A Comparison of the Levels of Nitrate, Nitrite and Phosphate in Homemade brews, Spirits, in Water and Raw Materials in Nairobi County Using UV-Visible spectroscopy

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Abstract

This study was carried out to determine the levels of nitrate, nitrite and phosphorus in homemade brews, spirits and the raw materials used using the UV-Visible spectroscopy. Nitrate and nitrite were analyzed using a SHADZU (Model No. UV-2550), phosphate was analyzed using and a Philips, Scientific equipment, UP-8700 Series UV-Visible spectrophotometer, measurements were based on ISO Method (N0.6635). This was done by reducing the nitrate to nitrite in the presence of Zn/NaCl. Nitrite produced was subsequently diazotized with sulphonilic acid then coupled with anthranilate to form an azo dye which was then measured at 493 nm. Orthophosphate and molybdate ions condense in acidic solution to form molybophosphoric acid (phosphomolybdic acid). Upon selective reduction with hydrazinium sulphate, a blue color is produced due to molybdenum blue of uncertain composition. The intensity of the blue colour is proportional to the amount of the heteropoly acid at 830 nm. One hundred and thirty two (132) home - made alcoholic beverages, and eighteen (18) raw materials (maize, millet, for sorghum, honey, jaggery and muratina) samples obtained from various parts of Nairobi County were analyzed. Some samples contained high levels of nitrate though slightly lower than the limits. The concentrations of nitrate varied from non detectable (ND) to 46.3 ± 1.404 mg/l, the levels of nitrite ranged from 0.93m/l to 2.82mg/l and phosphorous from 0.14 ± 0.008 to 4.16 ± 0.62 mg/l. The recommended maximum contamination levels set by Kenya Bureau of Standards/World Health Organization is 50 mg/l; Nitrate, 0.03 mg/l; nitrite and 2.2 mg/l; phosphate. Most of the homemade brews and spirits analyzed for nitrate in this study had values slightly lower than the levels recommended by the World Health Organization. Levels of nitrite and phosphorus were found to be generally higher than the MCL in the brews/spirits and the raw materials used. These raw materials may also have contributed in elevating the levels of these nutrients in the homemade brews. The results indicate that the UV-Visible spectroscopy method is suitable for analyzing the presently studied samples. This is a reliable and cheap method for routine analysis of a large number of homemade brews/spirits samples.

Key Words: Homemade brews, Nitrate, UV-Visible Spectroscopy

1.0 INTRODUCTION

1.1 Nitrate, its sources and health effects

In recent years, an increasing interest in the determination of nitrate levels in food products has been observed, essentially due to the potential reduction of nitrate to nitrite, which is known to cause adverse effects on human and animal health. Nitrate is a naturally occurring compound that is part of the nitrogen cycle, as well as an approved food additive. It plays an important role in the nutrition and function of plants. Nitrate is an important component of vegetables due to its potential for accumulation; this can be affected by a number of biotic and abiotic factors [1]. Human exposure to nitrate is mainly exogenous through the consumption of vegetables, and to a lesser extent water and other foods [1]. Nitrate is also formed endogenously. In contrast exposure to its metabolite nitrite is mainly from endogenous nitrate conversion [1].

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 [2]. They may also enter water via fertilizers from agricultural

runoffs. They can also be formed during thunderstorms and lightening [2]. 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 [2]. To protect those at risk, the maximum contamination level (MCL) for nitrate in water is 50 mg/l [3].

Intake of large doses of nitrate can be tolerated by adults but not infants and very young children who may suffer from methemoglobinaemia. The primary health hazard from drinking water with nitrate occurs when nitrate is transformed to nitrite in the digestive system. Bacteria in the infant's digestive tracts may convert the relatively harmless nitrate to nitrite [4]. The nitrite oxidizes iron in the haemoglobin of the red blood cells to form methemoglobin, which lacks the oxygen – carrying ability of haemoglobin. This creates the condition known as methemoglobinemia (sometimes referred to as "blue baby syndrome"), in which blood lacks the ability to carry sufficient oxygen to the individuals body cells causing the veins and skin to appear blue [5]. Most humans over one year of age have the ability to rapidly convert methemoglobin to haemoglobin within red blood cells so that it remains low in spite of relatively high levels of nitrate/nitrite uptake. However, in infants under six months of age, the enzyme systems for reducing methemoglobin to oxyhaemoglobin are incompletely developed and methaemoglobineamia can occur. This also may happen in older individual who have

