A Research Paper On The Analysis Of The Levels Of Nitrate In Homemade Brews, Spirits, In Water And Raw Materials In Nairobi County

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Abstract: Increased cases of deaths as a result of taking home made brews/spirits have been a major concern in our country. Recently, many lives have been lost due to the ignorance of the brewers and their patrons. This study was carried out to determine the levels of nitrate in home - made brews, home - made spirits, raw materials and water. Four hundred and forty (440) home - made alcoholic beverages, one hundred and ten (110) water and eighteen (18) raw materials samples obtained from various parts of Nairobi slums and its environs were analyzed for nitrate. UV-visible Spectrophotometry was used in the research. Some samples contained analyte values above limits set. The concentrations of nitrate varied from non detectable (ND) to 46.3 ± 1.404 mg/l. The recommended maximum contamination levels set by KEBS/WHO for nitrate in alcohols is nitrate 50 mg/l. Most of the home made brews and spirits analyzed in this study had values slightly lower than the levels recommended by the World Health Organization. Values of nitrate were observed to be generally high in the brews/spirits and the raw materials used. The raw materials may have contributed in elevating the levels of these pollutants in the brews. These findings are therefore useful since they provide a justified cause for the Kenyan Government to fight the selling of local alcoholic beverages.

Key Words: Nitrate, UV-Visible Spectroscopy, Brews, Spirits, Raw materials

1.0 INTRODUCTION

1.1 Nitrate, its sources and health effects

Nitrates may be found naturally in water or enter the supplies through a number of sources. Sources of nitrate pollution include; use of fertilizers, animal wastes, municipal and industrial waste, lightening among other sources. Nitrates are the products of aerobic stabilization of organic nitrogen [14]. They may also enter water via fertilizers from agricultural runoffs. They can also be formed during thunderstorms and lightening [14]. The concentrations of nitrates in surface and ground water vary within wide limits depending on geochemical conditions, human and animal waste management practices and on industrial discharge of nitrogen compounds [14]. To protect those at risk, the maximum contamination level (MCL) for nitrate in water is 50 mg/l as nitrate [7].

1.2 Alcohol drinking in developing countries

Almost every month, there are horror stories in the African press about locally produced alcohol, which has poisoned some unfortunate drinkers [5]. In Kenya, the making and selling of any kind of alcohol by ordinary people is entirely illegal, though widely practiced, allegedly because of widespread corruption and non-enforcement of the law. At least 137 people died in the Kenyan capital of Nairobi after drinking an alcoholic brew laced with methanol [3]. A further 500 people were hospitalized across the capital, with some serious condition, and there are reports that 20 people became blind [3]. In August 1998, 85 people died after drinking methanol contaminated liquor and in 1999, 17 people died [3]. Over the last two years 100 people have been blinded as a result of consuming the drink [3]. It was therefore disturbing to read similar stories from Kabale in Uganda where adulterated waragi had blinded 20 people while claiming the lives of 80 innocent Ugandans in one day making a total of 114 deaths of Ugandans in different parts of the country in the last eight months [11]. Alcoholism is a national disease we must tackle.

1.3 Ultraviolet and visible absorption spectroscopy (UV-Vis)

Nitrate was analyzed using this method. Ultraviolet and visible (UV-Vis) absorption spectroscopy was the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels, and UV-Visible spectroscopy was usually applied to molecules or in organic complexes in solution. The UV-Visible spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements [1]. Determination of the nitrite based on the reactions involving sulfanilic acid with methyl anthranilate as the coupling agents followed by reduction using Zn/NaCl and diazotization has been applied successfully to determine trace amounts of nitrite and nitrate in water and pharmaceutical preparations [8].

2.0 MATERIALS AND METHODS

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 arsenic, nitrate, nitrite and phosphorus. 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.

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

2.3 UV-visible spectroscopy instrument

Nitrate was analyzed using, a SHADZU (Model No. UV-2550) UV-Visible spectrophotometer with 1 cm matching quartz cell were used for the absorbance measurements. A WTW pH 330 pH meter was used [8].

