Analysis of Some Contaminants Commonly Found in Local Alcoholic Beverages

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***Abstract*: Analysis of common contaminants namely: methanol, 5-Hydroxy methyl furfural (HMF) and lead in local alcoholic liquors were carried out using the GC-FID, HPLC-UV and AAS methods respectively.**
The levels of contaminants present in local alcoholic liquor were determined and compared with Distilled liquor sample prepared in the laboratory and standard limit such as National Agency for Food and Drug Administration and Control, NAFDAC and Ministry of Health- Vietnam. All results found to be higher than standard limit.
Keywords: Local Alcoholic Beverages, Toxicity of Wine,

INTRODUCTION:

Wine (Arage) is an alcoholic beverage made from fermented dates or other fruits. Yeast consumes the sugars in the fruits and converts them into alcohol. Wines made from fruits besides grapes are usually named after the fruit from which they are produced (for example, pomegranate wine, apple wine and elderberry wine) and are generically called fruited wine.

Archaeological evidence suggests that the earliest known production of wine, made by fermenting grapes. However, the spread of wine culture westwards was most probably due to the Phoenicians who were centered on the coastal strip of today's Lebanon – itself one of the world's oldest sites of wine production. The wines of Byblos were exported to Egypt during the old Kingdom (2686 BC–2134 BC) and throughout the Mediterranean. Evidences include two Phoenician shipwrecks from 750 BC discovered by Robert Ballard, whose cargo of wine was still intact.

A 2003 report by archaeologists indicates a possibility that grapes were mixed with rice to produce mixed fermented beverages in China in the early years of the seventh millennium BC. (patrick, 2003).
Although excessive alcohol consumption has adverse health effects, epidemiological studies have consistently demonstrated that moderate consumption of alcohol and wine is statistically associated with a decrease in cardiovascular illness such as heart failure.(Ezra, 2008).

While evidence from laboratory and epidemiological (observational) studies suggest a cardio protective effect, no controlled studies have been completed on the effect of alcoholic beverages on the risk of developing heart disease or stroke. Excessive consumption of alcohol can cause cirrhosis of the liver and alcoholism; A study of women in the United Kingdom, called The Million Women Study, concluded that moderate alcohol consumption can increase the risk of certain cancers, including breast, pharynx and liver cancer.(Allen, *et al.* 2009).

Lead is a highly poisonous metal (whether inhaled or swallowed), affecting almost every organ and system in the body, The component limit of lead (1.0 µg/g) is a test benchmark for pharmaceuticals, representing the maximum daily intake an individual should have, Even at this level, a prolonged intake can be hazardous to human beings. (Heavy Metals Testing by Usp 2012).

5-Hydroxymethylfurfural (HMF) regularly occurs in foods and in alcoholic beverages and wine. However, the previous studies of HMF in wine its very few, so that this study will provide the first quantitative risk assessment of HMF in wine.

Objectives:

To identify and quantify some contaminants such as: methanol, lead, HMF in local alcoholic liquor.
To compare commercial local wine with distilled liquor sample prepared in laboratory.
To compare these contaminants in two samples with the standard limits.

Chemical Composition of Wine

The number of compounds identified in wine increased dramatically since the development of gas chromatography (GC), high pressure liquid chromatography (HPLC), a thin layer chromatography (TLC), infrared spectroscopy (IRS), and nuclear magnetic resonance (NMR). (George, *et al*. 1998).The interface of mass spectrometry (MS) to GC and to HPLC has been especially valuable in identifying unknown compounds. More than 500 compounds have been recognized in wine so far, of which 160 are esters. Wines generally contain 0.8–1.2 g of aromatic compounds per liter, of which the most common are fusel alcohols, volatile acids, and fatty acid esters. Fusel alcohols often constitute 50% of all volatile substances in individual wines. Carbonyls, phenols, lactones, terpenes, hydrocarbons, sulfur, and nitrogen compounds, although present in much lower concentrations, are more important qualitatively and contribute specific sensory characteristics relevant to the fragrance of a wine.
The taste and mouth-feel sensations are due primarily to the few compounds that occur individually at concentrations > 100 mg/L. These include water, ethanol, organic acids, sugars, and glycerol. Tannins occur in red wine and rarely in content and their precipitation during pressing. (Brun, *et al*. 1989).

