Modern Analytical Approaches for Ethanol Process and Quality Monitoring

National Technical Seminar
18th October, 2024
Bangalore

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Outlines

- **Process & Quality Imperatives**
  - Beverage Alcohol - Analytical & Sensory Quality consistency
  - Fuel Ethanol - Maximum yield & Consistent Biproducts

- **Rapid Analytical Methods**
  - Quality Consistency – Raw Materials /Process/Products
  - Cost & Time Savings

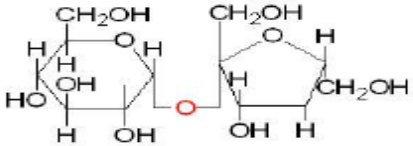
Rapid Analysis Requirements for Quality & Profits

- Quality control of Raw Materials & Products
- Yeast Assessment
- Fermentation Monitoring
- Co-product quality control – Congeners , DDGS

Raw Materials for Alcohol – Fuel & Beverage

RAW MATERIALS FOR ALCOHOL PRODUCTION

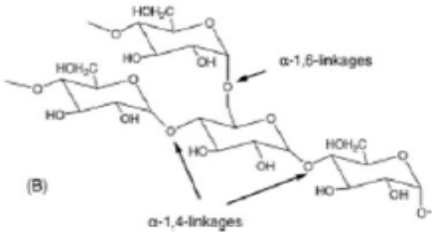
Simple → Sucrose



Single Stage Hydrolysis

Cane juice ; Beet juice; Molasses

Moderate → Starch

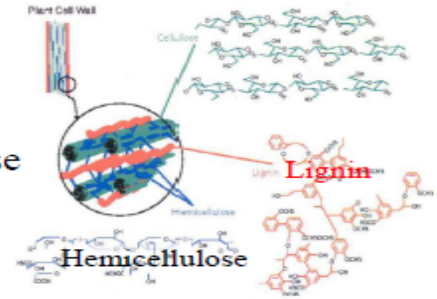


Complex

Two Stage Hydrolysis

Cassava; wheat rice; sorghum; millets

Most Complex → Cellulose



Pretreatment Followed by Liquefaction

Bagasse; wheat & rice straw; grass

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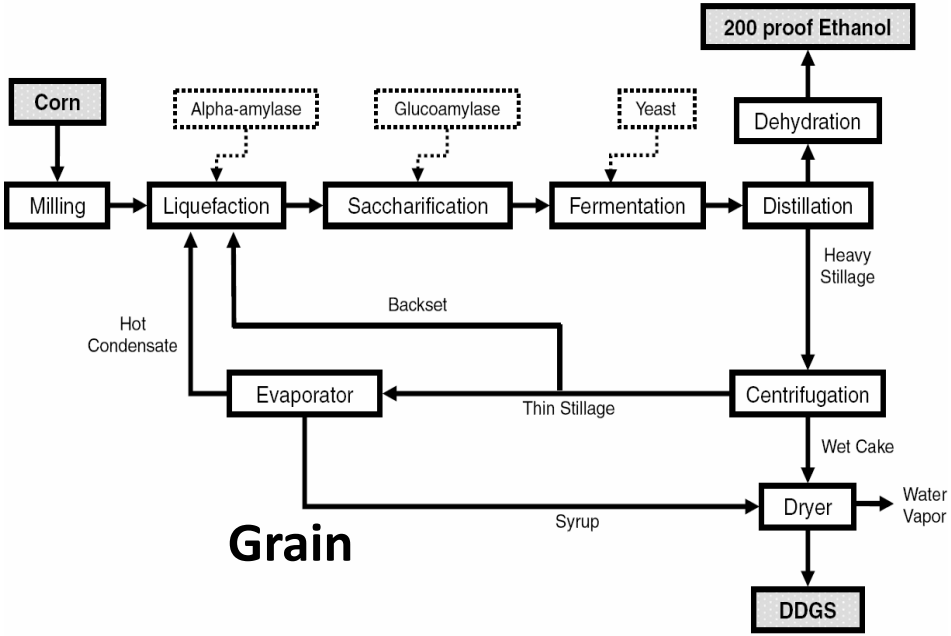
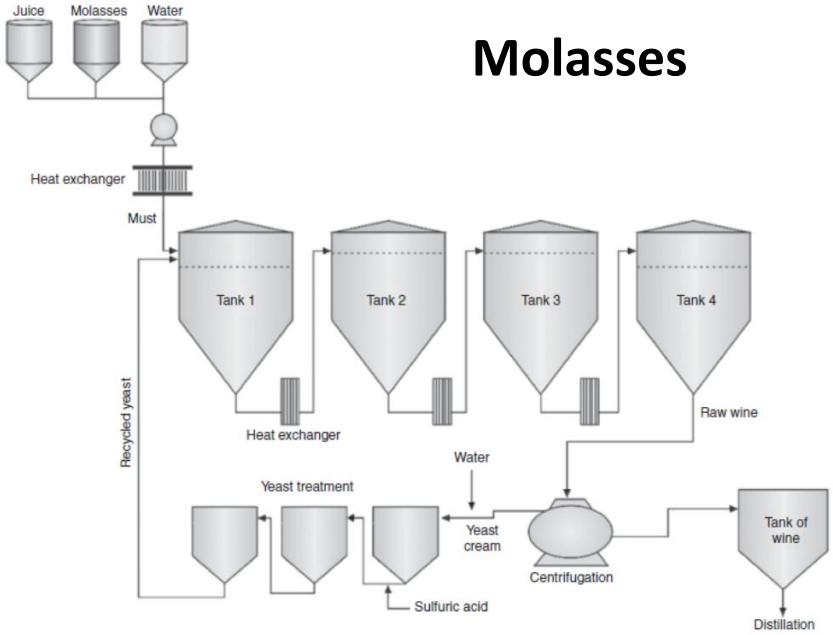
Process for Alcohol Production – Typical Example

