NITRITE, NITRATE AND NITROSAMINE CONCENTRATIONS IN COMMON WINE BRANDS, MALT DRINKS AND FRUIT JUICES IN MAKURDI, BENUE STATE

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Abstract: The nitrite, nitrate and nitrosamine concentration in ten (10) wine brands, five (5) malt drinks and five (5) fruit juices commonly consumed in Makurdi, Benue state were analyzed using UV-Vis spectrophotometry, with the aim of determining them and comparing the concentration values with World Health Organisation (WHO) and NAFDAC standards. The results showed that wine brands consumed in Makurdi, Benue state contained between 0.80±0.01 to 2.52±0.13 mg/L nitrites, 7.41±0.14 to 13.05±0.07 mg/L nitrates and nitrosamines were not detected in any of the samples. Malt drinks contained concentrations of nitrites within close range (0.71±0.08 to 0.80±0.01 mg/L) and nitrates (9.65±0.14 to 10.35±0.14 mg/L) suggesting similarity of raw materials and methods of processing. Nitrosamines were not detected in any of the malt samples. Fruit juices contained the lowest levels of nitrites (0.57±0.07 to 0.80±0.01 mg/L), moderately high levels of nitrates (8.72±0.08 to 15.47±0.21 mg/L) particularly in juices containing apples or coconut. Nitrosamines were not detected in the fruit juice samples studied. All concentrations obtained were below the acceptable concentration levels stipulated by the World Health Organization (WHO) hence, the beverages under study were verified safe for consumption. The statistical tests performed showed significant difference in the nitrite and nitrate concentrations of wine brands and fruit juices but no statistical difference in the concentrations of nitrite and nitrate in the malt drinks. This was attributed to wider variations in methods of processing, choice/quantity of preservatives and nature of raw materials in the manufacture of wine and fruit juices, which is probably absent in malt drink making. The parameters under study require regular checks and monitoring hence, it was recommended by the researcher, that this and similar research work be periodically carried out by NAFDAC and also, as research work by other students of Analytical chemistry or Food Science and Technology.

Keywords: Beverages, blue-baby syndrome, carcinogenic, juice, Makurdi

Introduction

Historically, there have been two main safety concerns around the presence of nitrate and nitrite in the diet. Those related to the reaction of nitrite (from the reduction of nitrate or occurring by itself) with haemoglobin to form methaemoglobin which can reduce oxygen transport in the blood, and a theoretical possibility of the potential for carcinogenicity through the formation of N-nitroso compounds (especially nitrosamines) in foods or in humans *in vivo* (Menard *et al.*, 2008; Grosse, 2006).

Naturally, nitrates are found in fruits and vegetables than in any other dietary source (Maynard *et al.*, 1976). Naturally occurring nitrates are not harmful but are actually beneficial. They play important roles in immune function, cardiovascular health and exercise performance. Naturally occurring nitrates have been proven to be so beneficial because of the accompanying inhibitors (e.g., ascorbic acid and vitamin E) preventing its conversion to harmful substances. Nitrates become troublesome when taken above acceptable standards, especially when artificial and in the absence of inhibitors to prevent its reduction to nitrite. They are easily converted to nitrites, especially in acidic medium. Nitrate is reduced by nicotinamide-adenine dinucleotide phosphate (NADPH) to nitrite in the presence of the enzyme nitrate reductase (NR).

Equation: $\text{Nitrate} + \text{NADPH} + H^+ \xrightarrow{NR} \text{Nitrite} + NADP^+ + H_2O$

Sources of nitrates and nitrites in beverages, in summary, include:

a. The fertilizer used in cultivating the grapes, barley or fruits used in making wine, malt drinks and fruit juices (Maynard *et al.*, 1976).
b. Contamination present in water used for the manufacture of these beverages, as a result of leakage from farms, sewage, run-off from chemical plants, etc. (Gangolli *et al.*, 1994).
c. Direct addition of nitrates (as sodium/potassium nitrate) and nitrites (as sodium/potassium nitrite) to the beverages as preservatives or colour enhancers. (Chung *et al.*, 2004).

Nitrosamines are chemical compounds of the chemical structure $R^1N(-R^2)-N=O$ most of which are carcinogenic. There is an increasing concern about the occurrence of N-nitroso compounds (NOCs) in food, because these compounds have been demonstrated to be carcinogenic in several species of animals and are therefore likely to be related to human cancer (Sen *et al.*, 1993). This, together with the fact that humans are exposed to trace amounts of N-nitroso compounds from several sources, has initiated the research on their occurrence, formation and biological activity.