genetically impaired enzyme systems for metabolizing methemoglobin [5]. This is because of nitrites, which are produced by reduction of nitrates by microbial action either in the environment or in the body. Children aged between 12 and 14 years who drank water with a nitrate level of 105 mg/l were noted to have a slightly delayed reaction to light stimuli compared to control children drinking water with a nitrate level of 8 mg/l whose methaemoglobin level averaged 0.75 percent [5].

1.2 Nitrite

Nitrite is mainly produced as a result of oxygen depletion, anaerobic biological conditions dominate and reduction sets in [5]. 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. Concentrations of 45 mg/l produce methaemoglobinemia [5]. The maximum contamination limit for nitrite is 0.03 mg/l [3]. Sources of nitrite pollution are generally the same as those of nitrate pollution.

1.3 Phosphorus and phosphate

Sources of phosphorus include; detergents, phosphorus acids, fire works, munitions, rat poisons among others [6]. Presence of these contaminants in high levels in local alcoholic beverages could cause problem to the consumers. Phosphates are one of the most common nutrients to move through the ecosystem in large quantities. Phosphates enter the waterways through run off from natural sources such

as phosphate – containing rocks and from human sources such as fertilizer, pesticides, detergents, and industrial wastes.

2.0 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 down 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. During the nineteenth century, in most parts of East Africa, the most used grain 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 [7]. 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 [7].; 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 [6].

2.1 Studies and health hazards of nitrate and nitrite

The high concentration of nitrate has adverse effects on environment, animals and humans:

a. Environment: the high concentrations of nitrate in water causes a phenomenon known as "Eutrophication", which means an excessive growth of the algae in water which consumes the oxygen gas dissolved in water causing the death of fishes in that water [14].

b. Animals: especially ruminant animals such as cows, sheep and goats [14]. When ruminants consume feed with high nitrate levels, the nitrate can be converted to nitrite, which causes both nitrate and nitrite accumulation in the rumen [14].

As a result of the accumulation of both nitrate and nitrite in animal rumen, it causes acute and chronic symptoms which run as reduction in weigh gain, reduction in milk production, low appetite, aborted breathing, blue coloring of mucus membrane, rapid heartbeat, abdominal pain, vomiting, reproductive problems, abortions, and premature death of calves [14]. The acute nitrate poisoning causes death, because the nitrate is reduced to nitrite in the rumen by bacteria. Nitrite is highly toxic because it combines with hemoglobin and form methemoglobin which is enabling to carry oxygen [14]. A death occurs within few hours after the ingestion of a high nitrate feed [14].

c. Human: Nitrate itself is not toxic; however, the conversion of nitrate to nitrite in human and animal bodies is very dangerous if it accumulates in high concentrations. On the other hand, the following could be occurring as a result of the accumulation (or uptake) of large dose of nitrate for human health:

Methemoglobenemia: drinking water and vegetables are the major sources of nitrate consumed by human stomach [14]. The toxicity of nitrate in humans is due to the body's reduction of nitrate to nitrite. This reaction takes place in saliva of humans at all ages and in the gastrointestinal tracts of infants during the first three months of life [12]. Nitrate becomes toxic when it is reduced to nitrite, a process that can occur in the stomach as well as in the saliva. Infants are especially susceptible because their stomach juices are less acidic and therefore conducive to the growth of nitrate reducing bacteria [9].

Nitrate is reduced to nitrites which combine with hemoglobin to form methemoglobin (metHP). The best-known effect of nitrite is its ability to react with haemoglobin (oxyHb) to form methaemoglobin (metHb) and nitrate:

$$NO_2^- + oxyHb(Fe^{2+}) \rightarrow metHb(Fe^{3+}) + NO_3^-$$
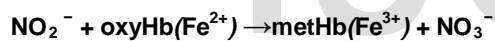
As a consequence of the formation of metHb the oxygen delivery to tissue is impaired.

