Methanol contamination in indigenous fermented alcoholic beverages

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Incidences of methanol contamination

- Beverage ethanol production via fermentation is an age-long tradition in many parts of the world.
- In the tropical world, indigenous/rural people including women are involved in the value chain of traditional alcoholic fermentation
- In Africa, Asia and South America, there are increasing incidences of methanol contamination in traditionally fermented alcoholic drinks
- For instance, in 2008, more than 180 people were killed in Bangalore and in 2009, 136 people were killed in Gujarat (India)
- In 2009, 25 persons died in Indonesia after consuming fermented palm wine containing methanol.
- 130 Indian villagers die from alcohol poisoning in 2011
- In June 2015, 27 persons died after consuming toxic alcohol in India.
- Between April and June 2015, 89 persons died in Nigeria following the consumption of alcohol beverage produced from palm wine

WHO (2014) Report

- There have been numerous outbreaks of methanol poisoning in recent years in several countries, including Cambodia, Czech Republic, Ecuador, Estonia, India, Indonesia, Kenya, Libya, Nicaragua, Norway, Pakistan, Turkey and Uganda. The size of these outbreaks has ranged from 20 to over 800 victims, with case fatality rates of over 30% in some instances.
- Concentrations of 6-27 mg/L have been measured in beer and 10-220 mg/L in spirits. In these concentrations methanol is not harmful.
- The informal and illicit production of alcoholic drinks is practiced in many parts of the world, including countries where alcohol is banned. Some common names for these drinks include: hooch/ moonshine (USA), chang'aa/kumi kumi (Kenya), tonto/waragi (Uganda), tuak/tapai (Malaysia), samogon (Russia), and talla (Ethiopia).

Symptoms of methanol poisoning

- Blurred vision, blindness
- Loss of consciousness
- Weight loss
- Headache, weakness, dizziness
- Breathing difficulties
- Abdominal pains, nausea, diarrhoea, vomiting

Source: Methanol Institute (2013)

Regulatory limits of methanol in beverages

Country	Maximum methanol value*	Reference
Brazil	0.5% (0.5 ml/100ml)	Mendonca et al (2011
Thailand	0.024% (240 mg/l)	Chaiyasut et al (2013)
Australia/ New Zealand	0.8% (8 g/l)	Chaiyasut et al (2013)
USA	0.1%	FDA (Federal Food, Drug and Cosmetic Act 21 USC 34 (a)(2)(C)
Vietnam	0.3%	Socialist Republic of Vietnam (2010)
Nigeria	0.0005% (5 mg/l)	NAFDAC 2005

*concentration of methanol in ethanol

Possible sources of methanol in fermented beverages?

- unscrupulous vendors might have deliberately spiked the beverages with methanol to increase the alcohol content,
- methanol might have been produced by contaminating microbes during fermentation.
- Methanol could be produced by microbes during the fermentation of fruits high in pectins
- Methanol could also be produced during fermentation in unclean containers

Traditionally/informally fermented beverages 1

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content	Remarks (Reference)
Palm wine	oil palm, Raffia palms, date palm, the palmyra, jaggery palm, *kithul* palms, or *nipa* palms. coconut palms, *Borassus*	Yeast (Saccharomyces cerevisiae, Saccharomyces ludwigii, Candida parapsilosis, Candida fermentati, Pichia fermentans, Schizosaccharomyces romyces pombe, Schizosaccharomyces romyces bailli, Kluvyeromyces africanus, Hansenula auvarum, Kloeckera apiculata, Torulaspora delbrueckii) & Lactic Acid Bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*), acetic acid bacteria (Acetobacter, Aerobacter)	Most African and Asian countries	>10%	Ogbulie et al (2007, Rokosu and Nwisienyi, 1980), Karamoko et al (2012)
Local gin (ogogoro)	Palm wine	(Saccharomyces cerevisiae) & Bacteria (Lactobacillus)	Most African and Asian countries	40-60% Ethanol	Ohimain et al (2012)