NOCs may be present in food because of their:

- Formation as a result of the use of nitrate/nitrite additives
- Formation during processing
- Contamination from secondary sources such as packaging materials or other ingredients (e.g. waxes) (Sen *et al.*, 1993).

Due to all this, the concentration of nitrates, nitrites and nitrosamines in common wine brands, malt drinks and fruit juices in Makurdi, Benue state needs to be ascertained and compared with approved standards, to ensure that consumers of these beverages are not exposed to the harmful effects of these substances.

Materials and Methods

Materials

Chemicals/Reagents

All chemicals used were of analytical reagent grade (99.9% purity), and were obtained from various companies (mostly from Sigma Aldrich) through Emole Nigeria Limited, Makurdi. Doubly distilled, de-ionized water was used in the preparation of all solutions in the experiments. Methyl anthranilate was prepared by a simple esterification reaction between anthranilic acid and methanol, with conc. H_2SO_4 as a catalyst and dehydrating agent.

Nitrite solution (1000 µgmL^{-1}) was prepared by dissolving 0.1500 g sodium nitrite in water and diluting to 100 mL. Nitrate solution (1000 µgmL^{-1})was prepared by dissolving 0.7220 g potassium nitrate in water and diluting to 100 mL. Working standard solutions were prepared by appropriate dilution (described below at 3.5.1).

Sulfanilic acid (0.5 g in 100 mL water) and methyl anthranilate (0.5 mL in 100 mL of alcohol) were used. The following reagents were prepared by dissolving appropriate amounts of reagents in water: 2 molL^{-1}HCl and 2 molL^{-1}NaOH.

Apparatus

A CALSPEC (Model No: UV-2550) UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements. Several standard volumetric flasks of 50, 100, 250 ml and 1 L capacity were used. Other pieces of apparatus used include, Whatman No 41 filter paper, funnels, beakers, measuring cylinders, stirring rod, dropping pipette and a time piece.

Methods

Sampling

Samples were collected at random from major supermarkets, wine bars and road-side shops within Makurdi town.

Determination of nitrite

5 ml of a blank solution (containing 0.0 mg/L nitrite) and 5 ml aliquots of stock solution containing 0.2-5.0 µgmL^{-1} (or mg/L) of nitrite were transferred in to series of 10 mL calibrated flask. To each flask, 1 mL of 0.5% sulfanilic acid and 1 mL of 2 molL^{-} hydrochloric acid solution were added and the solution was shaken thoroughly for 5 min to allow the diazotization reaction to go to completion. Then, 1 mL of 0.5% methyl anthranilate and 2 mL of 2 mol L^{-1} sodium hydroxide solutions 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 calibration graph was constructed.

5 ml each, of the samples of wine, malt drink or fruit juice, after decolorization with activated charcoal, were put into series of 10 ml standard flasks where 1 mL of 0.5% sulfanilic acid and 1 mL of 2 mol L^{-1}HCl solution were added and shaken thoroughly for 5 minutes for the diazotization reaction to go to completion. Then, 1 mL of 0.5% methyl anthranilate and 2 mL of 2 mol L^{-1} sodium hydroxide solutions 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 new red colored dye was measured at 493 nm. The value of absorbance for each sample was recorded and each analysis was performed in triplicate. The concentration of nitrite in each sample was determined from the construction of a calibration curve of the standard solutions and use of the method of least squares to obtain values for the expression: **y = mx + c**

Where, y is the absorbance,

m is the slope,

x is the concentration and,

c is the intercept.

Determination of nitrate

10 mL of nitrate stock solution was pipetted out into a beaker, 5 mL of Conc. HCl and 2 mL of Zn/NaCl granular mixture were added, and it was allowed to stand for 30 min with occasional stirring to form nitrite, then the solution was filtered into a 100 mL standard flask using Whatman No 41 filter paper and diluted up to the mark.

5 ml of a blank solution (containing 0.0 mg/L of reduced nitrate) and aliquots of stock solution containing 0.20-10.20 µgmL^{-1} (or mg/L) of reduced nitrate were transferred in to series of 10 mL standard flasks. 1 mL of 0.5% sulfanilic acid and 1 mL of 2 mol L^{-1}HCl solution were added and shaken thoroughly for 5 min for the diazotization reaction to go to completion. Then, 1 mL of 0.5% methyl anthranilate and 2 mL of 2 mol L^{-1} sodium hydroxide solutions 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 and a calibration graph was constructed.

