Development of a Sensitive Method for the Determination of Methanol in Fermented & Distilled Products

Dwight E. Matthews
Departments of
- Chemistry &
- Medicine
University of Vermont
Ethanol Is Produced By Biological Fermentation of Sugars

- Organisms from yeast to humans degrade sugars (glucose) for energy using O$_2$ to produce CO$_2$
- Under *anaerobic* conditions, yeast will stop the process short, producing ethanol, a 2-carbon alcohol
- Humans discovered and began making ethanol via fermentation for consumption even before discovering how to make soap
Ethanol Metabolism in Humans

- After consumption, ethanol distributes across body water and into tissues
- The enzyme, alcohol dehydrogenase, in the liver converts ethanol to acetaldehyde, which is subsequently converted to acetic acid

![Chemical structures](image-url)
Methanol As A By-Product of Fermentation

- **Methanol**
  - Is a one-carbon alcohol
  - Also distributes across body water and all tissues
  - Is also metabolized in the liver by the same enzymes that metabolize ethanol

- **Methanol metabolism produces toxic compounds**
  - Formaldehyde – embalming fluid
  - Formic acid – neurotoxic
    - Causes headache, nausea, vomiting in lower doses
    - Is toxic to the optic nerve and can cause blindness ("white lightning")
Legal Limits for Methanol

- European methanol (MeOH) limit in ethanol (EtOH):  
  - 10 g MeOH/L EtOH

- US ATF methanol limit in ethanol:  
  - 7 g MeOH/L EtOH → 7 mg MeOH/ml EtOH  
  - 2.8 mg MeOH/ml (40% EtOH) → 250 ml = 0.7 g MeOH  
  - 1.0 mg MeOH/ml (14% wine) → 750 ml = 0.75 g MeOH

- International Organization of Vine and Wine (OIV)  
  - 0.2-0.4 mg MeOH/ml wine
Most Sugar in Plants is in the Form of a Complex Carbohydrates, i.e. Polysaccharides

- Yeast digests only simple sugars (glucose, fructose, ...)
- Polysaccharides in plants:
  - Cellulose – not digestible
- Polysaccharides in seeds and fruits of plants (starch):
  - Amylose – 1,4 linked glucose
  - Amylopectin – 1,4 and some 1,6 linked glucose
Seeds and Fruits Contain Enzymes to Digest Starch

- Amylase enzymes digest both amylose & amylopectin to glucose
  - The enzymes are active in ripening fruit or can be activated by warming (e.g. grains)
Methanol Is Not Produced By Yeast

- Yeast metabolizes glucose to end-products, but not to methanol
- Plants (especially fruits) also contain the polysaccharide pectin
- Pectin is 1,4-linked α-D-galacturonic acid (oxidized galactose)
- Most of the galacturonic acids in pectin have been methylated
- Pectin found in a variety of fruits
- Pectin is isolated from fruit and used as a gelling agent for foods
Pectin Releases Methanol When Digested

- Enzymes in fruits that digest pectin
  - Pectinase – hydrolyzes the 1,4-linkages in pectin
  - **Pectinesterase** – hydrolyzes the methyl ester on galacturonic acid

\[
\text{α-D-Galacturonic acid} + \text{H}_3\text{C-OH} \rightarrow \text{Methanol}
\]
Methanol Content of Fermented and Distilled Beverages

- Methanol is naturally produced during preparation of fruits for fermentation
- Plant fruit sources are expected to produce more methanol than grain sources
- Methanol (bp: 65 °C) is not always/usually separated from ethanol (bp: 78 °C) during distillation

- Vermont and the northeastern states produce fermented fruit beverages (e.g. hard cider and fruit wine) and distilled products (e.g. apple & pear brandy/schnapps)

- Methanol content is not routinely determined by
  - small operation fermenters and distillers
  - home fermenters
Measurement of Methanol in Different Alcoholic Beverages

Measurement of Methanol in Different Alcoholic Beverages (Normalized to Ethanol Content)

Measurement of Methanol in NY Fermentation Products (Normalized to Ethanol Content)

Methods to Measure Methanol

- Colorimetric
- Titrimetric
- Enzymatic
- Biosensor

- High performance liquid chromatography (HPLC) – refractive index detector
- Gas chromatography (GC) – flame ionization detector

- Gas chromatography-mass spectrometry (GCMS)

*new method*
Gas Chromatograph Measurement of Methanol
Measurement of Methanol in an Ethanol/Water Solution by Gas Chromatography-Mass Spectrometry

m/z 33: Methanol

m/z 36: $^2$H$_3$-Methanol
Internal Standard
Final Points

- Methanol limits are archaic and should be reviewed/revised
- Methanol is found in human blood, but very little is understood about normal background production and disposal of methanol
  - E.g. aspartame produces methanol when ingested
- Northeastern & Vermont fermenters and distillers are likely to produce high methanol products
  - Small changes in methods can make big differences
- We are devising a new, rapid & sensitive method to measure methanol & ethanol

- People performing the work
  - Edward Gakpe, graduate student in chemistry
  - Tristan Baldwin, undergraduate chemistry major

- We are looking for VT fermenters and distillers for collaborations understanding methanol presence and problems related to it
Extras
Measurement of Methanol in New York State Fruit Fermentations

Methanol (µg/ml)

Niagra pomace  Riesling pomace  Niagra grappa  Riesling grappa  Empire  Jonagold  Empire  Jonagold

NY Grapes  Hard Cider  Eau-de-vie

A Gas Chromatograph-Mass Spectrometer (GCMS)

GC Column & Oven

Vacuum