Traditionally/informally fermented beverages 2

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content	Remarks (Reference)
Arak	Grape	Yeast	Israel, Lebanon, Iraq Syria Jordan Palestine, Cambodia,	40-63%	
Plum wine	Japanese Plum (Prunus salicina Linn)	Yeast	India	175mg/l Methanol	(Joshi et al 2009)
Cholai	rice, sugar-cane, juice of date tree, molasses, and fruit juice (pineapple and jackfruits)	Sacharomyces cerevisiae	India	14.5% alcohol	Islam et al(2014)

Traditionally/informally fermented beverages 3

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content	Remarks (Reference)
Cachaca (banana pulp wine)	Banana	Sacharomyces cerevisiae	Brazil	Ethanol (5.34-7.84%), methanol (0.65-0.189%)	Mendonca et al (2011)
Cachaca	Sugarcane	Sacharomyces cerevisiae and wild yeasts (Pichia sp & Dekkera bruxelensis)	Brazil	Methanol (0-0.5%)	Dato et al (2005)
Noni	Morinda trifolia	Lactobacillus plantarum & L. casei	Thailand	853 mg/l methanol	Chaiyasut et al (2013)

Traditionally/informally fermented beverages 4

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content	Remarks (Reference)
Pito (local beer)	Sorghum or maize	Bacteria (Pediococcus halophilus, Lactobacillus) & yeast (Saccharomyces cerevisiae, Candida tropicalis, Schizosaccharomyces romyces pombe, Kluvyeromyces africanus, Hansenula anomala, Kloeckera apiculata, Torulaspora delbrueckii)	West Africa	2-3% Ethanol	Orji et al (2003), Sefa-Dedeh et al (1999); Iwuoha and Eke (1996)
Burukutu	Sorghum	Sacharomyces cerevisiae, Streptococcus, Lactobacillus, Aspegillus, Fusarium, Penicillium	Nigeria, Ghana	1.63% ethanol	Eze et al (2011); Iwuoha and Eke (1996)

Traditionally/informally fermented beverages 4

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content	Remarks (Reference)
Ogi	Maize, sorghum or millet	Sacharomyces cerevisiae, Lactobacillus plantarum, streptococcus lactis	Nigeria	?	Iwuoha and Eke (1996)
Kwunu-zaki	Millet	Sacharomyces cerevisiae	Nigeria	?	Iwuoha and Eke (1996)
Cocoa sap wine	Cocoa sap	Sacharomyces cerevisiae	Nigeria	?	Iwuoha and Eke (1996)

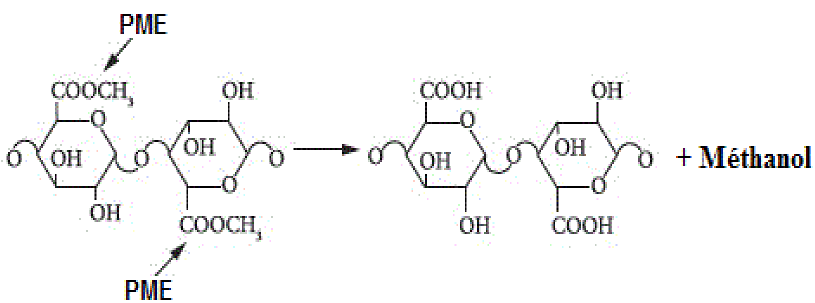
Pectins

- Pectin is a structural hetero-polysaccharide contained in the primary cell walls of terrestrial plants.
- It is used in food as a gelling agent, particularly in jams and jellies. It is also used in fillings, medicines, sweets, as a stabilizer in fruit juices and milk drinks, and as a source of dietary fiber (Wikipedia).
- PME hydrolyses pectin to polygalactoronic acid and methanol.