The wine, malt drinks and fruit juices were stirred for five minutes to give off any CO_2 in them. 2 g Zn/NaCl granular mixture was added to each of them and it was allowed to stand for 30 minutes with occasional stirring to form nitrite, then the solution was filtered into a 100 mL standard flask using Whatman No 41 filter paper.

The reduced samples of wine, malt drink or fruit juice were put into series of 10 ml standard flasks where 1 mL of 0.5% sulfanilic acid and 1 mL of 2 mol L^{-1}HCl solution were added and shaken thoroughly for 5 minutes for the diazotization reaction to go to completion. Then, 1 mL of 0.5% methyl anthranilate and 2 mL of 2 mol L^{-1} sodium hydroxide solutions 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 new red colored dye was measured at 493 nm. The value of absorbance for each sample was recorded and each analysis was performed in triplicate. The concentration of total nitrite in each sample was determined from the construction of a calibration curve of the standard solutions and use of the method of least squares to obtain values for the formula: **y = mx + c**

Where: y is the absorbance; m is the slope; x is the concentration and; c is the intercept.

This nitrite concentration value is a measure of total nitrite (Nitrite + Nitrate). Sample nitrate concentration = total nitrite – free nitrite (gotten in 3.3.3).

Determination of nitrosamine

The Greiss reaction revised by Fox (1979) and used by both Telling *et al.* (1981) and Ogunmodede (2012), for the determination of nitrosamines using UV-Vis spectrophotometry, was applied.

The samples were first stirred for some minutes to liberate any CO_2 present. Ammonium sulphamate was added to 50 ml of the wine, fruit juice and malt drink samples to stabilize any N-nitrosamine and also as a free nitrite scavenger. An aqueous sodium chloride solution was then added to liberate the nitrosamine from the mixture.

For each sample, 3% hydrobromic acid in glacial acetic acid was added and stirred for denitrosation to occur. 0.5% sulphanilamide was added and stirred for some time for diazotization to occur then, N-(1–naphthyl)ethylenediamine reagent was added. This gave a purple colouredazo dye whose absorbance was measured at 540 nm. The analysis was repeated thrice for each sample.

Nitrosamine concentrations were calculated manually from absorbance readings, using Beer-Lambert's law: **A = ƐlC**

Where,

A = Absorbance (Arbitrary unit)

Ɛ = Molar absorptivity coefficient (Lmol^{-1}cm^{-1}) = 1.48 x 10^4 L.mol^{-1}cm^{-1} for NDMA at λ_{max}=540 nm (Stefan and Bolton, 2002),

l = cell path length (cm) = 1cm,

C = Concentration (molL^{-1}).

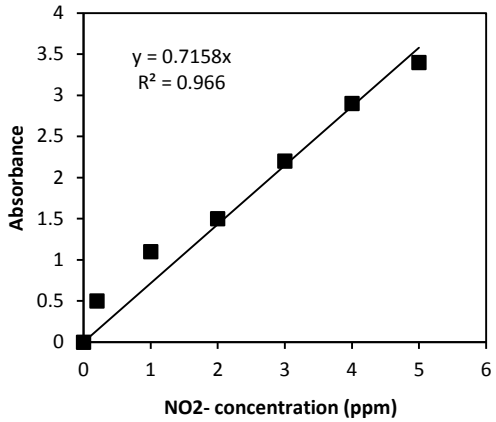
Results and Discussion

Nitrite concentration

Six standard solutions of known nitrite concentration and one blank solution analyzed, gave the following absorbance readings (Table 1).

Table 1: Absorbance of standard solutions- nitrite determination

Solution	Concentration (mg/L)	Absorbance
S_0	0.00	0.0
S_1	0.20	0.5
S_2	1.00	1.1
S_3	2.00	1.5
S_4	3.00	2.2
S_5	4.00	2.9
S_6	5.00	3.4



Fig/ 1: Calibration curve for UV-Vis spectrophotometric determination of NO_2^- (at λ_{max} = 493 nm)

The value for m was obtained electronically from the calibration plot of Absorbance against Concentration as 0.715 using Ms Excel; while the intercept c was taken as 0 since it is a straight line graph cutting through the x-axis at the origin.Hence, the general equation for calibration is: **A=0.715C + 0** for the calculation of concentration of individual samples of wine, malt drinks and fruit juices.
After the UV analysis of samples of each brand, the following concentrations were obtained (Table 2).