Methemoglobin is a compound that cannot combine with oxygen, and that decreases the capacity of the blood to transport oxygen from lungs to body tissues causing a condition known as "Blue Baby Syndromes" or "methemoglobenemia" (Abu-Dayeh, 2006). The normal met HP level in humans is less than 3% in infants under three months of age. However hypotoxic signs may develop at about 20%, while death occurs at 50% metHP or higher [14]. Because of their high stomach acidity, infants less than one year old (3-6 months old), are highly

infected by the methemoglobenemia, this acidity increases the conversion of nitrate to nitrite by providing an appropriate environment for the nitrate reducing bacteria [14]. Other groups that may be risk to form met HP are pregnant women, which may cause birth defects and miscarriages [14]. Also people who have deficiency in glucose-6- phosphate dehydrogenase or metHb reductase may be at risk to form metHP [14]. Adults with lower gastric acidity can be infected with metHP. Fatalities have been reported after single intake of 4-50 g of nitrate (equivalent to 67-833 mg of nitrate per Kg of body weight) [14]. The symptoms related to high levels of metHP in blood include bluish coloration of skin, headache, dizziness difficulty in breathing, in severe cases damage to brain and death may occur [14].

2. Carcinogenicity: nitrite reacts in stomach with nitrosatable compounds to form N-nitraso compounds [14]. These compounds have been found to be carcinogenic [14]. The US National Research Council found an association between high nitrate intake and gastric and esophageal cancer [14]. High levels of nitrate intake were also linked with the Non-Hodgkin's lymphoma, bolder cancer, pancreatic cancer and stomach cancer [14]. High levels of nitrate were also linked with the infection with the diabetes, the occurring of some birth defects, and miscarriages [14].

The objective of this research was to;

   a) determine the amounts and compare the variability in the nitrate, nitrite and

phosphate contents in homemade brews and the raw materials used in Nairobi using UV-visible spectroscopy.

b) Evaluate the relative safety of these homemade brews based on the MCL set by the WHO and EPA standards.

The Kenya water quality regulation has adopted the 50.0, 0.03 and 2.2 mg/l standard as the maximum contamination levels (MCL) for nitrate, nitrite and phoshate for regulated public water systems [3].

2.2 Phosphate, sources and health effects

Phosphorus is an essential nutrient for plants and animals in the form of ions PO_4^{3-} and HPO_4^{2-}. It is a part of DNA molecules, of molecules that store energy (ATP and ADP) and of fats of cell membranes. Phosphorus is also a building block of certain parts of the human and animal body such as bones and teeth. Natural sources of phosphorus are mainly derived from the weathering of phosphorus bearing rocks and the decomposition of organic matter [17]. Domestic waste water (particularly wastewater containing detergents), industrial effluents, and fertilizer run off, contribute to elevated levels in surface waters, making them major pathways of phosphorus transformation and residue decomposition [17].

In most natural surface waters, phosphorus concentrations range from 0.005 to 0.020 mg/l [17]. To control eutrophication, the EPA has made the following recommendations; total phosphorus should not exceed 0.05 mg/l as phosphorus in streams that do not discharge directly into lakes or reservoirs [17].

Phosphate levels greater than 1.0 mg/l may interfere with coagulation in water treatment plants [17]. The recommended maximum for rivers and streams is 2.2 mg/l [3].The maximum contamination level for foods is 5000 mg/kg [16].Total phosphorus concentrations in wade able streams in the U.S ranged from undetectable <1 μg/l) to more than 5000 5g/l. Thresholds for davorable or unfavorable water quality vary from one part of the country to another. The toxic effects of high levels of nitrites and nitrates calls for their levels to be continuously monitored in the environment.

2.3 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].

Nitrite, nitrate and phosphorus were 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 (Brain, 2000). 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 [20].

2.4 Phosphate (PO_4^{-3}) determination

Orthophosphate and molybdate ions condense in acidic solution to form molybophosphoric acid (phosphomolybdic acid). Upon selective reduction with hydrazinium sulphate, a blue color is produced due to molybdenum blue of uncertain composition. The intensity of the blue colour is proportional to the amount of the heteropoly acid. If the acidity at the time of reduction is 0.5 M in sulphuric acid and hydrazinium sulphate is the reductant then the resulting blue complex exhibits a maximum absorbance at 820-830 nm [21].

