A Comparison of IRMS and CRDS for the Detection of Economic Adulteration of Lemon Juice and Honey

Madhavi Mantha, John R Urban and Kevin M. Kubachka
US FDA, Forensic Chemistry Center, Cincinnati, OH

ASITA June 2016, Philadelphia, PA

FORENSIC CHEMISTRY CENTER

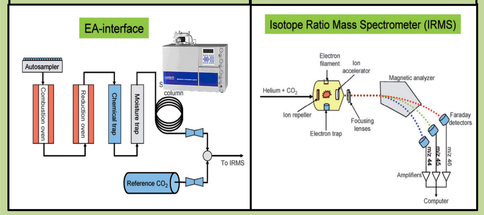
Introduction

In the last several years, economically motivated adulteration (EMA) of foods has received increased attention. Two popular targets are lemon juice and honey. For both of these types of adulteration analyses, the FCC is evaluating the use of Cavity Ring Down Spectroscopy (CRDS) to establish its suitability in comparison to the well established technique Isotope Ratio Mass Spectrometry (IRMS)

- Addition of C4 material will alter natural C3 $^{13}C/^{12}C$ ratio, indicative of adulteration.
- lemon Juice adulterated with exogenous citric acid and/or low cost sweeteners → C4 plant source
- Honey adulterated with low cost sweeteners → C4 plant sugars.
- Lemon juice samples are imported, part of an FDA assignment.
- Honey samples bought from local markets.
- Basic Procedure: Citric acid precipitate from lemon juice or bulk honey and protein precipitate → combusted in IRMS and CRDS for determination of $^{13}C/^{12}C$ (converted to per mil basis ($\delta^{13}C$(‰)).
- $\delta^{13}C$ citric acid less negative than -23‰ is indicative of lemon juice adulteration.
- $\delta^{13}C_{Protein}$ - $\delta^{13}C_{Honey}$ less negative than -1‰ is indicative of honey adulteration.

Instrument and Operation EA-IRMS

Costech Elemental Combustion System (ECS) 4010	THERMO Delta V Advantage



Samples are introduced into combustion oven at 980 °C, produces gases, then into reduction oven at 650 °C, get reduced. The gases pass through chemical trap and moisture trap, into the separating column, where CO_2 is separated from N_2 and other gases, and into the detector via electron impact ionization. Ions with m/z 44, 45 and 46 are collected in separate Faraday Cups and a complex algorithm calculates the $^{13}C/^{12}C$ ratio.

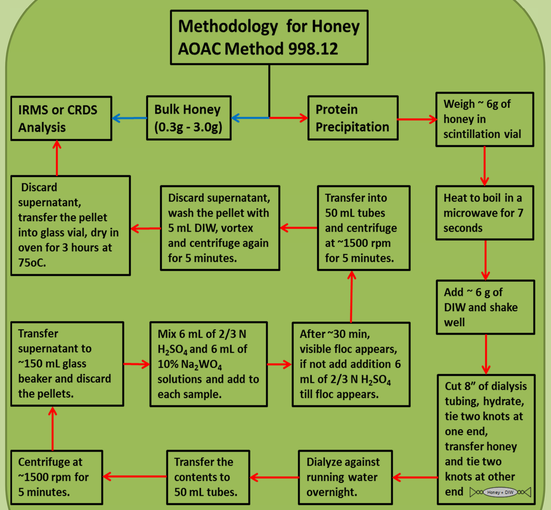
$$\delta \frac{\text{Sample}}{\text{Reference}} = \left(\frac{R_{Sam} - R_{Ref}}{R_{Ref}}\right)10^3 = \left(\frac{R_{Sam}}{R_{Ref}} - 1\right)10^3$$