Table 2: Determination of nitrite in beverage samples using methyl anthranilate as a coupling agent

Sample	Absorbance Reading	Nitrite Conc. (mg/L)
Wine Brand A	0.63±0.058	0.84±0.08
Wine Brand B	0.93±0.015	1.24±0.20
Wine Brand C	0.60±0.001	0.80±0.00
Wine Brand D	1.97±0.208	2.52±0.13
Wine Brand E	0.97±0.058	1.28±0.08
Wine Brand F	0.77±0.058	1.02±0.07
Wine Brand G	1.07±0.153	1.41±0.20
Wine Brand H	0.60±0.002	0.80±0.00
Wine Brand I	0.67±0.058	0.89±0.08
Wine Brand J	0.70±0.100	0.93±0.13
Malt Drink A	0.56±0.058	0.75±0.08
Malt Drink B	0.53±0.015	0.71±0.08
Malt Drink C	0.60±0.001	0.80±0.00
Malt Drink D	0.53±0.058	0.75±0.08
Malt Drink E	0.60±0.001	0.80±0.00
Fruit Juice A	0.40±0.100	0.57±0.07
Fruit Juice B	0.47±0.208	0.66±0.19
Fruit Juice C	0.53±0.115	0.80±0.00
Fruit Juice D	0.40±0.100	0.57±0.07
Fruit Juice E	0.47±0.153	0.66±0.13

Values: Mean±Standard deviation

Table 3 below compares the nitrite concentration of the samples analyzed to the WHO standard limit of 3.0 mg/L in beverages.

Table 3: Comparison of nitrite concentration values with acceptable standard

Sample	Nitrate Conc. (mg/L)	Above/Below Standard
Wine Brand A	0.84±0.08	Below
Wine Brand B	1.24±0.20	Below
Wine Brand C	0.80±0.00	Below
Wine Brand D	2.52±0.13	Below
Wine Brand E	1.28±0.08	Below
Wine Brand F	1.02±0.07	Below
Wine Brand G	1.41±0.20	Below
Wine Brand H	0.80±0.00	Below
Wine Brand I	0.89±0.08	Below
Wine Brand J	0.93±0.13	Below
Malt Drink A	0.75±0.08	Below
Malt Drink B	0.71±0.08	Below
Malt Drink C	0.80±0.00	Below
Malt Drink D	0.75±0.08	Below
Malt Drink E	0.80±0.00	Below
Fruit Juice A	0.57±0.07	Below
Fruit Juice B	0.66±0.19	Below
Fruit Juice C	0.80±0.00	Below
Fruit Juice D	0.57±0.07	Below
Fruit Juice E	0.66±0.13	Below

Values: Mean±Standard deviation

Nitrate concentration determination

Six standard solutions of known nitrate concentration (0.20-10.20 mg/L) and one blank solution analyzed, gave the following absorbance readings (Table 4).

Table 4: Absorbance of standard solutions- nitrate determination

Solution	Concentration	Absorbance
S_0	0.0	0.0
S_1	0.2	0.8
S_2	2.2	1.6
S_3	4.2	3.2
S_4	6.2	4.5
S_5	8.2	6.8
S_6	10.2	7.3

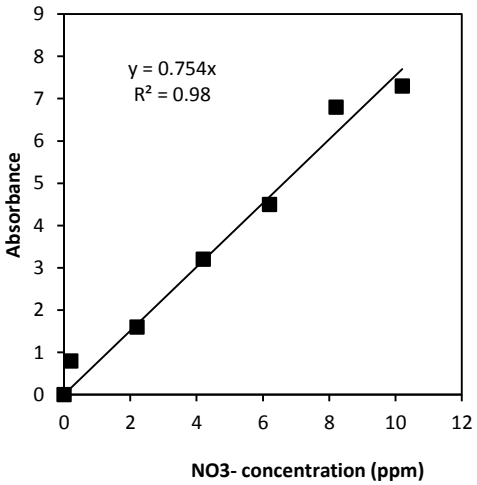


Fig. 2: Calibration curve for UV-Vis spectrophotometric determination of NO_3^- (at λ_{max} = 493 nm)

The value for m was obtained electronically from the calibration plot of Absorbance against Concentration; using msExcel, as 0.754 while the intercept c was taken as 0 since it is a straight line graph cutting through the x-axis at the origin.Hence, the general equation for calibration is:

A=0.754C + 0for the calculation of nitrate concentration in individual samples of wine, malt drinks and fruit juices.
After the UV analysis of samples of each brand, the following concentrations were obtained (Table 5).