Water:
Water is the major chemical constituent of wine and is critical in establishing its fundamental characteristics. It is an essential component in many of the chemical reactions involved in grape growth and juice fermentation and in wine aging.
(Amerine and Roessler 1982)

Sugars:
The principal grape sugars are glucose and fructose, and they occur in roughly equal proportions at maturity, whereas over mature grapes often have a higher proportion of fructose. Sucrose is rarely found in wine, and other sugars are found in insignificant amounts. (Amerine, Roessler 1982). The primary wine yeast, Saccharomyces cerevisiae, derives most of its metabolic energy from glucose and fructose and has limited ability to ferment other substances. Residual sugars in dry wines, generally below 1.5 g/L, consist mostly of pentose such as arabinose, rhamnose, and xylose.
Generally, sweetness is detected at levels higher than 1 Brix and this is influenced by other constituents such as ethanol, acids, and tannins. In addition to being absolutely essential for fermentation and production of ethanol, sugars are metabolized to higher alcohols, fatty acid esters, and aldehydes, which give different wines their individual aromatic character. High sugar concentrations also can increase the volatility of aromatic compounds. (Crippen, Morrison 1986).

Acids:
In wine, acids are divided into two categories: volatile and fixed. The first refers to acids that can be readily removed by distillation, whereas the latter refers to the carboxylic acids. The most common volatile acid in wine is as acetic acid. Quantitatively, carboxylic acids such as tartaric, malic, lactic, succinic, oxalic, fumaric, and citric acids control the pH of wine (Robinson 2006).

Ethanol:
The most important and abundant alcohol in wine is ethanol. Under standard fermentation conditions, ethanol can accumulate to ~14–15%, but generally ethanol concentrations in wine range between 10–13%. The primary factors controlling ethanol production are sugars temperature, and yeast strain.
Ethanol is crucial to the stability, aging, and sensory properties of wine. As its production increases during fermentation, it increasingly limits the growth of most microorganisms, allowing Saccharomyces cerevisiae to dominate the fermentation process. The inhibitory activity of ethanol, combined with the acidity of the wine and the added potassium metabisulfite, allows wine to remain sound for years in the
Absence of air. During skin fermentation of red grapes, ethanol acts as an important solvent in the extraction of pigments and tannins. It also influences the types and amounts of aromatic compounds produced by affecting the metabolic activity of yeasts. Furthermore, ethanol acts as an essential reactant in the formation of volatile compounds produced during fermentation and those formed during aging in wood cooperage. The dissolving action of ethanol probably reduces the evaporation of aromatic compounds along with carbon dioxide during fermentation. (Deibner, *et al.*, 1965). Ethanol plays several roles in the aging of wine. Together with other alcohols, it slowly reacts with organic acids to produce esters and influences their stability. Moreover, it also reacts slowly with aldehydes to produce acetals.

Methanol:
Methanol is a minor constituent of wine (0.1–0.2 g/L) and has no direct sensory effect. It is predominantly generated from the enzymatic breakdown of pectins. On degradation, methyl groups associated with pectins are released as methanol. Pectolytic enzymes added to juice or wine to aid clarification inadvertently increase the methanol content of wine.
Oxidation of methanol in the body produces formaldehyde and formic acid, which are toxic to the central nervous system. Wine has the lowest concentration of methanol of all fermented beverages. (Lee, et al. 1979).
Other potentially significant higher alcohols in wine are the straight-chain alcohols: 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol. (Rapp, Mandery 1988). The formation of higher alcohols occurs as a by-product of yeast fermentation and is markedly influenced by vilification practices such as temperature, presence of oxygen, suspended solids, and yeast strain. (Sponholz, 1988). Higher alcohols may originate from amino acid deamination and grape-derived aldehydes, and by the reductive denitrification of amino acids. (Chen, 1978).

