SPECIATION OF LEAD AND COPPER CONTENTS IN NON-ALCOHOLIC WINES FROM MARINA DISTRIBUTION CO. LTD, ACCRA, GHANA

TECHNICAL REPORT

BY

ESSEL KOFI KWEGYIR

JULY, 2014.

DECLARATION

I declare that the technical report was written by me, Kofi Kwegyir Essel and has not been submitted anywhere for any purpose. I am the brainchild of this technical report. The analyses for Copper and Lead were done by me in the Food Chemistry Laboratory of the Food Research Institute of the Council for Scientific and Industrial Research, Ghana.

DEDICATION

I dedicate this to my late grandmother, Ekua Kwegyirba Quagrinie. May her soul rest in perfect peace.

ACKNOWLEDGEMENT

My intellectual indebtedness goes to the God Lord Almighty for making this technical report possible. For giving me the intuition and the insight for this report to produce this technical report. May His name by praised forever.

ABSTRACT

The concentrations of two heavy metals (Cu and Pb) were determined in three brands of Ghanaian wines by flame atomic absorption spectrometer (FAAS) after digesting the wine samples with 0.1M HNO_3. The copper concentration in the three different brands of wine was in the range of 0.4-1.2 mg/L. The toxic lead metal was found to be below the detection (BDL) of 0.1 mg/L. The levels of Cu concentrations were found to be comparable to the permissible limit set by OIV, French and American Wines. The copper and lead identification sources were due to natural sources and anthropogenic sources during vinification processes such as the equipment used.

TABLE OF CONTENT

Content Page

TABLES

FIGURES

CHAPTER 1

1.0 Introduction

1.1 Importance of Wine

Wine is one of the most common alcoholic beverages consumed all over the world. Wine naturally contains 85-89 % water. Moderate wine consumption can reduce the risk of heart disease and individual components of wine (alcohol and principal polyphenols) can reduce the risk of coronary heart disease in certain individuals. It is important to know that polyphenols can reduce the rate of harmful cell oxidation and favourably affect other processes that, if unchanged, could lead to atherosclerosis and heart disease. Some studies indicated that the cardio protective compounds in grapes, polyphenolic antioxidants, reside in the skin and seeds (Gabriela, 2011).

Grape skins, which contain purple pigment, are crushed with pulp to make the red wine. The skins give red wine its coloration and contain the highest concentration of polyphenols which are potential antioxidants. Antioxidants are substances that protect cells from oxidative damage caused by free radicals. The polyphenols in red wine includes catechin, gallic acid and epicatechin. Laboratory experiments carried out showed that both wines were active against streptococci. The red had a stronger effect than the white, though the difference was not statistically significant. Organic acids in wine, such as acetic acid, citric acid, lactic acid, succinic acid and tartaric acids, are responsible for the antibacterial activity against oral streptococci. The finding suggests that wine "enhances oral health" (Daglia et al, 2007). Wine polyphenols displayed no activity against oral *streptococci or S. Pyogenes.*

Cell damage caused by free radicals has been implanted in the development of cancer. These chemicals can damage important parts of cells including protein, membranes and DNA.

Among other polyphenolic flavonoids, resveratrol is thought to be at least in part responsible for the possible anti-cancer effect of red wine. Resveratol has been found in at least seventy two plant species, a number of which are components of the human diet such as mulberries, grapes (Langcake Pryce, 1976; Sanders et al, 2000), red wine (Siemann and Creasy, 1992) and peanuts.

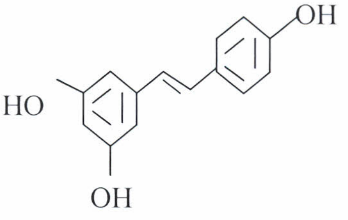

Figure 1: Structure of resveratol

Resveratol has also shown the ability to inhibit the growth of prostate cancer cells whilst leaving normal cells unaffected (Narayana et al, 2004).

Resveratol also decreases triglyceride levels as well as low density (LD) "bad" cholesterol levels. Raised levels of serum lipids are strongly associated with an increased risk of cardiovascular disease (Miura et al, 2003).