Table 5: Determination of nitrate in beverage samples using methyl anthranilate as a coupling agent

Sample	Absorbance Reading	Nitrate Conc. (mg/L)
Wine Brand A	5.83±0.153	8.16±0.21
Wine Brand B	6.23±0.058	8.72±0.08
Wine Brand C	5.40±0.100	7.55±0.14
Wine Brand D	9.33±0.058	13.05±0.07
Wine Brand E	6.57±0.115	9.18±0.16
Wine Brand F	5.30±0.100	7.41±0.14
Wine Brand G	8.53±0.058	11.94±0.08
Wine Brand H	6.27±0.058	8.76±0.08
Wine Brand I	5.63±0.058	7.88±0.08
Wine Brand J	6.27±0.058	8.76±0.08
Malt Drink A	7.23±0.306	10.12±0.43
Malt Drink B	7.00±0.100	9.79±0.14
Malt Drink C	7.17±0.058	10.02±0.08
Malt Drink D	7.40±0.100	10.35±0.14
Malt Drink E	6.90±0.100	9.65±0.14
Fruit Juice A	10.57±0.058	14.78±0.08
Fruit Juice B	11.02±0.153	15.47±0.21
Fruit Juice C	8.53±0.058	11.93±0.08
Fruit Juice D	9.53±0.058	13.34±0.08
Fruit Juice E	6.23±0.058	8.72±0.08

Values: Mean±Standard deviation

Table 6 below compares the nitrate concentration of the samples analyzed to the WHO standard limit of 50.0 mg/L in beverages.

Table 6: Comparison of nitrate concentration values with acceptable standard

Sample	Nitrate Concentration (mg/L)	Above/Below Standard
Wine Brand A	8.16±0.21	Below
Wine Brand B	8.72±0.08	Below
Wine Brand C	7.55±0.14	Below
Wine Brand D	13.05±0.07	Below
Wine Brand E	9.18±0.16	Below
Wine Brand F	7.41±0.14	Below
Wine Brand G	11.94±0.08	Below
Wine Brand H	8.76±0.08	Below
Wine Brand I	7.88±0.08	Below
Wine Brand J	8.76±0.08	Below
Malt Drink A	10.12±0.43	Below
Malt Drink B	9.79±0.14	Below
Malt Drink C	10.02±0.08	Below
Malt Drink D	10.35±0.14	Below
Malt Drink E	9.65±0.14	Below
Fruit Juice A	14.78±0.08	Below
Fruit Juice B	15.47±0.21	Below
Fruit Juice C	11.93±0.08	Below
Fruit Juice D	13.34±0.08	Below
Fruit Juice E	8.72±0.08	Below

Values: Mean±Standard deviation

This implies that, all the wine brands, malt drinks and fruit juices analyzed contain nitrates but at concentrations below the acceptable limit set by regulatory bodies.

Nitrosamine concentration determination

The following results were obtained from the analysis of nitrosamines in the samples (Table 7).

Table 7: Nitrosamine concentration in the beverages using UV-Vis spectroscopy

Sample	Absorbance Reading	Nitrosamine Concentration (mg/L)
Wine Brand A	0.0	ND
Wine Brand B	0.0	ND
Wine Brand C	0.0	ND
Wine Brand D	0.0	ND
Wine Brand E	0.0	ND
Wine Brand F	0.0	ND
Wine Brand G	0.0	ND
Wine Brand H	0.0	ND
Wine Brand I	0.0	ND
Wine Brand J	0.0	ND
Malt Drink A	0.0	ND
Malt Drink B	0.0	ND
Malt Drink C	0.0	ND
Malt Drink D	0.0	ND
Malt Drink E	0.0	ND
Fruit Juice A	0.0	ND
Fruit Juice B	0.0	ND
Fruit Juice C	0.0	ND
Fruit Juice D	0.0	ND
Fruit Juice E	0.0	ND

ND: Not detected within the limit of analytical method used

Below is a comparison of the concentration of nitrite, nitrate and nitrosamine in all the beverage samples analyzed using UV-Vis spectrophotometry.