Levels of pectin in plants (fresh weight)

Citrus peels	**>20.0%**
Oranges	0.5–3.5%
Apples	1.0–1.5%
Carrots	1.4%
Apricots	1.0%
Cherries	0.4%

High Pectin Fruit	Moderate Pectin Fruit	Low Pectin Fruit
Apples, crisp and tart	Apples, ripe	Apricots
Blackberries, sour	Blackberries, ripe	Blueberries
Crabapples	Cherries, sour	Cherries, sweet
Cranberries	Chokecherries	Figs
Currants	Elderberries	Grapes (Western Concord)
Gooseberries	Grape Juice, bottled	Peaches
Lemons	Oranges	Pears
Loganberries	Plums (not Italian)	Plums (Italian)
Quinces		Raspberries
		Strawberries



Microbial production of methanol

- Methanol production in traditional fermented beverages can be linked to the activities of pectinase producing yeast, fungi and bacteria.
- Microbes producing pectin methyl esterase are able to produce methanol from fruits/juices containing pectin
- Under traditional/informal fermentation, alcoholic beverages produced by mixed microbial consortium probably lead to the production of mixed alcohols
- Microbes can also produce methanol via the oxidation of methane (biogas)

Microbial production of methanol (Siero et al 2012)

- Pectin enzymes are widely distributed in nature and are produced by yeast, bacteria, fungi and plants
- Proteolytic enzymes are classified into esterases and depolymerase (lyases and hydrolyses)
- Hydrolyses of pectin by lyases produces oligo- or mono-galacturonate
- Hydrolysis of pectin by esterases produces pectic acid and **methanol**

Literature /Research highlights 1

- Methanol is produced during fermentation by the hydrolysis of naturally occurring pectin in the wort (Tomoyuki et al 2000; Mendonca et al 2011)
- Alcohols made from mangoes, pears, banana and melon have been shown to contain methanol (Mendonca et al 2011)
- The total alcohol (ethanol and methanol) produced from orange juice was 3.19 % w/v with *S. cerevisiae var. ellipsoideus* and 6.80% w/v) with *S. carlsbergensis* (Okunowo and Osuntoki 2007).
- Chaiyasut et al (2013) reported factors affecting the methanol production in fermented beverages including raw material size and age, sterilization temperature, pectin content and pectin methyl esterase (PME) activity (Note that PME activity is optimal at 50-60^{o}C).
- During ripening, pectin in fruits is broken down by PME resulting in the formation of methanol (Chaiyasut et al, 2013: Michel 2001).
- PME (EC: 3.1.1.11) de-esterify pectin to low –methoxyl pectins resulting in the production of methanol (Chaiyasut et al, 2013: Michel 2001)

Literature / Research highlights 2

- Chaiyasut et al (2013) compared pectin levels in fermented beverage containing Morinda citrifolia (9.89%) with that of other fruits including guava (4.36%), tomato (0.3%), apple (0.5%), carrot (0.8%) and cherries (0.4%)
- During the production of sugarcane beverage called cachaca in Brazil, Saccharomyces cerevisiae produced no methanol while contaminating yeasts (Pichia silvicola and P. anomata) produced 0.5% methanol (Dato et al 2005)
- Pichia methanolica is able to utilize, pectin, polygalacturonic acid and methanol as sole carbon sources (Nakagawa et al 2005)
- Stringini et al (2009) studied yeast diversity during tapping and fermentation of oil palm wine from Cameroon and found Saccharomyces cerevisiae, Saccharomyces ludwigii, Schizosaccharomyces bailli, Candida parapsilosis, Pichia fermentans, Hanseniaspora uvarum and Candida fermentati in addition to lactic acid bacteria and acetic acid bacteria

Literature / Research highlights 3

- Pectin is typically found in the intracellular regions and cell walls of most fruits and vegetables, with its greatest abundance in citrus particularly orange, grape, limes and lemons (Siragusa et al 1988)
- Citrus contains 7-10% pectin (Siragusa et al 1988)
- Human colonic bacteria, Erwinia carotovora is able to degrade pectin releasing methanol (Siragusa et al 1988)
- Anaerobic bacteria, Clostridium particularly C.butyricum, C. therocellum, C. multifermentans, and C. felsineum produce methanol from pectin (Ollivier and Garcia, 1990)
- Nakagawa et al (2000) found that Candida boidinii can grow on pectin or polypalactoronate as a carbon source producing methanol
- Schink and Zeikus (1980) reported various pectinolutic strains of Clostridium, Erwinia and Pseudomonas

Methanol contamination: the Nigerian case

- In the Nigerian methanol poisoning case, fermented alcoholic beverage was found to contain 16.3 % methanol
- The blood methanol concentration of victims was found to be 1500-2000 mg/l.