Lead:
Lead is a highly poisonous metal (whether inhaled or swallowed), affecting almost every organ and system in the body. The component limit of lead (0.1 μg/g) is a test benchmark for pharmaceuticals, representing the maximum daily intake an individual should have. Even at this level, a prolonged intake can be hazardous to human beings. (Usp 2012). Much of its toxicity comes from how Pb^{2+} ions are confused for Ca^{2+} ions, and lead as a result gets into bones.

The main target for lead toxicity is the nervous system, both in adults and children. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system. (Lead in air 2016). Long-term exposure to lead or its salts (especially soluble salts or the strong oxidant PbO_2) can cause nephropathy, and colic-like abdominal pains. It may also cause weakness in fingers, wrists, or ankles. Lead exposure also causes small increases in blood pressure, particularly in middle-

aged and older people and can cause anemia. Exposure to high lead levels can cause severe damage to the brain and kidneys in adults or children and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. Chronic, high-level exposure has been shown to reduce fertility in males. (Golub, *et al.*, 2005).

5-Hydroxymethyl Furfural (HMF):

5 - Hydroxymethyl furfural, is an organic compound derived from dehydration of certain sugars.(Andreia *et al.*, 2011; Jan van Putten *et al.*, 2013)This yellow low-melting solid is highly water-soluble. The molecule consists of a furan ring, containing both aldehyde and alcohol functional groups. HMF has been identified in a wide variety of heat-processed foods including milk, fruit juices, spirits, honey, etc. HMF, which is derived from cellulose without use of fermentation, is a potential "carbon-neutral" feedstock for fuels and chemicals (Jan, *et al.*, 2013)

HMF was found in alcoholic beverages and wine, for example, in fortified wine (maximum 840 mg/L), whiskey (maximum 55.9 mg/L). (Frischkorn, et al., 1982), and rum (maximum 43.5 mg/L), (Villalon *et al.*, 1987). Thus, the formation of HMF from sugar dehydration or due to caramel color addition in alcohol products could be a potential problem.

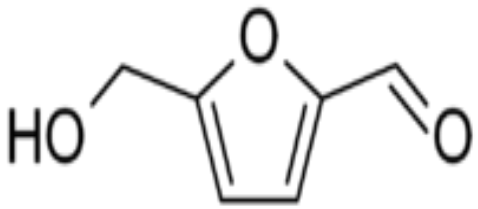


Figure (1): Chemical structure of 5-Hydroxymethyl Furfural (HMF)

Toxicity of HMF

HMF is considered an irritant and is irritating to eyes, upper respiratory tract, skin and mucous membranes. No positive or negative epidemiological studies or case reports associating HMF with a cancer risk in humans were identified in the available literature. The National Institute of Environmental Health Sciences nominated HMF for testing based on the potential for widespread exposure in the diet, evidence for carcinogenic potential of other members of this class, and the fact that little is known about HMF toxicity. NTP plans to develop protocols to investigate the metabolism, toxicity and carcinogenicity of HMF.

Metabolism and disposition studies of HMP are being performed in support of the NTP bioassay. Recent literature reports of in vitro studies indicate that sulfate conjugation of HMF leads to a positive result in Salmonella mutagenicity tests. NTP studies will investigate the possibility that HMF is metabolically converted to an alkylating agent in vivo by correlating the metabolites observed to what would be expected from an alkylating agent, e.g. mercapturic acid metabolites. Covalent binding to protein in metabolically active tissues will also be determined to assess production of reactive species. Comparison of metabolism and disposition in mice and rats will also be performed to aid in the design and interpretation of NTP studies (FPA, 2006).