3.1 Sample size and study site

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, 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. These 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.

3.2 Reagents, chemicals, solvents, standards and blanks

3.2.1 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 [21].

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.

3.3.2 Standard for phosphate analysis

The following reagents were also used for the determination of phosphate ions

(i)   12.5 g of analytical reagent sodium molybdate ($Na_2MoO_4.2H_2O$)

(ii)   Hydrazinium sulphate solution

(iii)   1.5 g of analytical reagent hydrazinium sulphate was dissolved in deionized water and diluted to 1000 ml [21]

(iv)   Standard phosphate solution (10 ppm P)

(v)   0.04393 g of analytical reagent potassium dihydrogen phosphate was dissolved in de ionized water and was diluted to 1000 ml

(I ml of solution = 0.01 mg P). This solution is to be prepared fresh on monthly basis [21].

(vi)   Calibration Solution

The phosphate stock solution containing 10 mg PO_4/l was used to prepare standard solutions of between 0-1 mg PO_4/l.

3.4 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.

3.4.1 UV-visible spectroscopy instrument

 Nitrite and nitrate were 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 [20].

Phosphate was analyzed using, the UV-visible spectrophotometer (Philips, Scientific equipment, UP-8700 Series). For the automated analysis of up to 270 samples, SDS-270 auto-sampler and the auto-sipper flow through cell can be used [21].

3.5 Cleaning of apparatus

Cleaning of apparatus was adopted from Mendham *et al*, (2002), and AOAC (2000) [28]. 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 bottles were then immersed in a hot (50- 60 °C) bath of 1 N trace metal grade HCl for at least 48 hours. The bottle were then thoroughly rinsed with reagent water and filled with 0.1 % (v/v) ultra pure HCl and double-bagged in new polyethylene zip-type bags until needed [22]. The apparatus were cleansed using detergents, water, rinsed with distilled deionized water and dried overnight in the oven at 100 °C.

3.6 Sample collection and pretreatment

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.

3.6.1 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.

3.6.2 Raw materials

In the sample pretreatment, modified procedures for washing and drying proposed by Santos *et al.* (2004) and Kawashima & Soares (2003) [23]., 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 [23].

3.7 Sample preparation

3.7.1. Sample preparation in the analysis of phosphate

In the phosphate analysis all samples of the brews, digested raw materials and standards (Including quality control solutions) were processed in the same manner. The pH of a well mixed sample was adjusted from 6.0 to 8.0 using 6 M NaOH and 1M HCL. 10 ml of ammonium persulphate $((NH_2)_2S_2O_8)$ solution and 0.25 ml of 5.4 M H_2SO_4 were added to each tube and mixed. The caps were initially tightened, inverted a few times to ensure good mixing, and then the caps were unscrewed until the seals had just become loose. The tubes in the racks were placed in either autoclave or pressure cooker. For the autoclave, the manufacturer's directions were used and the mixture was heated at 120 °C for 30 minutes. The tubes were finally removed from the autoclave or pressure cooker and cooled between 20-30 °C. A volume of 0.40 ml of 6 M NaOH was added followed by 1 drop of phenolphthalein solution to each sample and mixture. Thereafter 6 M NaOH was added into the solution until the solution turned pink and then 5.4 M H_2SO_4 until the pink color just cleared.

3.7.2 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 [18].

Table 1: Microwave digestion program [18]

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

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 automatically reduced power when the maximum pressure of 60 bars was applied [18].

3.7.3 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.

3.8 Sample analysis

Samples were analyzed using Hydride generation atomic absorption spectroscopy and UV-Visible spectroscopy. The maximum holding time for NO_3-N, NO_2-N and PO_4-P was 48 hours. The concentration of the nutrients in solution was determined by measuring the absorbance. Nitrite and nitrate were analyzed at 493 nm and phosphate at 830 nm respectively, and then applying the Beer-Lambert law the concentrations of the solutions were obtained.