Possible sources of methanol in fermented beverages?

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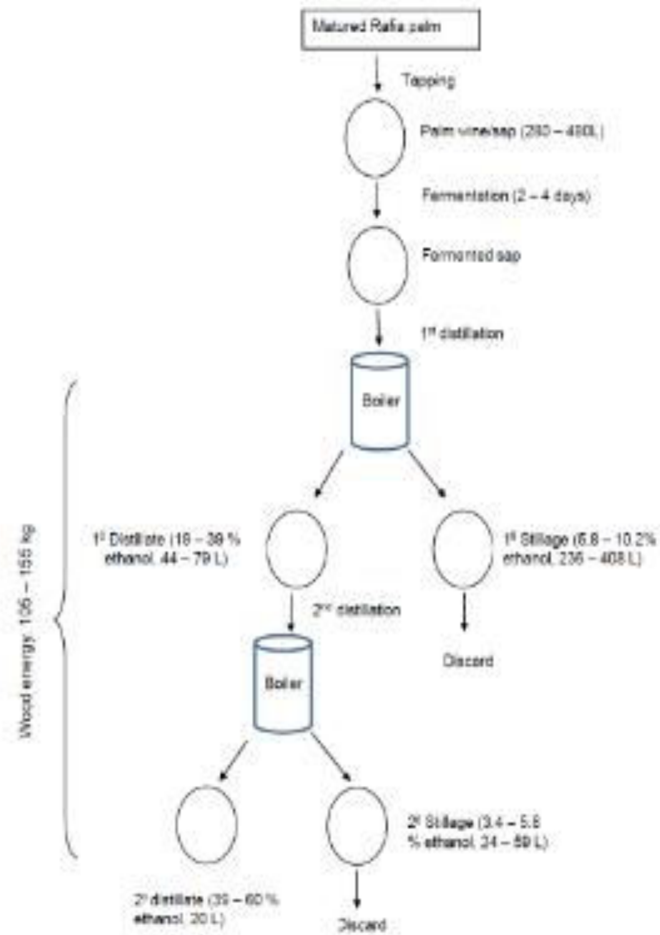
Price of alcohols

- Locally produced gin of 40-60% ethanol content costs N20 per shot of 30ml i.e. ₦670/litre i.e. £ 1.97/litre (£ 1.00= ₦340)
- 25 litre pure 99.85% methanol is £37.95 i.e. £1.52/litre (excluding importation and duty costs). The landing cost could be more than double
- Methanol is not currently produced in Nigeria (but there are at least 2 methanl plants under construction)

Traditional production of beverage ethanol from raffia palm (Ohimain et al 2012)



Physical properties of methanol, ethanol and gasoline (Modified from Kamboj and Karimi (2014), Methanol Institute (2011))