Although, the positive health effects of wine are many, the evidence is clear that these benefits are the highest for those who drink red wine in moderation (two drinks per day for men and one drink per day for women) over extended periods of time. Three or more drinks per day may increase the risk of neurodegeneration, depressive disorders, obesity, weakening of bones, hypertriglyceridermia, heart disease, hypertension, stroke, breast cancer, suicide and injuries (Saremi and Arora, 2008). Wine consumption in any amount is contraindicated for pregnant women, children, patients with liver disease and in combination with certain medications (Feher et al, 2005). Historically, wine has been used as an antiseptic, a pain killer and to treat dermatological conditions and digestive disorders (Feher, et al; Robinson, 2006)

Regular wine consumption should be used with caution in individuals predisposed to alcoholism, organic diseases, cirrhosis of the liver, migraine, headaches and allergies. Red wine, de-alcoholized red wine and grape juice consumption have lowered blood pressure in patients with coronary artery disease or hypertension (Foppa et al, 2002; Park et al, 2004; Karatzi et al, 2005; Jimenez et al, 2008).

1.2 Objective

The objective of this technical report is to determine lead and copper contents in white, red and rose wines.

CHAPTER 2

2.0 Literature Review

2.1 Effects of Heavy Metals in Wine on Humans

The identification of heavy metals in wine is a subject of increasing interest since complex atom may reduce their toxicity and bioavailability (Cocchi et al, 2004). Metals in wine can originate from both natural and anthropogenic sources and its concentration can be a significant parameter affecting consumption and conservation of wine. Since metallic ions have important roles in oxido-reductive reactions resulting in wine browning, turbidity, cloudiness and astringency, wine quality depends greatly on its metal consumption.

Consumption of wine may contribute to the daily dietary intake of essential metals e.g. copper, iron and zinc, but can also have potentially toxic effects if metal concentrations are not kept under permissible limit (Tariba, 2011).

Copper has a definite negative effect on the organoleptic properties of wine (Gennaro et al, 1986). Copper is both an essential and potentially toxic element for humans when in excess (Scheinberg, 1991). When wine or wine products are consumed in large quantities, the toxic effect of their pollutants may have an additive effect to induction of alcoholism. A typical example is the presence of lead in wines (Roses et al, 1997). Several investigators have shown that accumulation of lead in mitochondria of mice occurs with the simultaneous ingestion of small doses of lead and alcohol (Brivet et al, 1990); Nation et al, 1990). Trace metal composition of grapes, wines and other alcoholic products is influenced by the type of soil, wine processing equipment vinification methods, the composition of fungicides, insecticides, fertilizers etc (Eschnauer and Neeb, 1988) used in the wine industry.

Laurie et al (2010) undertook a research titled "Analysis of Major Metallic Elements in Chilean Wines using Absorption Spectroscopy". They concluded that all metal concentrations were within normal ranges as compared to previously reported data from other world wine areas. They also observed that the concentration of Na was higher in the wines produced in the northern parts of the country.

Woldemariam and Chandravanshi (2011) studied the concentration of essential and non-essential elements in selected Ethiopian wines. They concluded that wet digestion method and the determination of selected metals at trace levels in wines by flame atomic absorption method were found to be efficient, precise and accurate. It was also observed that Pb concentrations in Ethiopian wines were found in the range of 0.14-0.31 mg/L and these Pb values were somehow larger than the limit set by International Organization for Wines and Grapes (OIV) as 0.2 mg/L. They also said that moderate wine consumption contributes to the daily nutritional requirements of many essential metals. The OIV for Cu is 1 mg/L. The content of some metals can be used to identify the geographic region in which the grapes were grown due to the direct relationship with soil composition (Pyrzynska, 2007).

CHAPTER 3

3.0 Method

The wines were analyzed for two heavy metals by AAS and were done in triplicates.