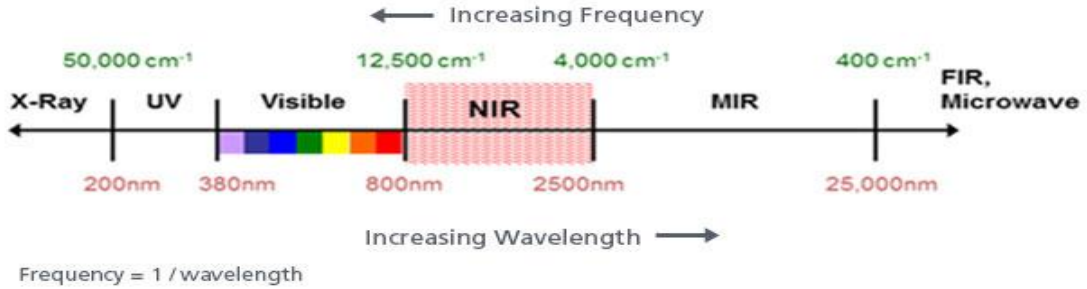


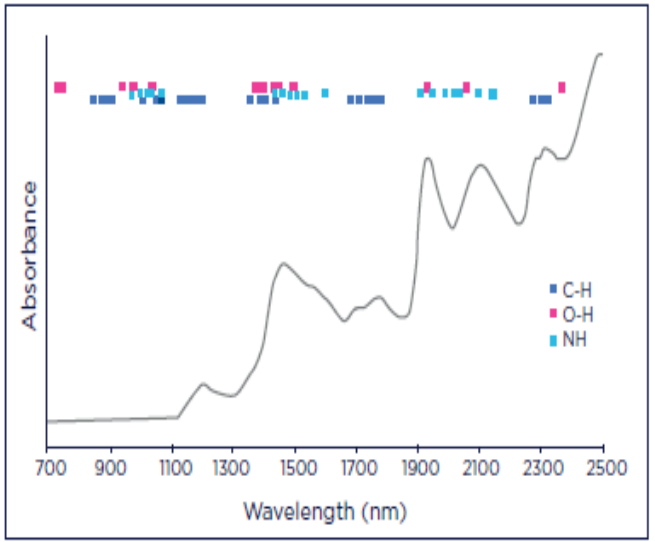


NIR & FT NIR for Quality & Process Control

- Rapid, non-destructive analysis of Grains using a diffuse reflectance measurement
- Measures moisture, protein and starch in seconds
- Fermentation monitoring online or offline
- Ethanol , carbohydrates, acids & glycerol monitored at different stages of fermentation
- Also applied for Sugar Cane & Molasses analysis
- Realtime process monitoring by NIR spectroscopy can be used to evaluate enzymes, yeasts & additives used in fermentation and to improve the process yield
- Constant quality control is vital for DDGS (a potential protein source in feeds for aquaculture). Protein, fat, starch, and other nutrients can be analyzed within seconds