2.4 Brews

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.

2.5 Raw materials

In the sample pretreatment, modified procedures for washing and drying proposed by Santos *et al.* [13] and Kawashima & Soares [6], 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 °C 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 [12].

2.6 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 H₂SO₄ (Fischer Chemicals, UK), 75ml of fuming H₂SO₄ (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.

2.7 Sample analysis

Samples were analyzed using UV-Visible spectroscopy. The maximum holding time for NO_3 -N was 48 hours. The concentration of the nutrients in solution was determined by measuring the absorbance. Nitrate was analyzed at 493 nm, then applying the Beer-Lambert law the concentrations of the solutions were obtained.

2.8 Sample analysis for nitrate in UV-visible spectroscopy

In the analysis of nitrate 10 ml sample was pippeted out of the stock solution into a beaker, followed by 5 ml of HCl and 2 ml of Zn/NaCl granular mixture added. This was allowed to stand for 30 minutes with occassional stirring to form a nitrite. The final mixture was filtered into a 100 ml standard flask using what man No. 41 filter pap and diluted up to the mark. Aliquots of stock solution containing 0.26-10.7 µg/l of reduced nitrate were transferred in to series of 10 ml standard flask. 1 ml of 0.5 % sulfanilic acid and 1 ml of 2 mol/I HCl solutions were added, shaken thoroughly for 5 minutes for the diazotization reaction to go to completion. Followed by, 1 ml of 0.5 % methyl anthranilate and 2 ml of 2 M NaOH solution were added to form an azo dve and the contents were diluted to 10 ml with water. After dilution to 10 ml with water, the absorbance of the red colored dye was measured at 493 nm against the corresponding reagent blank [8].

2.9 Data analysis

The quantitative relationship between absorbance and concentration was first done by using a standard curve (calibration curve). The concentration of each solution was calculated based on the regression equation for data analysis. P-values, t –tests and ANOVA were used in data analysis.

3.0 RESULTS AND DISCUSSION

3.1 Method validation

The parameters for method validation are specificity, selectivity, precision, repeatability, intermediate precision, reproducibility, accuracy, trueness, bias, linearity range, limit of detection, limit of quantization, robustness and ruggedness [2]. In this study the following were considered; research apparatus and standard analytical reagents as recommended by Association of Official Analytical Chemists (AOAC) were used, standard solutions were prepared using the standard methods, standard analytical methods were applied, significant values were considered using the (ANOVA test, t-test and p-values), instrumental calibration was done before use, blanks, external calibration graphs were prepared and international MCL standards were also considered.

3.2 Concentration of analytes in various homemade brews, spirits, tap water and the raw materials used

3.2.1 Concentration of nitrate in various home - made brews, spirits and tap water

The levels of nitrate-N in home - made brews/spirits and water was determined using UV-visible spectroscopy and the result obtained for various stations are presented in Table 3.2.2 and Figure 3.2.2. From the Table 3.2.2, the average levels of nitrates were generally high in homemade brews/spirits. The highest levels of nitrate were obtained in Muratina from Gikomba which had the concentration of 46.50 ± 5.42 mg/l. The lowest nitrate levels were obtained in Muratina from Githurai with a concentration of 13.10 ± 1.06 mg/l. Busaa from Kibera, Kariobangi, Kawangware, Gikomba, Githurai, Uthiru, Mathare and Kangemi had high levels of nitrate ranging from 32.70 ± 0.46 mg/l from Githurai to 44.10 ± 0.87 mg/l from Mathare. Busaa could not be obtained in areas like Kiambu, Kilimani, Embu, Siakago, Baricho, Runda, Sagana and Kibwezi were not analyzed. The nitrate levels in the brews/spirits were generally lower than the recommended levels of 50 mg/l for water [7], Kenya Bureau Of Standards does not have standards foe nitrates in alcoholic beverages. These levels were also found to be lower than the maximum limit of 500 mg/l set by Alcohol Measures for Public Health Research Alliance (AMPHORA) for alcohol [4]. The mean concentration of nitrates in the home made brews and spirits was calculated and the results were used to plot a graph of concentration against home - made brew/spirit as shown in the Figure 1. Kangara had the highest mean concentration of NO₃-N at 40.90 ± 1.05 mg/l, followed by Busaa at 39.6 ±1.27 mg/l, Chang'aa had 37.70 ±1.79 mg/l. Water showed no detectable levels of these ions. All the brews indicated concentrations below the maximum allowable limit set by Kenya Bureau of Standards [7] of 50 mg/. The levels are also higher than what is found in uncontaminated water, but higher than the USEPA value of 10 mg/l. This could be due to the use of nitrogenous fertilizers in growing raw materials, river waters in some cases and also use of additives in the brewing process. From Table 3.2.2 and Figure 3.2.2, the levels of nitrate in the home made brews and spirits varied from 13.10 ±1.06 mg/l to 46.30 ± 1.40 mg/l, water did not contain any detectable amounts of nitrates.