Table 8: Comparison of nitrite, nitrate and nitrosamine concentrations in common wine brands, malt drinks and fruit juices in Makurdi using uv-vis spectroscopy

Sample	Nitrite Conc. (mg/L)	Nitrate Conc. (mg/L)	Nitrosamine Conc. (mg/L)	Toxicity
Wine Brand A	0.84±0.08	8.16±0.21	ND	Non-toxic
Wine Brand B	1.24±0.20	8.72±0.08	ND	Non-toxic
Wine Brand C	0.80±0.00	7.55±0.14	ND	Non-toxic
Wine Brand D	2.52±0.13	13.05±0.07	ND	Non-toxic
Wine Brand E	1.28±0.08	9.18±0.16	ND	Non-toxic
Wine Brand F	1.02±0.07	7.41±0.14	ND	Non-toxic
Wine Brand G	1.41±0.20	11.94±0.08	ND	Non-toxic
Wine Brand H	0.80±0.00	8.76±0.08	ND	Non-toxic
Wine Brand I	0.89±0.08	7.88±0.08	ND	Non-toxic
Wine Brand J	0.93±0.13	8.76±0.08	ND	Non-toxic
Malt Drink A	0.75±0.08	10.12±0.43	ND	Non-toxic
Malt Drink B	0.71±0.08	9.79±0.14	ND	Non-toxic
Malt Drink C	0.80±0.00	10.02±0.08	ND	Non-toxic
Malt Drink D	0.75±0.08	10.35±0.14	ND	Non-toxic
Malt Drink E	0.80±0.00	9.65±0.14	ND	Non-toxic
Fruit Juice A	0.57±0.07	14.78±0.08	ND	Non-toxic
Fruit Juice B	0.66±0.19	15.47±0.21	ND	Non-toxic
Fruit Juice C	0.80±0.00	11.93±0.08	ND	Non-toxic
Fruit Juice D	0.57±0.07	13.34±0.08	ND	Non-toxic
Fruit Juice E	0.66±0.13	8.72±0.08	ND	Non-toxic

Statistical analysis

The ANOVA results and mean graphs shown below reveal that there is no significant difference in the nitrate concentration between each sample (sig. (.440) > 0.05). The same holds for Nitrite concentration (sig (.987) > 0.05).
For the Nitrosamine concentration, the mean graph below reveals the uniformity of its concentration in each sample. The mean graphs for nitrate and nitrite concentrations are also given below.

Table 9: One-sample T-test nitrite determination

	Test Value = 3					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Nitrite Conc	-21.177	19	.000	-2.06000	-2.2636	-1.8564

Table 10: One-Sample T-test Nitrate Determination

	Test Value = 50					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Nitrate Conc.	-74.828	19	.000	-39.72100	-40.8320	-38.6100

Table 11: ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Nitrate Conc	Between Groups	65.240	11	5.931	1.134	.440
	Within Groups	41.838	8	5.230		
	Total	107.078	19			
Nitrite Conc	Between Groups	.855	11	.078	.227	.987
	Within Groups	2.741	8	.343		
	Total	3.596	19			
	Within Groups	.000	8	.000		
	Total	.000	19			