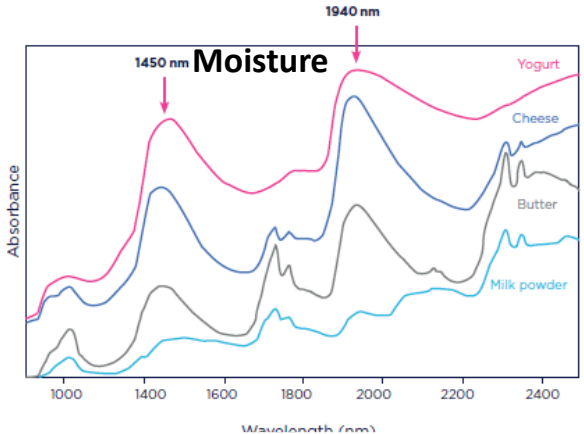
NIR & FT NIR Instruments

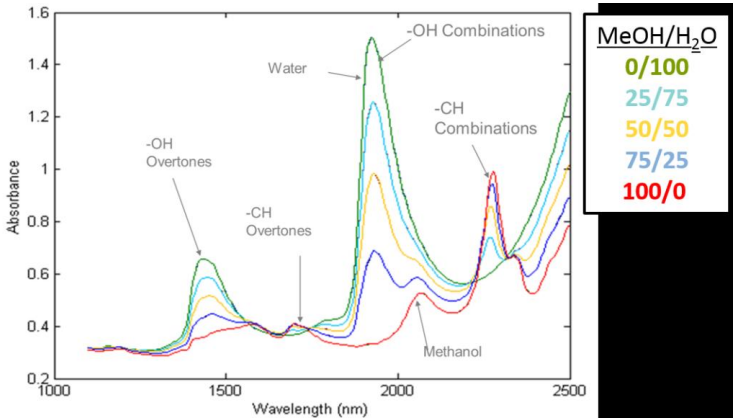


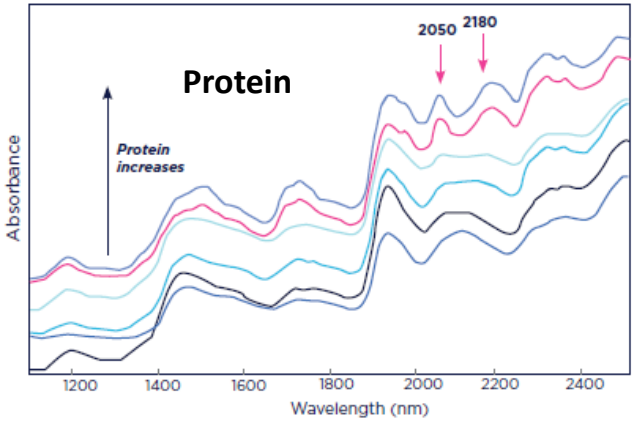


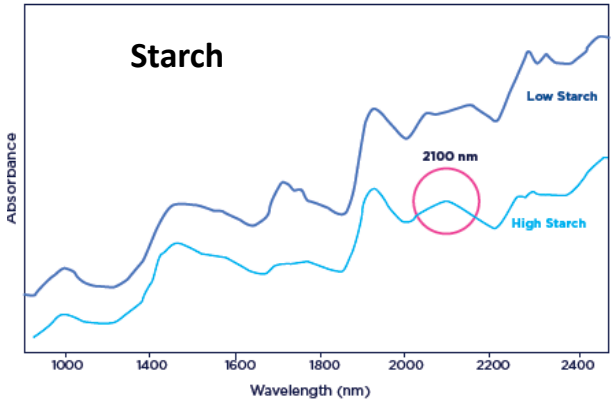
NIR absorbance region corresponds to overtones and combinations of the fundamental vibrations of O-H, N-H and C-H bands. These characteristics result in spectra with broad and overlapping bands First and second derivatives are used to sharpen the peak of NIR spectra and to reduce baseline offsets resulting from light scattering due to increases in cell concentration