3.8.1 Sample analysis for phosphate using UV-visible spectroscopy

A volume 50 ml of alcohol samples and blanks were shaken and filtered sample were put into 125 ml Erlenmeyer and 1 ml of 40 % concentrated sulphuric acid was added to the mixture followed by 600 ml of distilled water, cooled and diluted to 1 liter. This was evaporated on a hot plate for 30 minutes, cooled, diluted to 50 ml with distilled water and 8 ml of the reducing agent [prepared by mixing in the following order, 50 ml of 2.5 M H_2SO_4, 5 ml of potassium antimonyl tartarate (BDA chemicals Ltd, Poole, England) solution, 15 ml ammonium molybdate (Aldrich Chemical Co. Inc., USA) solution and 30 ml of ascorbic acid (Fisher Chemicals, UK) solution] added the solution thoroughly mixed. The standards and the blanks were treated in the same manner. After 30 minutes the absorbances of the test solutions and the standards were measured at 830 nm against the reagents blank as reference using a UV – visible spectrophotometer (Philips, Scientific equipment, UP – 8700 series).

3.8.2 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 contents 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 [20].

3.8.3 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/l 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 dye 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 [20].

4.0 Concentration of nitrate, nitrite and phosphate in various homemade 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 2 and Figure 1.

From the Table 2, the average levels of nitrates were generally high in homemade brews/spirits.

The mean nitrate levels in the brews/spirits were generally lower than the recommended levels of 50 mg/l for water [3]. Nitrite and Phosphate levels were generally higher than the MCL levels of 0.03 and 2.2 mg/l respectively. Kenya Bureau of Standards does not have standards foe nitrates in alcoholic beverages. The mean concentration of nitrate in the home made brews and spirits were calculated and the results were used to plot a graph of concentration against home made brew/spirit as shown in the Figure 1. Karubu had the highest mean concentration of NO_3-N at 40.90 ± 1.05 mg/l, followed by Busaa at 39.6 ±1.27 mg/l and Muranina had the lowest mean level at 33.71 ± 11.81 mg/l.

Kangara had the highest mean concentration of nitrite NO_2-N at 6.85 ± 0.40 mg/l, followed by Muratina at NO_2-N at 6.18 ± 5.35 mg/l and Chang'aa had the lowest mean level at NO_2-N at 0.12 ± 0.22 mg/l.

Karubu was not analyzed. Busaa had the highest mean concentration of PO_4-P at 3.28 ± 0.37 mg/l, followed by Chang'aa at 3.04 ±0.10 mg/l and Kumikumi had the lowest mean level at 0.13 ± 0.01 mg/l.

Some brews such as Chang'aa, Busaa, Kumi kumi, Miti and Muratina are normally 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 nutrients in the home made brews may have been due to the use of untreated waters used in the slum areas.

From ANOVA one way test on nitrate levels in the homemade brews; the probability of the between-treatments MS being ≥ 0.783 times the within-treatments MS, if the null hypothesis is true, is p = 0.570. The mean was 36.8343, the median 38.3 and the SD was 6.447. We can therefore conclude that there were no statistically significant differences between the concentrations of nitrates in the brews means as determined by one-way ANOVA (F = 0.783, p = 0.570).

The ANOVA one way test on nitrite in the raw materials used gave the following results; 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. An ANOVA two way test was also done for nitate and nitrite. Since the calculated $F_{statistics}$ (1,5) =383.003, was greater than $F_{critical}$,= 16.258 we know that there was a statistically significant difference between the nitrate and nitrite concentrations, $p < 0.01$. Thus, the null hypothesis was rejected.

The following ANOVA one way test results were found for Phosphate in homemade brews; the probability of the between-treatments MS being ≥ 5.122 times the within-treatments MS, if the null hypothesis is true, is p = 0.000. The P-value of 0.000 is less than the significance level (0.01), so we can reject the null hypothesis and safely assume that phosphate concentration affects the health of the users. The mean was 2.945, the median 3.11 and the SD was 0.9494. We can therefore conclude that there were no statistically significant differences between the concentrations of phosphates in the brews means as determined by one-way ANOVA (F = 91.251, p = 0.000). F (91.251) is greater than F crit (2.95), so again, we can reject the null hypothesis. All levels were considered at 95 % confidence interval. In all the homemade brews the value of $p < 0.000$, implying that there were significant differences in the levels of

phosphate in the homemade alcoholic beverages. From these values we can conclude that the levels were also lower than the maximum contamination levels of 2.2 mg/l.