Method Development and Validation for lemon juice

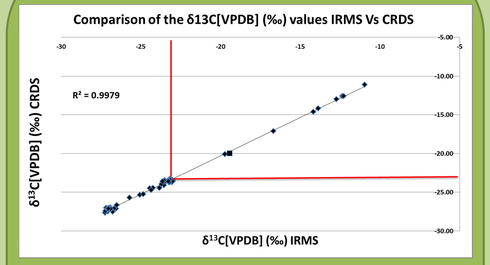
- Doner L., "Carbon Isotope Ratio in Natural and Synthetic Citric Acid as Indicators of Lemon Juice Adulteration, *J. Agric. Food Chem., 22(1985) 770-772*
- Method development to optimize calcium citrate precipitation
  - Varied amounts of $Ca(OH)_2$
  - Heating time
  - Changing the pH with NaOH and NH_4OH
- Measureable CO_2 background signal
  - From carbonate contamination in $Ca(OH)_2$ reagent present in calcium citrate precipitate.
- On achieving dissatisfactory results, focus was shifted to
  - Using $Ca(NO_3)_2$ and $CaCl_2$
  - Varying the conditions as mentioned above
- Finally using 2 mL of 3M $CaCl_2$ solution at pH greater than 8.5 and heating at 60°C for 2 hours, produced calcium citrate precipitate, free of carbon contamination

- Validation
  - Two commercially available citric acids
  - Three locally purchased lemon juice from concentrates
  - Freshly squeezed locally purchased lemons

Methodology for Honey AOAC Method 998.12

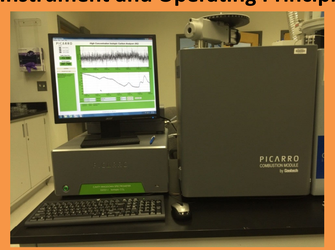


IRMS Vs CRDS for Lemon Juice



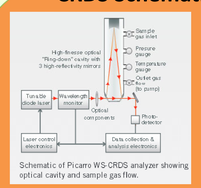
- 69 samples were analyzed by CRDS and IRMS and the results were compared.
- From IRMS analysis, 9 were Adulterated, 7 were Inconclusive and 53 were Not Adulterated
- From CRDS analysis, 9 were Adulterated, 1 was inconclusive and 59 were Not Adulterated
- The difference in $\delta^{13}C$[VPDB] (‰) values between IRMS and CRDS ranged from -0.13 ‰ to + 0.58 ‰.

CM-CRDS Instrument and Operating Principle

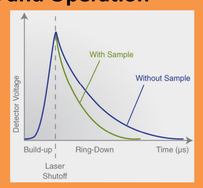


- PICARRO Combustion Module by Costech with G2121i Isotopic CO_2 analyzer.
- At start, N_2 carrier gas is switched to a volume of O_2 depending on size and composition of the sample.
- Sample in tin capsule is dropped into combustion reactor, combusts at 1700-1800°C, breaks down into N_2, CO_2, H_2O and SO_2.
- Cu wires absorb excess O_2, SO_2 adsorbed by oxidation catalysts water adsorbed by Water Trap.
- N2 + CO_2 → GC column →Liaison Interface module → CO_2 analyzer → δ $^{13}C/^{12}C$ measurement.
- CO_2 analyzers use time based, optical absorption spectroscopy

CRDS Schematic and Operation



Schematic of Picarro WS-CRDS analyzer showing optical cavity and sample gas flow.



- Light from a tunable semiconductor diode laser is directed into an optical resonator cavity containing the analyte gas.
- When the optical frequency matches the resonance frequency of the cavity, energy builds up in the cavity.
- When the build-up is complete, the laser is shut off.
- The energy decays from the cavity exponentially in time, or "rings down," with a characteristic decay time. This energy decay is measured, as a function of time, on a photodiode.
- The ring down time is measured at several different wavelengths as the laser is tuned across the molecular signature of the analyte gas.
- CRDS is a measurement of time not of absorbance. When the laser is at a wavelength where the gas in the cavity is strongly absorbing, the ring down time is short; when the wavelength is such that the gas does not absorb, the ring down time is long.
- The concentration is proportional to the difference in these ring down time.