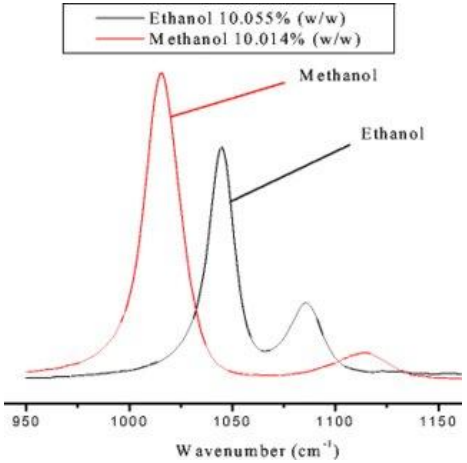
NIR FT NIR – Examples



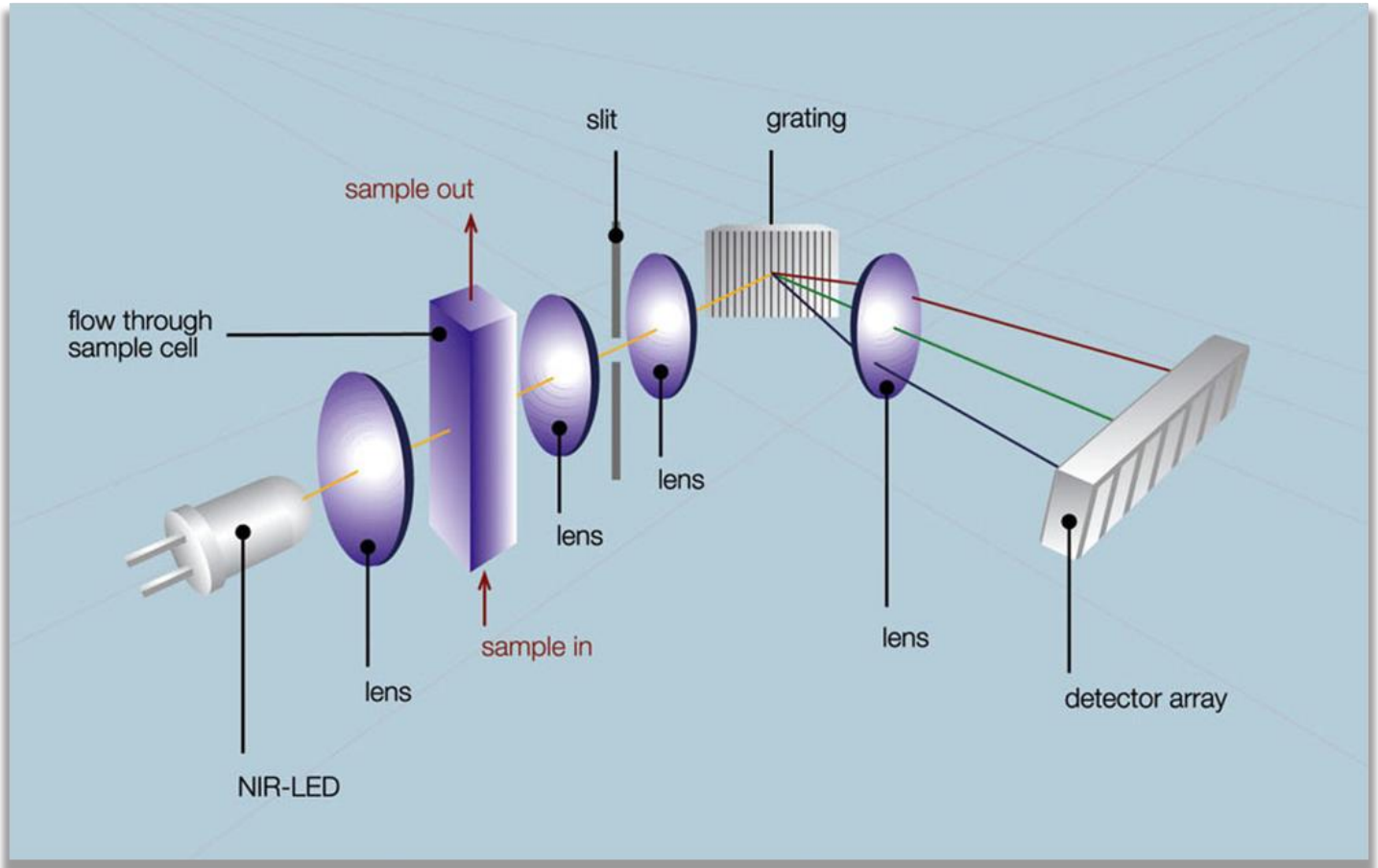








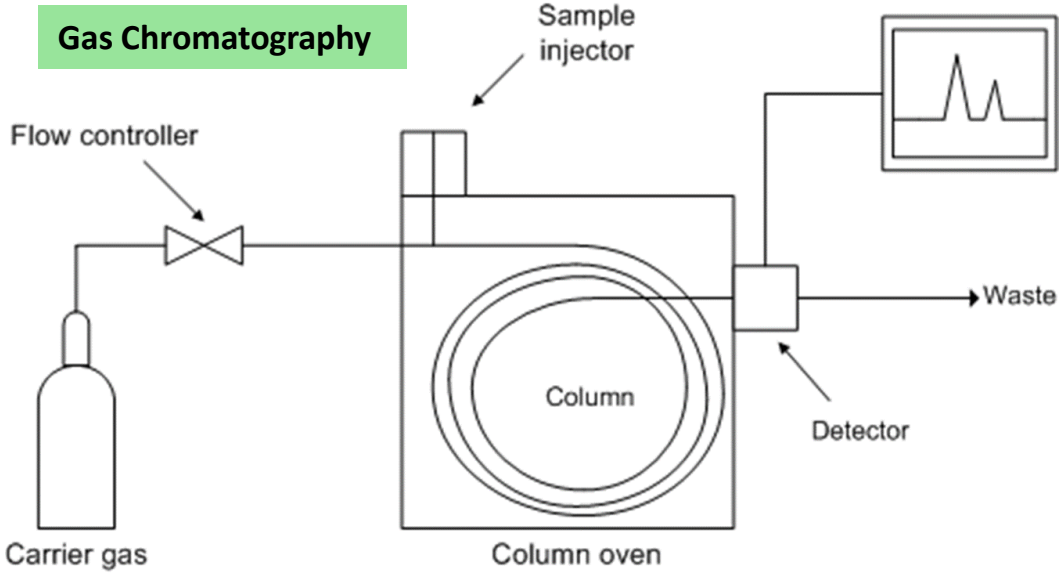
Measuring Principle – Optical Setup

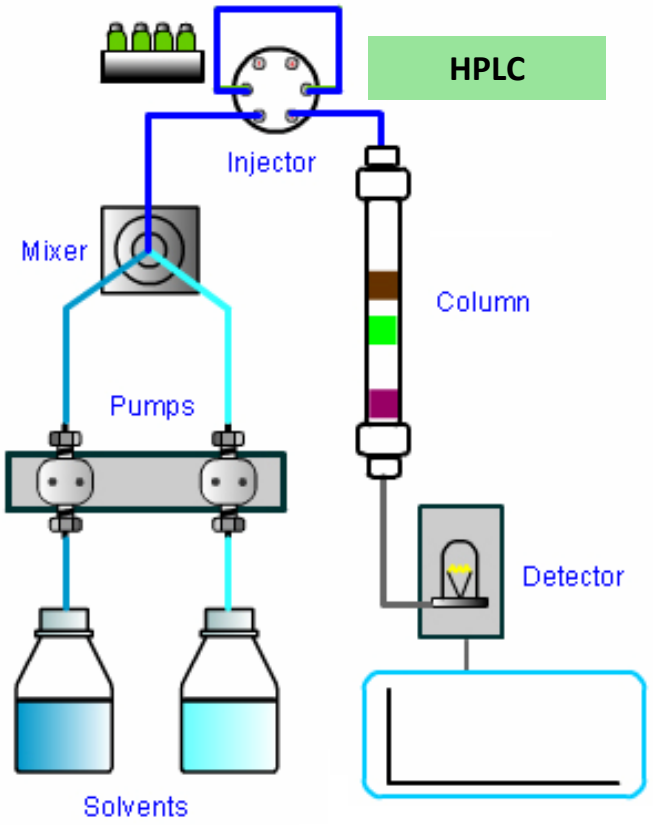


NIR & FT NIR Instruments

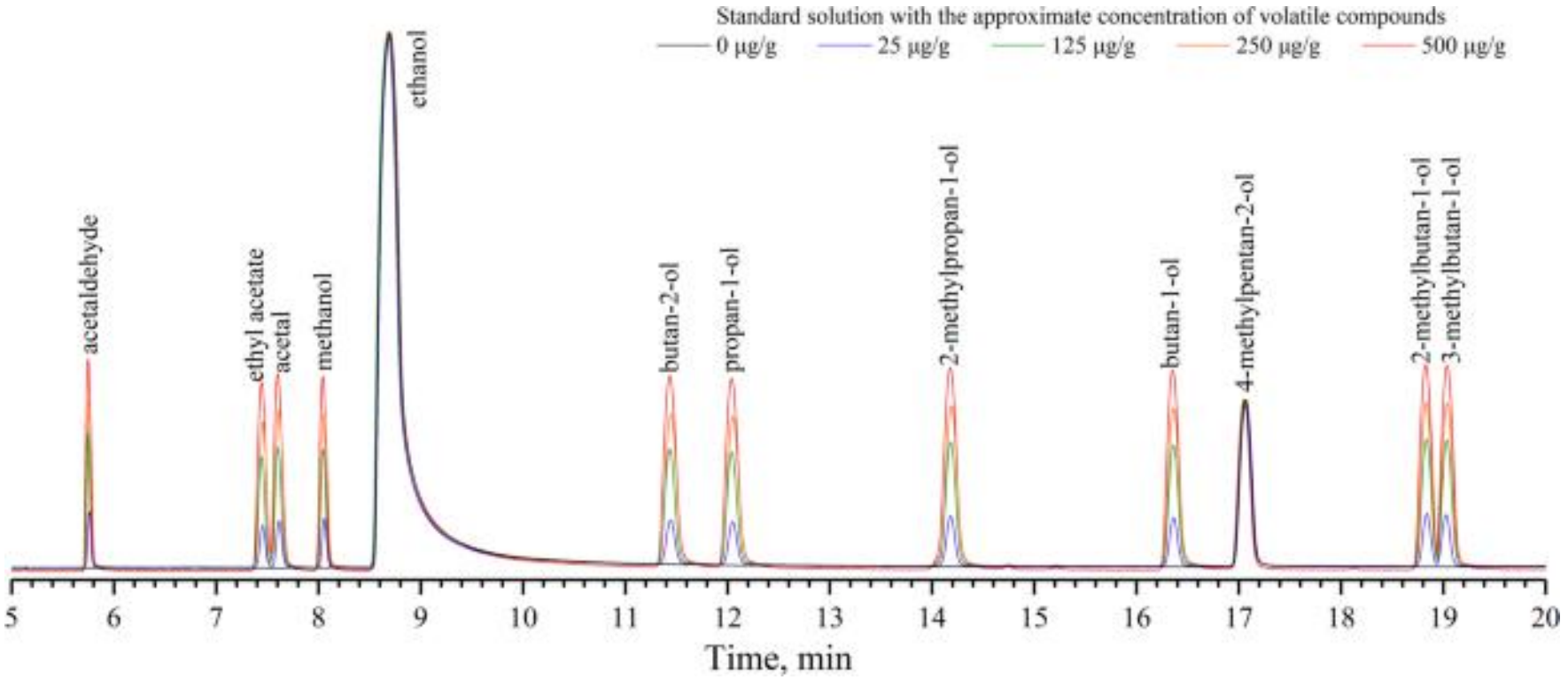