The concentration of nutrients were generally above the maximum allowable limit set by KEBS and WHO [3]. Kenya Bureau of Standards did not have standards for nitrites in alcoholic beverages. Hence they pose health danger to the consumer. Their source could be from 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.

4.1 Concentrations of nitrate, nitrite and phosphate 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.

Presented here in Table 3 and Figure 2, the mean nutrient levels were generally high in the raw materials used. Nitrate had values ranging from 215.50 ± 33.00 to 326.20 ± 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 Maize had the lowest mean concentration of 215.50 at 281.30 ± 10.44 mg/kg, Maize had the lowest at 215.50 ± 18.31 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 [11]. An Acceptable Daily Intake (ADI) for nitrate of 3.7 mg/kg b.w./day, equivalent to 222 mg nitrate per day for a 60 kg adult was established by the former Scientific Committee on Food (SCF) and was reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002. The CONTAM Panel noted that no new data were identified that would require a revision of the ADI [1].

An ANOVA one way test was done on nitrate levels in the homemade brews gave the following results; the mean was 276.233, the median 276.85 and the SD was 37.104. A P-value of 0.000 is less than the significance level (0.01) and F (14.535) is greater than F critical (14.47103), hence the concentrations of the raw materials used did not affect significantly the concentrations of nitrate.

The ANOVA one way test done on the mean levels of phosphate in the homemade raw materials used gave the following results; the mean was 276.233, the median 276.85 and the SD was 37.104. A P-value of 0.000 is less than the significance level (0.01) and F (279.083) is greater than F critical (3.197), hence the concentrations of the raw materials used did not affect significantly the concentrations of nitrate. From this we can deduce that there were significant differences between the phosphates levels in all the raw materials used.

The ANOVA two way test was also done on nitrate and nitrite. The F$_{statistics}$ (1,5) =39.665, was greater than $F_{critical}$,= 16.258 we know that there was a statistically significant difference between the nitrate and nitrite concentrations, p < 0.01. Thus, the null hypothesis can be rejected.

From table 3, 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 Millet seeds had the lowest concentration of nitrite at 116.00 ± 41.93 mg/kg. 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.

The same test was repeated for nitrite levels in the raw materials and the following results were obtained; 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.

The mean concentrations of phosphate-P in the raw materials were found to be generally low in fact below the maximum contamination level of 5000 mg/kg (Codex, 2001). Concentration ranged from 104 ± 6.08 to 285 ± 23.80 mg/kg.

Jaggery had the highest concentration of 285.00 ± 23.80 mg/kg, followed by sorghum at 190.00 ± 6.45 mg/kg and maize seed had the lowest level of 104.00 ± 6.08 mg/kg. These levels are low and therefore do not pose risk to the health of the consumers. This indicates that phosphate pollution is low in the raw material analyzed hence the raw materials could not have contributed in elevating the levels of this nutrient in the brews and spirits.

This implies that the raw material whose nutrients concentrations were generally high for nitrate, nitrite and phosphate contributed positively towards elevating the levels of these nutrients in the brews/spirits. Hence, it is also possible that slum dwellers may be using polluted river waters in brewing.

Table 2: Average concentrations (mg/l) of nitrate, nitrite and phosphate in various homemade brews and tap water [Mean ± SD]
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Nutrient		Nitrate	Nitrite	Phosphate

Brew	Number (n)			
Busaa	24	39.54 ± 3.26	4.95± 4.725	3.28 ± 0.37
Chang'aa	33	37.58 ± 3.84	0.12 ± 0.22	3.04± 0.10
Miti	24	37.10 ± 2.63	1.02 ± 0.72	2.93± 0.29
Muratina	33	33.71 ± 11.81	6.18 ± 5.35	2.73 ± 0.22
Kumikumi	3	35.00 ± 1.27	0.39 ± 0.50	0.13 ± 0.01
Kangara	3	35.00 ± 0.1	6.85 ± 0.40	2.12 ± 0.11
Karubu	12	40.90 ± 1.06	NA	2.61± 0.06