Table 3.2.2: Average concentrations (mg/l) of nitrate in various homemade brews and tap water [Mean ± SD]

	Tiernade brews and tap water [Mean ± 0D]				
BREW PLACE	BUSAA [n = 24]	CHAN G' AA [n = 33]	MITI [n = 24]	MURATI NA [n = 33]	
KIBERA	41.10 ±1.07	37.30 ± 1.67	40.00 ± 0.16	42.70 ±2.58	
KARIOBA NGI	38.90 ±0.25	35.90 ± 1.65	37.10 ± 5.10	37.60 ± 1.42	
KAWANG WARE	40.00 ± 1.07	38.30 ± 2.01	38.40 ±3.74	46.30 ± 2.26	
GIKOMBA	39.70 ± 1.31	39.3 ± 0.424	36.7 ± 6.72	46.5 ± 5.42	
GITHURAI	32.70 ± 0.464	33.40 ± 3.37	40.30 ± 1.95	13.10 ± 1.06	
UTHIRU	38.60 ± 2.57	38.9 ± 1.37	37.4 ± 4.85	24.2 ± 6.09	
KANGEMI	41.20 ±2.57	32.50 ± 1.34	34.20 ± 4.55	31.90 ± 6.04	
MATHARE	44.10 ± 0.87	46.30 ± 1.40	32.70 ± 5.80	27.40± 1.25	
KIAMBU	NA	35.40 ± 2.17	NA	NA	
KILIMANI	NA	38.50± 2.17	NA	NA	
EMBU	NA	NA	NA	NA	
SIAKAGO	NA	NA	NA	NA	
BARICHO	NA	NA	NA	30.60 ±0.49	
RUNDA	NA	NA	NA	NA	
SAGANA	NA	38.30 ± 2.08	NA	31.90±6.0 4	
KIBWEZI	NA	NA	NA	31.2 ±1.08	
MEAN	39.55 ± 1.27	37.66 ± 1.79	37.12 ± 4.11	33.03 ± 4.09	
P- values	0.05	0.06	0.05	0.048	

BREW	KUMI KUMI [n = 3]	WATER [n = 0]	KANGA RA [n = 3]	KARUBU [n = 12]
FLACE	[11 = 3]			
KIBERA	35.00 ± 1.27	ND	NA	NA
KARIOBA NGI	NA	ND	NA	NA
KAWANG WARE	NA	ND	NA	NA
GIKOMBA	NA	ND	NA	NA
GITHURAI	NA	ND	NA	NA
UTHIRU	NA	ND	NA	NA
KANGEMI	NA	ND	NA	NA
MATHARE	NA	ND	NA	NA
KIAMBU	NA	ND	NA	NA
KILIMANI	NA	ND	NA	NA
EMBU	NA	ND	NA	31.80 ± 3.19
SIAKAGO	NA	ND	NA	25.60 ± 1.30
BARICHO	NA	ND	NA	NA
RUNDA	NA	ND	40.9 ± 1.06	NA
SAGANA	NA	ND	NA	31.30 ± 1.18
KIBWEZI	NA	ND	NA	31.40 ± 1.08
MEAN	35.00 ± 1.27	ND	40.90 ± 1.06	30.03 ± 1.66
P- values	0.00	ND	0.00	0.045