- FT-NIR (Bruker, Thermo, Buchi, Galaxy)
- Dispersive Infrared Instruments (Foss, Unity)
- Diode array (Perten, Zeiss, Foss)

Chromatographic Processes – GC & HPLC

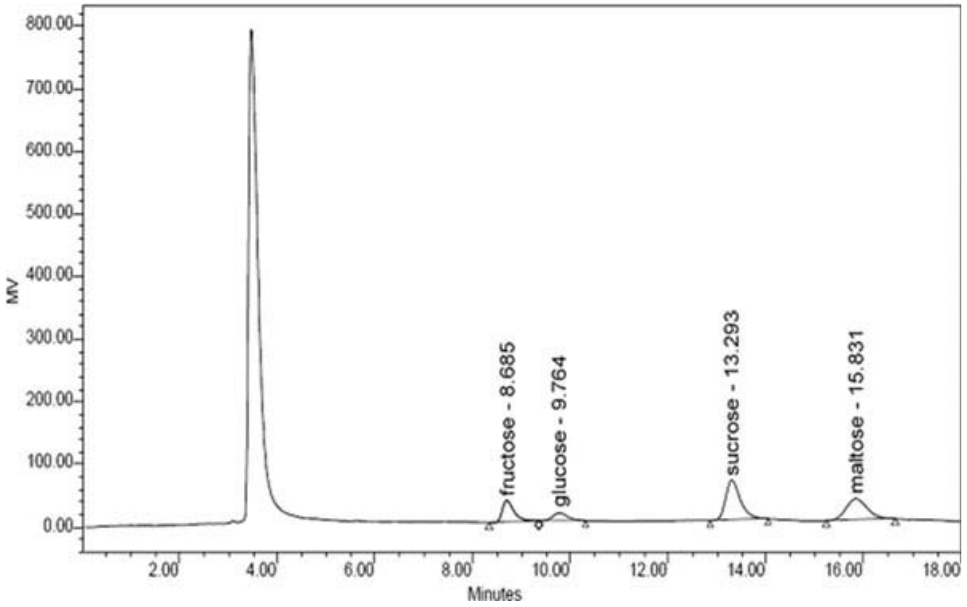




Typical GC analysis of volatiles congeners

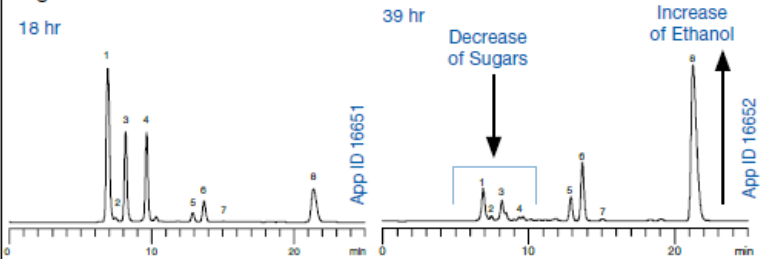


HPLC for Quality & Process Control – Examples



HPLC-RID chromatogram of three carbohydrates in molasses with a maltose internal standard. Mobile phase - acetonitrile and water (75: 25, v/v), flow rate 1.0 mL/min, column temperature 30◦C, and RID temperature 35◦C.