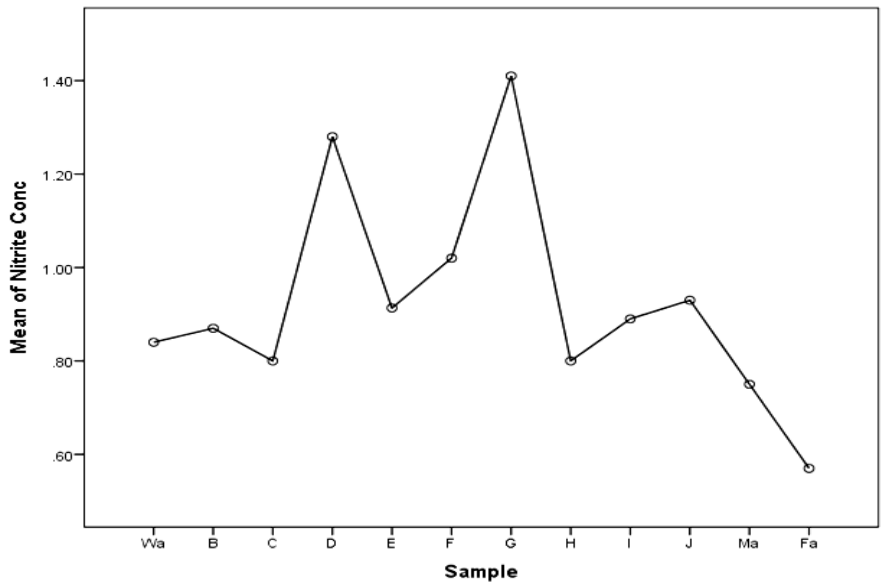


Fig. 3: Mean graph for nitrite concentration

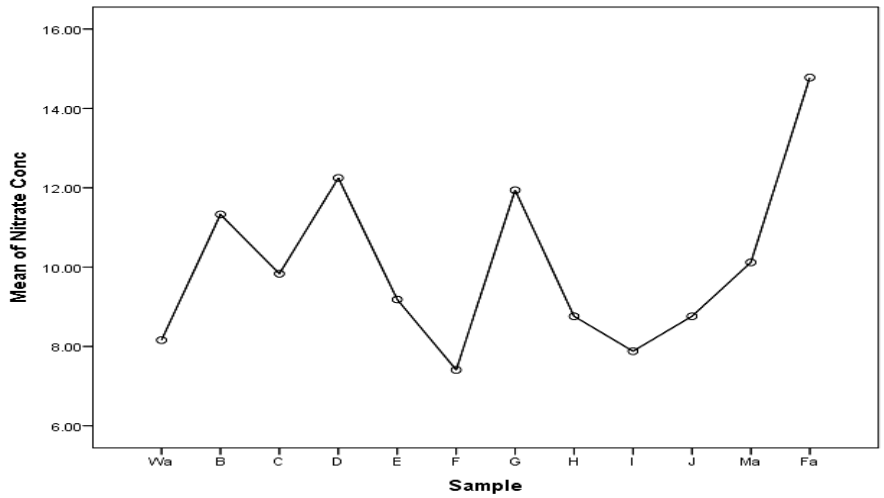


Fig. 4: Mean graph for nitrate concentration

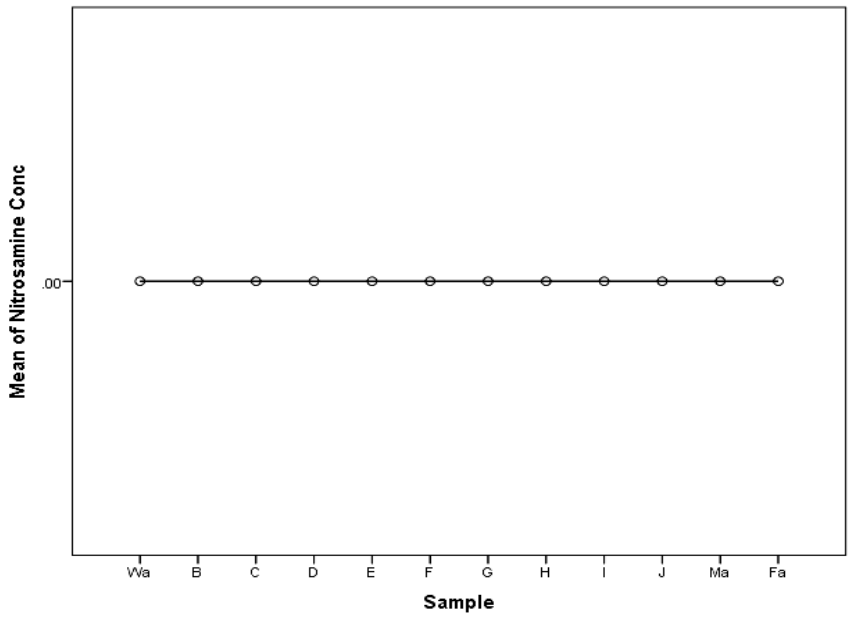


Fig. 5: Mean graph for nitrosamine concentration

Nitrite and nitrate concentration

From the results obtained, the nitrite concentration in fruit juice samples is lower than that in the malt drinks and wine brands. This suggests a relationship between the level of ascorbic acid and prevention of the reduction of nitrates to nitrites. Also worthy of note is the fact that all the malt drinks had almost equal absorbance readings (and consequently concentrations). This probably suggests that the raw materials, methods for handling the barley and preservatives used are similar, if not exactly the same.

The results also showed that, the nitrate concentration in fruit juice samples and malt drinks is higher than that in the wine brands. It is particularly high in fruit juices that contain a mix of apples or coconut juice. The concentration of nitrates in fruit juices may be a result of using more of high amounts of nitrate preservatives than nitrite preservatives or from the fertilizer used in cultivation of the fruits not going through processing as much as the barley (for malt) or grapes (for wines). However, the concentrations obtained being below or above the maximum acceptable limit is what matters, as discussed earlier. Again, the malt drinks showed similar values for nitrate concentration.