3.1 Analyses by AAS and Flame Photometer:

The metals Cu and Pb were determined using Atomic Absorption Spectrophotometer, Perkin Elmer Model 200A using an air/acetylene flame. Distilled water was used to prepare 0.1M HNO_3 acid for dilution of wine solutions. All glasswares were thoroughly washed and were completely dried before use.

3.2 Sampling:

For this study, three most popular Ghanaian wine brands consisting of white wine, red wine and rose wines were selected. Seven brands of white wines, six brands of red wines and two brands of rose wines were selected randomly from Marina Distribution Co. Ltd at different sites of Accra, the capital of Ghana. Each bottle of the same three brands was shaken slightly to ensure mixing.

3.3 Ashing

Crucibles were dried in the furnace to a constant weight for 30 minutes. It was crucibles were then cooled to room temperature. The sample was ashed in a muffler furnace after taking 5.0 ml of the wine samples and evaporation on a hot plate at 550 ^{0}C for 6-8 hours. The samples in the crucibles were cooled to room temperature after which digestion was carried out.

3.4 Digestion of Wine Samples

From the bulk samples, 5.0 ml of the wine samples were wet ashed (digested) to decompose the organic substances and make clear solution in duplicates. After digestion was completed, the crucible with the digest was removed from the hot plate and was left to cool (for 10 mins). The cooled digest was transferred quantitatively into 50 ml volumetric flask after the addition of 0.1 M HNO_3. The volume of the solution was diluted to the mark with 0.1M HNO_3. The digest was kept in the refrigerator until the analysis by AAS.

CHAPTER 4

4.0 Results and Discussion

The Lead (Pb) contents in wines found in Marina Distribution Company in Accra were found to be below the detection limit of 0.1 mg/L in all the fourteen wine samples. These lower concentrations of Pb found in the local wines showed that they are of good nutritional value and are of good economic importance if consumed. Pb could be toxic when ingested (Lara et al, 2005). Wine is susceptible to lead contamination from the seals on wine bottles and the linings of wine casks.

The concentrations of copper in the studied area ranged between 0.40-1.20 mg/L. This could be compared to the limit set from Marina Distribution Co. Ltd did not contain any lead (Pb).

Table 4.1: Analytical wavelengths, detection limits, correlation coefficients and correlation equations of the calibration curves for the determination of metals in wine samples by FAAS.

These values were below the standard set by OIV and other countries.

Metal	Wavelength (nm) detection limit	Instrument detection limit	Method coefficient of calibration curve	Correlation for calibration curve	Equation
Cu	324.7	0.02	0.03	0.9998	Y= 0.0893x - 0.0017
Pb	283.2	0.100	0.10	0.9995	Y= 0.0058x + 0.001

The analytical wavelengths, the correlation coefficients and correlation equations of the calibration curves for the determination of heavy metals in wine samples by FAAS are given in Table 4.1. The correlation coefficients of all the calibration curves were > 0.999 and these correlation coefficients showed that there was very good correlation (relationship) between concentration and absorbance. The method detection limits for both lead and copper were

<0.1 mg/L, which clearly indicate that the method is applicable for the determination of metals at trace levels.

Table 4.2 Results of mean concentrations of heavy metals in Ghanaian White, Red and Rose wines (mg/L)

Sample	Cu	Mean	Pb	Mean
White wine	0.58-0.89	0.76	BDL	-
Red wine	0.40-0.84	0.67	BDL	-
Rose wine	0.50-1.20	0.85	BDL	-

From Table 4.2, the concentrations of white wines ranged from 0.58-0.89 mg/L with a mean of 0.76 mg/L. The red wine concentrations ranged from 0.40-0.84 mg/L with a mean of 0.67 mg/L. The concentration of the Rose wine range between 0.50-1.20 mg/L with a mean of 0.85 mg/L. The lead contents in white, red and rose wines were found to be below the detection limit of 0.1 mg/L.

The rose wine contains Cu in the highest amounts with concentration 1.2 mg/L. The lowest concentration was found in red wine with copper concentration of 0.40 mg/L.

TABLE 4.3 Comparison of the concentrations of heavy metals in wines of different countries.