Figure 2.
Different fermentation timepoints run on a Rezex 300 x 7.8 mm ROA column. Note the depletion in the early sugar peaks as fermentation continues. The ethanol peak increases throughout the fermentation as sugar is converted into ethanol.



Conditions for both runs:

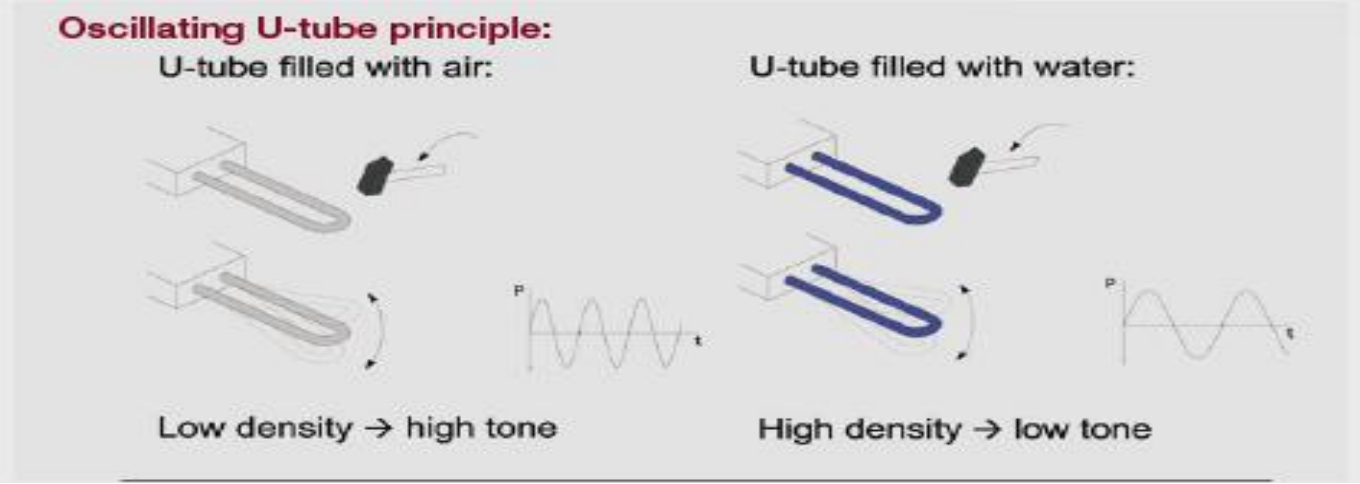
Column: Rezex ROA-Organic acid
Dimensions: 300 x 7.8 mm
Part No.: 00H-0138-K0
Mobile Phase: 0.005 N Sulfuric acid
Flow Rate: 0.6 mL/min
Temperature: 60 °C
Detection: RI @ 40 °C
Instrument: Shimadzu® Prominence™ LC-20A System
Sample: 1. Dp4+
2. Dp3
3. Maltose
4. Glucose
5. Lactic acid
6. Glycerol
7. Acetic acid
8. Ethanol

Alcohol Strength

Automated Density Measurement – Anton Paar & Mettler

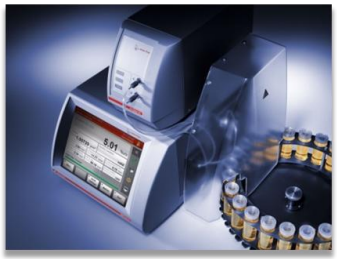
The oscillating U-tube density meter is based on the principle of a U-tube which has a resonant frequency that is inversely proportional to the square root of its mass. The volume of the tube is given; the density of the liquid sample filled into the U-tube is calculated from its resonant frequency.

Density: (kg/m3 or g/cm3) - m / V - whereby m is the mass and V the volume of a substance.



Density Meters

Alcohol Analyzers



Alcolyzer Plus Spirits – Anton Paar

Alcohol Content , Density , pH , colour

A highly alcohol-specific range of the NIR spectrum is evaluated using a highly stable high-resolution spectrometer and suitable Algorithms. The color measured at 430 nm according to the EBC and ASBC standards.