NA = Not analyzed   ND = Not detected
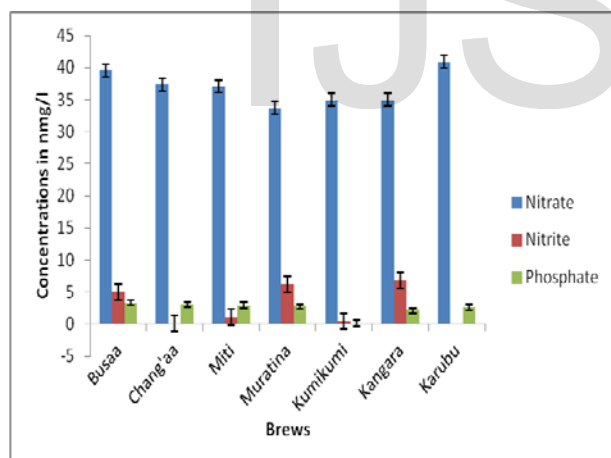
Figure 1: Mean concentrations (mg/l) of nitrate, nitrite and phosphate in various homemade brews

Table 3: Mean concentration of nitrate in various raw materials in mg/kg [Mean ± SD]

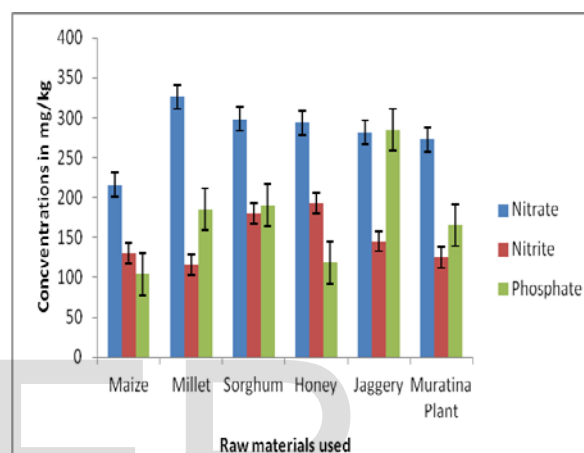| Raw materials [n = 18]	Nitrate	Nitrite	Phosphate
Maize	215.50 ± 18.31	130 ± 7.69	104.0 ± 6.08
Millet	326.20 ± 14.93	116 ± 41.93	185.00 ± 5.16
Sorghum	298.50 ± 10.44	180 ± 16.02	190.00 ± 6.45
Honey	263.50 ± 23.34	193 ± 18.52	118.00 ± 18.17
Jaggery	281.30 ± 14.99	145 ± 47.79	285.00 ± 23.80
Muratina fruit	272.40 ± 16.38	125 ± 14.90	165.00 ± 24.68

Figure 2: Mean concentrations (mg/kg) of nitrate, nitrite and phosphate in various raw materials used with standard error bars

CONCLUSION

The nitrate-N levels were generally below the maximum contamination levels of 50 mg/l set by the WHO. In the raw materials the levels were relatively high. 105 out of 180 samples analyzed for nitrite had levels above the maximum contamination levels of 0.03 mg/l level for water. Phosphate-P were also found to be high in the homemade brews, 102 out of 180 samples analyzed had levels above the maximum contamination levels of 2.2 mg/l for water.

The levels of nitrate levels in raw materials used in the preparation of the homemade alcoholic beverages

were generally higher than the MCL of 5 mg/kg and the LOD of 8 mg/kg; Nitrite levels in the raw material were higher than the MCL of 2 mg/kg and the LOD of 0.6 mg/kg, but lower than the MCL of 5 000 mg/kg for phosphates.

These levels of nitrate, nitrite and phosphate in the sample areas exceeded the maximum levels recommended by the World Health Organization [11]. Nutrient levels observed in the home made alcoholic beverages most likely reflect the levels of these nutrients in the raw materials used in the brewing processes and the fertilizer used in farming.

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