Metal Concentrations of heavy metals (mg/L) in different countries

Country/Metal	Copper (Cu)	Lead (Pb)
Czech	0.012-6.827	0.010-1.253
French	ND-0.48	0.006-0.023
German	0.02-0.71	-
Greek	0.2-1.65	ND-0.62
Hungarian	0.15-2.57	-
Italian	0.001-1.34	0.01-0.35
Spanish	ND-3.1	0.001-0.096
American	0.05-0.58	-
Ghanaian	0.4-1.2	BDL

The comparison of heavy metals in Ghanaian wines and published data on wines from different countries is given in Table 4.3. The Cu contents in Ghanaian wines were in the range of 0.4-1.20 mg/L. The Ghanaian wines had lower levels of Cu than that reported in Czech, Greek, Hungarian, Italian and Spanish wines. However, the Ghanaian Cu contents in wine are comparable to that reported in the French, German and American wines. But the Ghanaian wines contain lower amount of Cu than that reported in Czech, German, Greek, Hungarian, Italian and American wines.

Table 4.4 Comparison of the levels of metals in Ghanaian wines to permissible levels of some metals (mg/L) in some countries and by OIV* (Benitez et al, 2002; Azenha, 2000) Bull. Chem. Soc. Ethiopia (2011, 25/2).

Country	Concentration of metals (mg/L)				
Metal	Na	Cu	Zn	Pb	Cd
Australia		5	5	0.2	0.05
Germany		5	5	0.3	0.01
Italy		10	5	0.3	0.01
OIV*	0.4-1.2		BDL of 0.1 mg/L		

* International Organization for Grapes and Wines (OIV)

Many countries have set maximum permissible limit of some heavy metals in wines considering both the enological and toxicological effects of the heavy metals in wines. The tolerable limits for some heavy metals in wines are also set by International Organization for Grapes and Wines (OIV). The maximum permissible levels in Australia, Germany, Italy and by OIV are given in Table 4.4 above.

The Ghanaian wines contained lower concentration of Cu (0.4-1.2 mg/L) than the limit set for Cu in Australia, Germany and Italy. However, the Ghanaian content of Cu in wine is comparable to that set for Cu (1 mg/L) by OIV. The Pb contents were below the detection limit in wines from Ghanaian market and cannot be compared to the permissible limits set by OIV and the countries in Table 4.4.

CONCLUSION

The Ghanaian wines were found to contain Copper lower than the ones set by the countries discussed in Table 4.3. Excerpt by the permissible limit set by OIV. This shows that wines could be a good source of Cu for humans. The toxic Lead was not detected in Ghanaian wines revealing that Ghanaian wines contain very low concentration of Pb (< 0.1 mg/L). In general the Cu content values in wine are in line with the previously reported data set by OIV, American and French wines. The Ghanaian wines are safe for consumption and for economic reasons.

REFERENCES

Antibacterial Activity of Red and White Wine against oral streptococci. Daglia, M; Papetto, A; Grsoli, P; Aceti, C; Dacarro, C; Gazzani, G.J. Agric Food Chem; (Article); 2007; 55 (13); 5038-5042. DO1 10.1021/Je0703529.

Cocchi, M.G. Franchini, D. Manzini, M. Manfredini, A. Marchetti and A. Ulrici, 2004. Journal of Agric Food Chemistry, 52(13): 4047-56.

Daglia, M; Papetto, A; Grsoli, P; Aceti, C; Dacarro, C; Gazzani, G.J. Agric Food Chem; (Antibacterial Activity of Red and White Wine against oral streptococci. Article; 2007; 55 (13); 5038-5042. DO1 10.1021/Je0703529.

Feher., J G. lengyal and A. Lugasi. 2005. Cultural history of wine , the theoretical background of wine therapy. Orv. Hetil. 146:2635-2639.