Infratec™ 1256 Beverage Analyzer- FOSS

Alcohol/Real Strength , Colour

Full Scanning NIR Technology
Wavelength range: 850-1050 nm
(570-1100 nm with colour module)

Optimisation of Yeast

Fermentation is critical for a profitable distillery…….

Yeast is key ingredient to produce alcohol from any substrate

Critical Quality assessment parameters are :

- **Higher alcohol yield**
- **High viability**
- **High vitality**
- **Faster fermentation**
- **Improved sugar : alcohol ratio**
- **Reduced water / effluent / energy consumption**
- **Use of suitable nutrient**

Yeast Analysis - 1

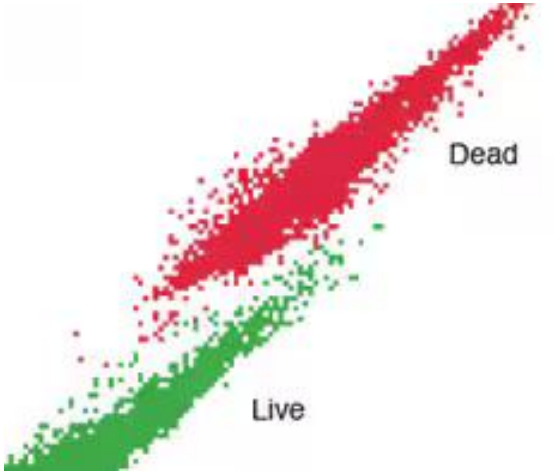
Aber Countstar

- Automated cell counter.
- Slide based image analysis instrument for yeast cells that can be used with same safe, traditional dyes used in microscopy.
- Disposable slides with 5 chambers.
- 20µl sample.
- Less than 20 seconds.

Yeast Analysis - 2

- Broad cell size range detected by Aber Countstar from 2 µm,
- Viability assessed from 5 µm.
- Samples need to be within the working cell range $5x10^4$ – $3x10^7$ cells per ml.
- Dilutions may need to be carried out
- Total Cell Concentration (cpm)
- Viability (%)
- Live Yeast Concentration (cpm)
- Average Diameter (µm)
- Average Compact
- Aggregate (%)

Yeast Analysis - 3



- CytoFLEX Flow Cytometers – Beckman Coulter
- Attune CytPix Flow Cytometer – Thermo Fisher

Flow Cytometry

A yeast viability cytometer, or flow cytometer allows clear separation of living and dead cells based on various cellular characteristics. Due to the inherent differences between prokaryotic and eukaryotic cells, specialized dyes must be used to differentiate live and dead cell populations in yeast.

Purity of Neutral Alcohol

- Any off note from impurities/contamination adversely affects
  - Product sensory quality
  - Aroma & flavour profile,
  - Consistency and stability
  - Effect on post-consumption well-being (Taste , Hangover, Toxicity)

- ❑ Neutral spirits can exhibit objectionable off notes beyond MDL of analytical instruments but fall within sensory threshold of panellists

- ❑ Structured sensory panellists training and regular proficiency calibration enhances trustworthiness of sensory quality decisions

Typical off notes in Potable Neutral Spirits

TABLE 2

Off- Notes	Associated Terms	Origins
Acetaldehyde	Green Apple Skin, Leafy, Grassy, Cardboard, Oxidised	Fermentation., Co-distilled with alcohol. Also formed on long storage / transportation due to oxidation
Diacetyl	Buttery, Butterscotch, Creamy, Toffee	By citrate-metabolising lactic acid bacteria and decarboxylation of a-acetolactic acid during fermentation
DMS	Sweet corn, Cooked Cabbage, Tomato, Shellfish, Oyster-like	DMS Sweet corn, Cooked Cabbage, Tomato, Shellfish, Oyster-like By products from biosynthesis of sulphur containing amino acids and from the reduction of sulphate salts in wort. Infection due to poor sanitation
H_2S Sulphury	Rotten Eggs	
Mercaptan	Sulphury, Drain Gas, Butane-like	
Iso Amyl Alcohol, Higher Alcohols / Fusel Oils	Impure Spirits, Fruity, Banana	Formed by yeast during fermentation by amino acid deamination and decarboxylation
Ethyl Acetate	Fruity, Nail Enamel, Solvent, Acetone, Paint, Glue	Formed during primary fermentation by enzymatic chemical condensation of organic acids and alcohols.
Isoamyl Acetate	Fruity, Banana	
4-Ethyl Phenol Brettanomyces contamination	Band-aid, Medicinal / Barnyard, Sweaty Horses	Decarboxylation of p-coumaric acid from grain by yeasts and bacteria
Plastic	Styrene, Acrylic, Solvent	Storage in recycled plastic containers

Thank You
Questions ?