(www.picarro.com)

Reference Materials (RM) and Quality Control

- NIST RM 8542 (Sucrose, $\delta^{13}C_{VPDB}$= -10.45±0.07‰)
- NIST RM 8573 (L-glutamic Acid, $\delta^{13}C_{VPDB}$= -26.39±0.09‰)
- Acetanilide - used to condition reactors and verify measurement performance for $\delta^{13}C$.
- *RMs are used to normalize instrument $\delta^{13}C$ to sample based $\delta^{13}C_{VPDB}$, using Excel for calculations*

Advantages and Disadvantages

- **EA-IRMS**
  - Well established technique with good linearity and precision
  - High maintenance
  - High degree of technical knowledge needed for operation
  - Very expensive
  - Used for $^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{18}O/^{16}O$, $^{34}S/^{32}S$ and $^{15}N/^{14}N$
- **CM-CRDS**
  - Relatively inexpensive
  - Robust
  - Simplified analysis technique
  - Relatively new technique with no officially defined methods.
  - Only works for $^{13}C/^{12}C$ analysis

IRMS Vs CRDS for Bulk Honey

Sample Number	IRMS Honey $\delta^{13}C$[VPDB]	IRMS Protein $\delta^{13}C$[VPDB]	IRMS Protein - Honey $\delta^{13}C$[VPDB]	CRDS Honey $\delta^{13}C$[VPDB]	CRDS Protein $\delta^{13}C$[VPDB]	CRDS Protein - Honey $\delta^{13}C$[VPDB]
15-1	-26.19	-26.00	0.19	-26.27	-26.33	-0.06
16-1	-26.18	-26.19	-0.01	-26.39	-26.00	0.40
16-2	-25.45	-25.86	-0.40	-25.48	-25.80	-0.32
16-3	-26.38	-26.16	0.22	-26.46	-26.70	-0.24
16-4	-25.66	-25.87	-0.21	-25.90	-26.40	-0.50
16-5	-25.19	-25.51	-0.33	-25.11	-25.56	-0.45
Suspect-A Sub 1	-25.78	-26.92	-1.15	-25.86	-27.02	-1.16
Suspect-A Sub 4	-25.71	-26.84	-1.13	-25.74	-26.89	-1.15
Suspect-A Sub 8	-25.74	-26.95	-1.21	-25.75	-27.01	-1.26
Suspect-B Sub 1	-25.64	-25.81	-0.17	-25.57	-26.00	-0.43
Suspect-C Sub 1	-24.71	-25.57	-0.86	-24.74	-25.68	-0.94

- Eleven honey samples were analyzed for Bulk Honey and Protein analysis and results were compared.
- Eight of the eleven samples had ($\delta^{13}C_{Protein}$ - $\delta^{13}C_{Honey}$) less negative than -1‰, indicative of no honey adulteration. Three samples were adulterated.
- The difference in $\delta^{13}C$[VPDB] (‰) values between IRMS and CRDS for Bulk Honey ranged from -0.07‰ to + 0.24‰.
- The difference in $\delta^{13}C$[VPDB] (‰) values between IRMS and CRDS for Bulk Honey ranged from -0.06‰ to + 0.54‰.

Conclusion

- For both IRMS and CRDS analysis for lemon juice and bulk honey and protein, the results were quite comparable.
- The average difference in $\delta^{13}C$[VPDB] (‰) values between IRMS and CRDS for lemon juice was 0.36‰.
- The average difference in $\delta^{13}C$[VPDB] (‰) values between IRMS and CRDS for bulk honey was 0.28‰.
- The above results indicate that the new CM-CRDS is quite suitable for adulteration analysis when applied to lemon juice and honey samples.

Future Work

- To explore the CM-CRDS instrument on more honey samples and extend to other matrices like maple syrup and other fruit juices.

Acknowledgements

PICCARO Support Group