Foppa, M. T.D. Fuchs, L. Preissler, A. Andrighetto, G.A. Rosito and B.B. Duncan. 2002. Red wine , the noon meal lowers post-meal blood pressure. A randomized trial in centrally obese, hypertensive patients. J.Stud. Alcohol 63:247-251

Karatzi, K.N. C.M. Papamicheal, E.N. Karatzi, T.G. Papaioannou, K.A. Aznaouridis, P.P. Katrichti, K.S. Stamatelopoulos, A. Zampelas, J; P. Lekakis and M.E. Mavrikakis, 2005. Red wine acutely induces favourable effects on wave reflections and central pressures in coronary artery disease patients. Am. J. Hypertens. 18:1161-1167.

Jimenez, J.P, J. Serrano, M. Tabernero, S. Arranz, M.E. Diaz-Rubio, L. Garcia-Diz, I. Goni and F. Saura-Calixto, 2008. Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. Nutrition 24:646-653.

Langcake, P. & Pryce, R, J. (1976). The production of resveratrol by Vitis vinifera and other members of the vitaceae as a response to infection or injury physiol. Pathol. 9:77-86.11.

Lara, R, S Cerutti, J.A. Salonia, R.A. Olsina and Argentine wines using ETAAS and USN-ICP-OES. A Journal of Food and Chemical Toxicology. 48:293-297.

Miura D. Miura Y. Yagasaki K (2003). Hypolipidermic action of dietary resveratol, a phytoalexin in grapes and red wine, in hepatoma- bearing rats.

Narayanan, N.K. Narayanan, B.A. Nixon DW, Resveratol-induced cell growth inhibition and apoptosis is associated with the modulation of phosphoglycerate mutase B in human prostrate cancer cells: two dimensional sodium dodecyl sulphate- polyacrylamide gel electrophoresis and mass spectrometry evaluation. Cancer Detect Prev. 2004; 28(6):443-52.

Park, Y.K. S.H. Lee. Park, S.S. Kim and M.H. Kang, 2009. Changes in antioxidant status, blood pressure in Korean hypertensive men. Double blind, placebo controlled intervention trial. Biofactors 22:145-147

Robinson, J. 2006. The Oxford companion to wine. Oxford University Press, New York.

Saremi A. Arora R. (2008) . The cardiovascular implications of alcohol and ed wine. Am J. Ther. 2008 May –June; 15(3): 265-77. Doi:10: 1097MJT. Ob013e3180 a5e61a

Sanders, T.H, McMichael, R.W; Jr. & Hendrix, K.W. (2000). Occurrence of resveratrol in edible peanuts. J. Agric. Food Chem. 48:1243-1246.12.

Slemani, E.H. & Creasy L.L. (1992) Concentration of Phytoalaxin resveratrol in wine. Am. J. Enol. Vitic. 43:49-52:13.

Tariba, B. 2011. Metals in wine. Impact on Wine Quality and Health Outcomes. Journal of Springer Science, 11: 2-7.

V. Felipe Laurie, Evelyn Villagra, Jamie Tapia, Jorge E.S. Sarkis and Marcos A. Hortellani (2010). Analysis of major metallic elements in Chilean wines by Atomic spectroscopy. Gen. Inv. Agr. 37(2): 77-85. 2010.

APPENDIX :Results of Copper and Lead contents in White, Red and Rose wines

Sample Description	Cu	Pb
Vinho Verde Blue Ocean White wine	0.58	BDL
Vinho Verde Monte Geko, White wine	0.87	BDL
Ferras Do Sado White wine	0.89	BDL
Alentejo Adega De Borba White wine	0.62	BDL
Douro cake Do Taha White wine	0.71	BDL
White wine	0.84	BDL
White wine	0.84	BDL
Ferras Do Sado, Red wine	0.65	BDL
Alentejo Galitos, Red wine	0.40	BDL
Alentejo Adega De Borba Premium Red wine	0.72	BDL
Alentejo Ageda De Borba, Reserva Red wine	0.74	BDL
Douro Zimbro, Red wine	0.72	BDL
Douro cake Do Tanha, Red wine	0.65	BDL
Vinho verde Blue Ocean Rose wine	0.50	BDL
Ferras Do Sado Rose wine	1.20	BDL

BDL – Below detection limit of 0.1 mg/L.