Toxicity of Wine

Wine contains ethyl alcohol, the same chemical that is present in beer and distilled spirits and as such, wine consumption has short-term psychological and physiological effects on the user. Different concentrations of alcohol in the human body have different effects on a person. The effects of alcohol depend on the amount an individual has drunk, the percentage of alcohol in the wine and the timespan that the consumption took place, the amount of food eaten and whether an individual has taken other prescription, over-the-counter or street drugs, among other factors. Drinking enough to cause a blood alcohol concentration (BAC) of 0.03%-0.12% typically causes an overall improvement in mood and possible euphoria, increased self-confidence and sociability, decreased anxiety, a flushed, red appearance in the face and impaired judgment and fine muscle coordination. A BAC of 0.09% to 0.25% causes lethargy, sedation, balance problems and blurred vision. A BAC from 0.18% to 0.30% causes profound confusion, impaired speech (e.g., slurred speech), staggering, dizziness and vomiting. A BAC from 0.25% to 0.40% causes stupor, unconsciousness, anterograde amnesia, vomiting, and death may occur due to inhalation of vomit (pulmonary aspiration) while unconscious and respiratory depression (potentially life-threatening). A BAC from 0.35% to 0.80% causes a coma (unconsciousness), life-threatening respiratory depression and possibly fatal alcohol poisoning, the main active ingredient of wine is alcohol, and therefore, the health effects of alcohol apply to wine. Drinking small quantities of alcohol (less than one drink in women and two in men) is associated with a decreased risk of heart disease, stroke, diabetes mellitus, and early death. Drinking more than this amount; however, increases the risk of heart disease, high blood pressure, atrial fibrillation, and stroke. Risk is greater in younger people due to binge drinking which may result in violence or accidents. (O'Keefe et al., 2014). About 3.3 million deaths (5.9% of all deaths) are believed to be due to alcohol each year (WHO, 2014).

In 2008, researchers from Kingston University in London discovered red wine to contain high levels of toxic metals relative to other beverages in the sample. Although the metal ions, which included chromium, copper, iron, manganese, nickel, vanadium and zinc, were also present in other plant-based beverages, the sample wine tested significantly higher for all metal ions, especially vanadium. (Hague *et al., 2008).*

Finally, there are many reports of contaminants in wines that pose potential health risks, including pesticide and fungicide residues, acetic acid, bacteria, lead, fungi and mycotoxins such as ochratoxin A (Guilford and Pezzuto, 2011), that may also be present in beer (Medina *et al.*, 2005). It is also known that alcoholic beverages may be adulterated or contaminated with methanol (Zhang *et al.*, 2012).

Materials and Methods:

Materials.

All chemicals and reagents used in analysis were analytical grade and were used with standard Ethanol and Methanol obtained from BDH laboratory supplies England, HMF (99.8%) and Lead standard (1000ppm) obtained from sigma – Aldrich China and Acetonitrile obtained from BDH laboratory supplies England Distilled water was used throughout the work.

Sample Collection: Fresh commercial sample (date wine) from Khartoum locality and Distilled liquor sample prepared in the laboratory.

Equipment: The equipment's used in this work are electronic balance (OHAUS, Switzerland), rotary evaporator and thermometer,

Instruments: all instruments were used from Japan (Shimadzu Company), GC-MS spectrophotometry technique model (GC/MS – QP2010-Ultra), (HPLC) has UV-VIS detector and Atomic Absorption Spectrophotometer (A.A).

Methods.

Preparation of sample:

One kilogram of dates was washed and placed in a plastic bottle, 2 liters of distilled water together with 10 grams of yeast were added and the mixture was stored at 40 °C for four days. The sample was filtered and distilled at 80 ° C (local method)

Chemical Instrumentation Analysis:

All the samples and standard were run in duplicate.

Determination of Ethanol and Methanol by GC according to (shabir, 2003).

HMF was measured by HPLC according to the method of (AOAC, 1990). Determination of lead by Atomic Absorption spectrophotometer according to (Sobia, *et al.;* 2015).