Ezeagu (2005) reported a likely connection between the alcoholic content of beverages with the concentration of nitrites and nitrates present in them. He detected high levels of nitrate (up to 22.5 mg/L in palmwine and 50.0 mg/L in beer) and 0.26-9.53 mg/L nitrite in beer and no nitrite was detected in palmwine. The alcoholic wine brands analyzed in this research work showed marked increase in nitrite and nitrate concentrations in comparison to the non-alcoholic wine brands. However, some fruit juices which were not alcoholic had higher concentrations, particularly of nitrates. This suggests that, though alcoholic content may play a role in the level of nitrite and nitrate concentrations in beverages, there are other stronger factors which come into play in determining these concentrations. The fact that no nitrite was detected in palmwine in his research also suggests that the source of nitrites in canned/bottled beverages is entirely from the preservatives which they contain.

Nitrosamine concentration

From the result obtained, no nitrosamine was detected in any of the samples analyzed. This implies that the wine brands, malt drinks and fruit juices do not pose any risk at all, of carcinogenicity from nitrosamines, to consumers of these beverages in Makurdi, Benue state. This will not be the first time beverages tested negative to the presence of nitrosamines. A limited survey carried out to determine the nitrosamine concentration of several varieties of alcoholic beverages (beer and ale, whiskey, wine, cider, etc.) sold in Canada in 2007, showed that of 22 samples different beers and ale analyzed, all but one contained no traces of N-nitrosodimethylamine (NDMA). Only one Canadian rye and one scotch whiskey out of 13 samples contained traces of nitrosamines and all 8 wines and 7 cider samples were negative (McPherson *et al.*, 2007).

Volatile nitrosamine (VNA) levels in local South Korean and imported beverages were determined between 1995 and 2002. A total of 147 beverages including lager beer, whiskey and traditional Korean beverages (Chungju, Takju and Soju) were analyzed for their VNA content by GC-TEA. Of eight VNAs (NDMA, NDEA, NPYR, NMOR, NDBA, NPIP, NDPA and NDPhA), only NDMA was detected.

In 1995, NDMA was detected in 79.3% of domestic beers, the average was 0.8 µkg/L. Seven years later, the average NDMA level for 18 domestic beers was 0.3 µkg/L and it was positive in 55.6% samples. However, in 2002, NDMA was not detected in any of the samples (Shin *et al.*, 2005). This suggests that over the years, improvement in production methods for beverages has occurred and the carcinogenic nitrosamines may have successfully been eliminated from our much cherished beverages. However, only periodic, random checks will confirm or contradict this stand.

Conclusion

The concentration of nitrite, nitrate and nitrosamine in common wine brands, malt drinks and fruit juices in Makurdi, Benue state was successfully tested using UV-Vis spectroscopy and proven not to pose any health risk to consumers in Makurdi, Benue state. Nitrate and nitrite were detected at concentrations below the maximum acceptable level stipulated by the World Health Organisation (WHO) and nitrosamines were not detected in any of the samples.

This confirms the reason why no case of blue-baby syndrome, or any illness associated with contamination from nitrite, nitrate and nitrosamines, has not been reported in Makurdi, Benue state in recent times.In view of the known carcinogenicity of N-Nitroso compounds, exposure to these compounds in food and drinks should be minimized by appropriate technological means such as;

a. Using low temperatures in the manufacturing process.
b. Lowering the nitrite and nitrate concentration in preserved foods and drinks to the minimum required to ensure microbiological safety.
c. Use of inhibitors of nitrosation like α-tocopherol (vitamin E) and/or ascorbic acid (vitamin C) as described above.

Recommendations

The nitrite, nitrate and nitrosamine concentrations of beverages consumed in Makurdi, Benue state and other locations should be periodically studied to ensure that their levels are maintained below the acceptable limit. Regulations should also be enforced by NAFDAC, to ensure that nitrosation inhibitors such as ascorbates and erythrobates are inculcated into packaged foods, in sufficient quantities.

Satchet water, smoked fish/pork, *'burukutu',* common vegetables like fluted pumpkin and *Amaranthusspp*, grown around the River Benue, and other food stuff typically consumed in Makurdi, Benue state should be analyzed as well for the levels of nitrates, nitrites and nitrosamines. This will ensure that consumers of these substances are not exposed to harmful contaminants in their consumables.

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