	34			

Table 4.5: Average levels of nitrite in various raw materials [Mean ± SD]

Raw materials	Nitrite (mg/kg) [n = 18]
Maize	130 ± 7.69
Millet	116 ± 41.93
Sorghum	180 ± 16.02
Honey	193 ± 18.52
Jaggery	145 ± 47.79
Muratina fruit	125 ± 14.90

Table 4.6 ANOVA values for Nitrites in Raw Materials

	SS	df	MS	F	p
Between	14804.500	5	2960.900	3.609	0.032
Within	9845.572	12	820.464		
Total	24,650.072	17			

Table 4.3 Descriptive Table Statistics for nitrite in homemade brews

Descriptive Statistics:	
Minimum	0.14
Maximum	12
Range	11.86
Count	30
Sum	123.52
Mean	4.117
Median	1.41
Mode	1.18
Standard Deviation	4.516
Variance	20.39

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Mid Range	**6.07**
Quatiles	**Quartiles:** **Q_1 --> 1.04** **Q_2 --> 1.41** **Q_3 --> 10.1**
Interquartile Range (IQR)	**9.06**
Sum of Squares	**591.3**
Mean Absolute Deviation:	**4.008**
Root Mean Square (RMS):	**6.055**
Std Error of Mean:	**0.8244**
Skewness	**0.7899**
Kurtosis:	**1.786**
Coefficient of Variation:	**1.097**
Relative Standard Deviation:	**109.7%**

Table 4.7 Descriptive Table Statistics for nitrite in raw materials

Frequency Table		
Value	**Frequency**	**Frequency %**
0.14	**1**	**3.33**
0.17	**1**	**3.33**
0.18	**1**	**3.33**
0.34	**1**	**3.33**
0.39	**1**	**3.33**
0.58	**1**	**3.33**
1.01	**1**	**3.33**
1.04	**1**	**3.33**
1.08	**1**	**3.33**
1.18	**4**	**13.33**
1.39	**1**	**3.33**
1.43	**1**	**3.33**
1.49	**1**	**3.33**
1.6	**1**	**3.33**
1.78	**1**	**3.33**
3.83	**1**	**3.33**
6.18	**1**	**3.33**
6.85	**1**	**3.33**
10.1	**1**	**3.33**
10.3	**1**	**3.33**
10.4	**1**	**3.33**
11.1	**1**	**3.33**
11.4	**1**	**3.33**
11.5	**2**	**6.67**
12	**1**	**3.33**

Table 4.8 Frequency Table for nitrite in homemade brews

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Descriptive Statistics:	
Minimum	116
Maximum	193
Range	77
Count	6
Sum	889
Mean	148.2
Median	137.5
Mode	130, 116, 180, 193, 145, 125
Standard Deviation	31.42
Variance	987

Mid Range	154.5
Quatiles	Quartiles: Q_1 --> 125 Q_2 --> 137.5 Q_3 --> 180
Interquartile Range (IQR)	55
Sum of Squares	4935
Mean Absolute Deviation:	25.56
Root Mean Square (RMS):	150.9
Std Error of Mean:	12.83
Skewness	0.4556
Kurtosis:	1.342
Coefficient of Variation:	0.212
Relative Standard Deviation:	21.2%

Table 4.4 Frequency Table for nitrite in raw materials used

Frequency Table		
Value	**Frequency**	**Frequency %**
116	1	16.67
125	1	16.67
130	1	16.67
180	1	16.67
193	1	16.67

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