Results and Discussion:

Determination of Methanol and Ethanol:

Table (1): Shows Concentrations of Ethanol and methanol in samples:

Sample Name	Ethanol %	Methanol mg/L
Fresh commercial sample (date wine)	23.33	22.93
Distilled liquor	59.15	0.094

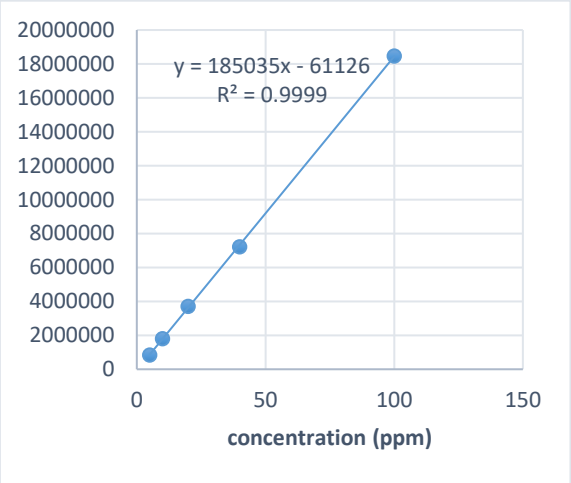
Methyl alcohol (also known as: methanol) is the simplest, lowest molecular weight alcohol, yet it is the most toxic of all, due to its metabolic products- formaldehyde and formic acid (Cortes, 2005). The concentrations of methanol in two samples are shown in table (1). the concentration of methanol in sample1(commercial sample) was found to be higher than sample 2 and the permissible standard set by National Agency for Food and Drug Administration and Control(Nigeria), NAFDAC (0.05mg/L), that refer to the bad fermentation without distillation.

Wine is composed of two main ingredients, water (generally over 80%) and ethanol (usually over 9%). Alcohol, the second major component of the wine, is primarily produced by yeasts during alcoholic fermentation. Ethanol in wine is the basic alcohol produced in the course of fermentation (Jackson, 2008).
Table (1) showed higher concentration of samples than the standard limits. In a prepared sample the high concentrations of ethanol (59.15%) may be due to sample concentration by distillation and the commercial sample (date wine) may be added during the production process to improve the taste and smell.

Determination of 5-HMF.

Table (2): Shows Concentration of Hydroxymethyl furfural in samples:

Sample	Concentration of HMF(ppm)
commercial	0.37
Distilled liquor	Not detected



There are three main methods in current use for measuring HMF: a colorimetric method (Winkler method), a direct measurement using HMF absorbance at 284 nm (White method) and an HPLC method with detector set at 284 nm. The HPLC method was used in this study, the HMF in wine samples was identified according to the retention time of HMF standard chromatograms (RT 6.8).The concentrations were calculated using the calibration curve of HMF, the linearity was 0.9999 (R^2), Table (**2**) showed the concentration of HMF in date wine samples. The presence of HMF in sample may due to decomposition of sugars in date by overheating during fermentation process

Determination of Lead:

Table (3): Concentration of lead in samples:

Sample	**Concentration(ppm)**
commercial sample	0.3706
Distilled liquor	0.1784

The concentrations of lead in commercial sample found to be (0.3706 ppm) higher than prepared sample (0.1784 ppm), and maximum limit of Ministry of Health- Vietnam (0.2 mg/L). The high level of lead may refer to addition of batteries by winemaker to increase the fermentation.

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Conclusion and Recommendations:

This study has shown that most of the local wines contain contaminants above the permissible level that has been set by regulatory bodies. And the adverse effects of drinking alcoholic beverages may not appear at the present time but in the future because these contaminants have tendency to bio-accumulate in the body

The limit of HMF content in wine and alcoholic beverages was not included in the previous studies because of a lack of documented data, so the extension of this study is identify the HMF in different type of wine and we will provide a reliable human exposure estimate as well as a risk assessment about HMF from alcohol consumption.

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