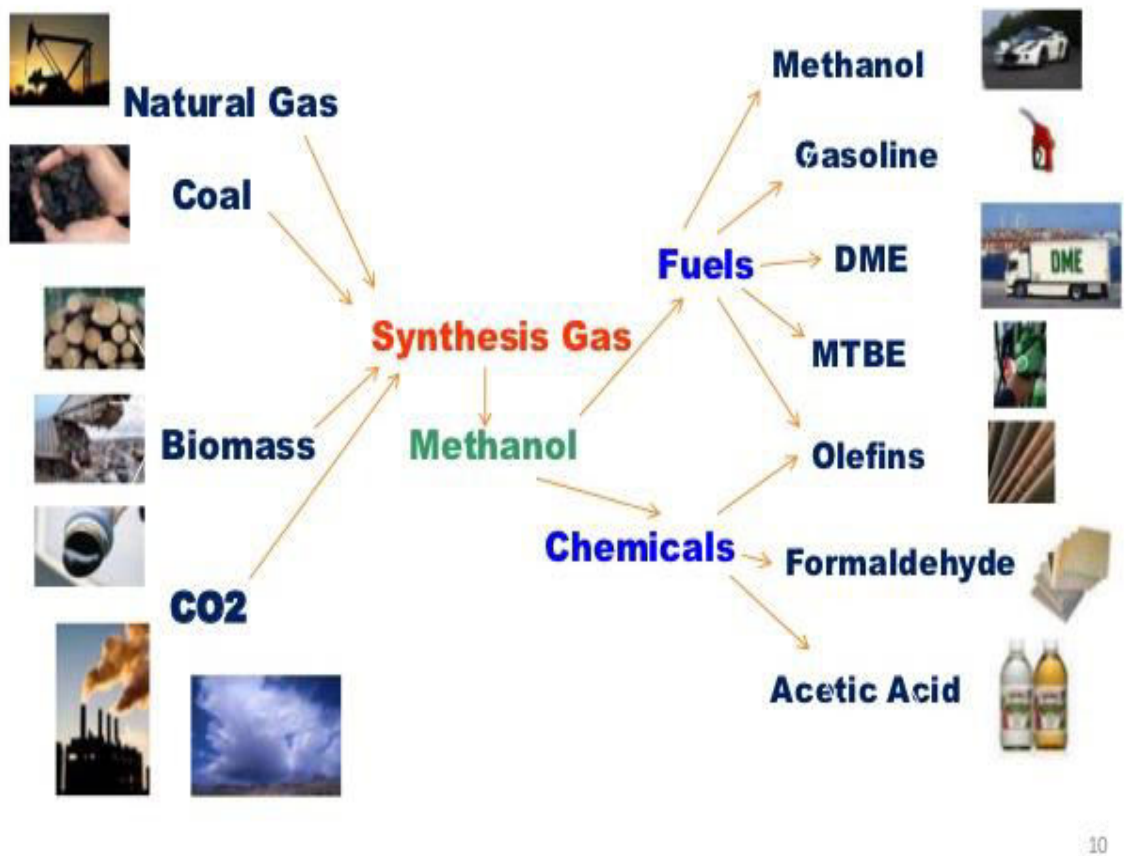
Property	Methanol	Ethanol	Gasoline
Chemical formula	CH_3OH	C_2H_5OH	
Molecular weight(Kg/kmol)	32.04	46.07	
Oxyzen present (wt %)	49.9	34.8	
Density (g cm_{-1})	792	789	740
Freezing point at 1 atm ($_0$C)	-97.778	-80.0	
Boiling temperature at 1 atm ($_0$C)	**64.9 (65)**	**74.4 (78)**	
Auto-ignition temperature ($_0$C)	463.889	422.778	
Latent heat of vaporization at 20$_0$C (KJ/Kg)	1103	840	
Stoichiometric air/fuel ratio (AFR)	6.47	9.0	
Lower heating value of the fuel (MJ/kg)	19.7	26.9	43.9
Research octane number (RON)	111	108	92
Motor octane number (MON)	92	92	
Fuel equivalence	0.48	0.677	1

Alternative uses of methanol

- Fuel use (direct fuel use, reagent for the production of biodiesel, synthesis of other carriers e.g. DME & MTBE, Direct Methanol fuel cells, fuel additives)
- Pesticide synthesis
- Chemical synthesis
- Solvent

Methanol Economy

Methanol Sources



Methanol Uses

http://www.slideshare.net/EMA_SIEW/dom-lavigne-methanol-fuels-safe-efficient-affordable-mature downloaded 18 Aug 2015

Alcohol fuels

http://www.thecuttingedgenews.com/index.php?article=74973
downloaded 18 Aug 2015

Conclusion

contaminated alcoholic beverages be converted for fuel use rather than out rightly banning the age –long traditional alcohol fermentation.

Future research directions & request for collaboration on:

- Physicochemical analysis of palm wine to detect the presence of pectins
- Microbial diversity of raffia palm wine using 16S rRNA gene sequencing and ITS2 rRNA region
- Assessment of methanol producing microbes in fermented beverages
- Genetic analysis to assess if Sacharomyces cerevisiae have picked up or developed capability to produce methanol
- Possible use of mixed alcohols (ethanol and methanol) for bioenergy applications (transportation or cooking fuel)