NA = Not analyzed

ND = Not detected

This trend was also observed in Chang'aa, Miti and Muratina. The nitrate levels in Chang'aa ranged from 32.50 \pm 1.34 mg/l for Chang'aa from Kangemi to 46.30 \pm 1.40 mg/l in the brew from Mathare, while for Miti the nitrate levels ranged from 32.70 \pm 5.80 mg/l in the brew from Githurai to 40.30 \pm 1.95 mg/l for the brew from Mathare. The nitrate levels in Muratina ranged from 24.20 \pm 6.09 mg/l for the brew from Uthiru to 46.50 \pm 5.42 mg/l for Muratina from Kawangware. Kumi kumi and Kangara had only station value analyzed hence they were not significant. Only one value of these samples could be obtained since handling these brews is illegal and one had to use a go between to buy samples. Analyzed water had nitrate at non detectable levels.

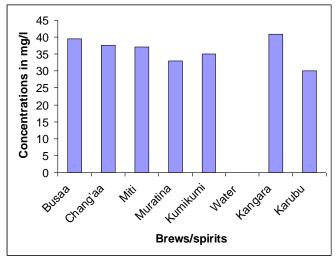


Figure 3.2.2: Mean concentrations (mg/l) of nitrate in various analytes

The source of the nitrate ions in brews may not have been water but could be from the use of untreated river water. Karubu had the lowest overall average levels of nitrate at 30.0 ± 1.66 mg/l. Though Kangara registered the highest average it is not significant since only one sample was obtained. This also applies to Kumi kumi since samples from only one station was considered in both cases. Some brews such as Chang'aa, Busaa, Kumi kumi, Miti and Muratina are prepared by the river bank to facilitate cooling. Waters from these rivers are also sometimes used in brewing process. Since they are generally polluted with industrial and domestic wastes, the nutrients end up in the brews. The other source of nitrate ions in the home made brews may have been due to the use of untreated waters from the slum areas. The mean levels of nitrates in various brews were used to determine whether there was any significant difference between the levels of nitrates in the various brews using the t-test. The results were, for busaa and chang'aa ($t_{cal} = 2.124$, df = 55, $t_{cal} > t_{tab}$); for miti and muratina (t_{cal} = 7.668, df = 55, t_{cal} > t_{tab}); in the case of muratina and karubu ($t_{cal} = 1.074$, df = 43, $t_{cal} < t_{tab}$) and for busaa and muratina ($t_{cal} = 1.209$, df = 55, $t_{cal} < t_{tab}$) all at 95 % confidence interval. From this we can deduce that there were significant differences in the nitrate levels between busaa and chang'aa as well as in miti and muratina. But there were no significant differences between the levels of nitrate in muratina and karubu, as well as in busaa and muratina. Cases where p > 0.05, meant there were significant differences in the levels of nitrate in most of the brews excepet tap water and kangara. The levels of nitrates in the brews were generally high depending on the type of brew and its source. From these values we can conclude that the levels were also lower than the maximum contamination levels of 50 mg/l [7]. This means that the values seen in waters were in order and that water was not the source of the high concentration of the nitrate registered in the brews.

3.2.3 Concentrations of nitrate in various raw materials

The levels of nitrates in the raw materials used to make the brews were determined using UV-Visible spectroscopy and the results are represented in Table 3.3.4. The mean levels

of nitrate in raw materials used in the home made brews and spirits are calculated and results represented in the Figure below.

Table 3.2.4: Mean concentration of nitrate in various raw

materials [Mean ± SD]

Raw materials	Nitrate (mg/kg) [n = 18]		
Maize	215.50 ± 18.31		
Millet	326.20 ± 14.93		
Sorghum	298.50 ± 10.44		
Honey	263.50 ± 23.34		
Jaggery	281.30 ± 14.99		
Muratina fruit	272.40 ± 16.38		

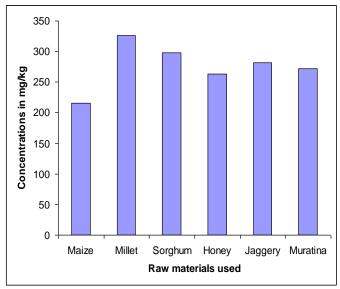


Figure 3.2.4: Mean concentrations (mg/kg) of nitrate in raw materials used in brewing the home made brews using UV-Visible spectroscopy

Presented here in Table 3.2.4 and Figure 3.2.4, the nitrate concentrations were generally high in the raw materials, ranging from 215.50 \pm 33.00 to 326.20 \pm 75.00 mg/kg millet had the highest concentration at 326.20 ± 75.00 mg/kg followed by sorghum at 298.50 ± 27.50 mg/kg, and jaggery at 281.30 \pm 10.44 mg/kg, Maize had the lowest at 215.50 \pm 33.00 mg/kg. The levels of nitrate in all materials were found to be well above the maximum allowable limits of 5 mg/kg of nitrate set by the World Health Organization [15]. Hence the raw materials could have been a source for the nutrient. This could be as a result of the soil levels where the raw materials were grown. Figure 4.2 illustrates the results obtained for; NO₃-N in the raw materials which may have contributed to the high concentration of this nutrient in the home made brews and spirits. Nguyo (2006) [9] explained that the high concentration of nitrate in river water may have been due to the use of fertilizers in agriculture.

Honey used in the preparation of miti and muratina had a mean level of 263.5 ± 23.34 mg/kg. All these levels were higher than those observed in the home made brews. The high concentrations of nitrates in the raw materials can be attributed to the use of fertilizers, environmental pollution and to some extent, the fermentation process in the breaking down of raw materials where amino acids are broken down and converted to nitrates. Raw materials may not have been from the same region, where the brews were being made. Hence the level of nitrates in the home made brews and spirits may have been elevated by the high levels of the nitrate ions in the raw materials. The mean levels of nitrate-N in the study were much higher in all the raw materials than the WHO recommended maximum levels of 5 mg/kg of Nitrate-N [14]. The mean levels of nitrates in various raw materials used were used to determine whether there was any significant difference between the levels of nitrates in the various raw materials using the t-test. The results were; maize and millet (t_{cal} = 13.92, $t_{cal} > t_{tab}$), for sorghum and honey ($t_{cal} = 3.06$, $t_{cal} > t_{tab}$) and for jaggery and muratina ($t_{cal} = 1.164$, $t_{cal} > t_{tab}$) all at the same degree of freedom (18) and confidence interval (95 %). From this we can deduce that there were significant differences between the nitrates levels in maize, millet, sorghum and honey. But the levels between jaggery and muratina plant were not significant. Nitrate was detected in all food groups except beverages and sugars and preserves at mean concentrations above the Limit of Detection (LOD) of 8 mg/kg.

3.3.5 Summary and Conclusion

The nitrate-N levels were generally low in the home made brews/spirits and were found to be below the maximum contamination levels of 50 mg/l set by the WHO, but higher than the USEPA level of 10 mg/l for all homemade alcoholic beverages analyzed. In the raw materials the levels were also high. Water contained non detectable levels of nitrates. This meant that the source of contamination for the brews/spirits may not have been water, but either the use of contaminated water, or any other additives placed in the brew/spirit during the brewing process. The results also verified that the levels of nitrate ions in the raw materials used were generally higher than the MCL of 5 mg/kg and the LOD of 8 mg/kg. These levels exceeded the maximum levels recommended by the World Health Organization [15]. Many developed countries routinely monitor drinking water quality [10], but this is not the case in developing countries. Contaminant levels measured in the home made alcoholic beverages more likely reflected the levels of nutrients in water and the raw materials used in the brewing processes.

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