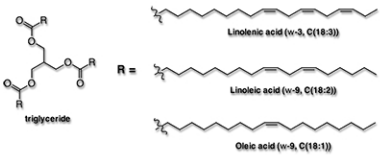
NMReady-60 Benchtop NMR Spectrometer for Detection of Soybean Adulteration in Olive Oil

Owing to attractive flavour & nutritional characteristics (*e.g.,* actively lowers LDL cholesterol[1], aids blood pressure & blood sugar regulation[2]), olive oil has long been vulnerable to fraud.[3]

Despite the efforts of regulation agencies, like the International Olive Council (IOC)[4] or the USDA[5], olive oil remains especially susceptible to fraudulent adulteration. Adulteration can be either innocuous, passing off cheaper, inferior oils as authentic extra virgin olive oil (EVOO), or lethal if oil is contaminated with toxic chemicals.[6]

Typical methods to determine adulteration in olive oil focus on gas or liquid chromatography-mass spectrometry (GC-MS or LC-MS). These methods can be time consuming - requiring extensive sample preparation and data interpretation. Although not commonly used in this context, NMR spectroscopy is an alternative technique that can provide useful information based on distinct chemical signatures within the proton NMR spectrum. The benefits of NMR include linear response factors, fast sample preparation and simple data analysis.

Oils are composed primarily of triglycerides with fatty acids chains, such as the unsaturated fatty acids shown here. Each type of olive oil will have an expected fatty acid composition.



		% Composition
Olive Oil	Saturated fatty acids	14.00
	Monounsaturated fatty acids	72.00
	Linolenic acid (ω-3)	<1.5
	Linoleic acid (ω-6)	9-20
	Oleic acid (ω-9)	
Soybean Oil	Saturated fatty acids	15.65
	Monounsaturated fatty acids	22.78
	Linolenic acid (ω-3)	7
	Linoleic acid (ω-6)	50
	Oleic acid (ω-9)	24

EVOO has fewer polyunsaturated fats than many other oils, including soybean. As such, ^{1}H NMR spectra of pure EVOO will have weaker *bis*-allylic resonance than those adulterated with soybean oil.

Specifications:

Frequency: ^{1}H 60 MHz
Magnet: permanent, cryogen-free
Stray field: confined within enclosure
Uses Stanard 5 mm NMR Tubes
Spectral resolution/Lineshape:
LW at 50%: <2 Hz
LW at 1%: <30 Hz
Power: 100-240 VAC, 50-60 Hz
Weight: 53 lbs
Dimensions: 11 x 11 x 19"
User Interface: Built-in Touchscreen
Connectivity: USB, Ethernet

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Experimental

Store bought soybean and authentic EVOO were used to prepare 0.5 M stock solutions of each oil in *d*-chloroform. Pure samples of each oil were prepared by transferring 0.5 mL of stock solution into a 5mm NMR tube. The adulterated EVOO samples were prepared at 5, 10, 20, 25, 30, 35, 40, 45, 50 & 60% soybean oil. 8 scan ^{1}H NMR spectra were measured in triplicate for each sample with a 12 ppm spectral width, 4k points, 5s delay and 0.1 Hz apodization. Raw data was exported from the NMReady to be phased, baseline corrected, referenced and integrated over pre-defined regions in MNova software.

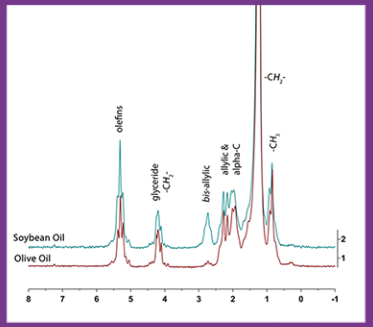

Figure 1: Representative ^{1}H NMR spectra outlining the integral regions for pure olive & soybean oil.

Results

Figure 1 shows a typical ^{1}H NMR spectrum for the pure oils. The integral regions were defined as:

1) olefin
   (δ 4.92-5.67 ppm)
2) glyceride -CH_2-
   (δ 3.82-4.50 ppm)
3) *bis*-allylic
   (δ 2.50-3.05 ppm)
4) allylic/alpha-C
   (δ 1.75-2.50 ppm)
5) alkyl
   (δ 0.46-1.75 ppm)

As is consistent with the expected composition of C(18:3) and C(18:2) fatty acids, there is a notable difference between the *bis*-allylic resonances of the two oil samples. By monitoring the relative integration of this region, it is possible to determine the percentage of soybean oil adulteration. See Figure 2 for a visual representation of changes within the *bis*-allylic region upon increased rate of soybean contamination.

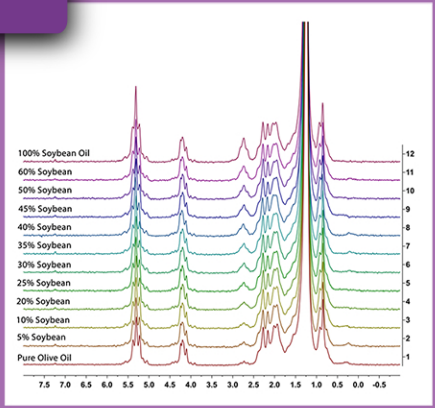

Figure 2: Stacked ^{1}H NMR spectra of manually adulterated olive oil-soybean oil samples.

For each spectrum, the ratio of *bis*-alylic protons to total integration was used to determined the percentage of *bis*-allylic component within the sample and therefore the degree of soybean oil adulteration. The *bis*-allylic:total integration ratio was plotted versus the known amount of soy adulteration to afford a calibration curve. Figure 3 shows the linearity of the data obtained on the NMReady over the adulteration range as well as the precision in each measurement.

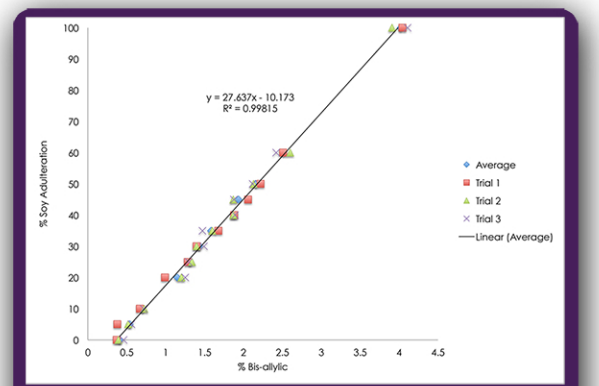

Figure 3: Amount of soybean oil adulteration as determined by the percentage of *bis*-allylic protons in each EVOO oil sample.

Conclusion

This work shows the efficacy of the NMReady for rapid determination of soybean oil adulteration into olive oil. NMR spectroscopy, although not commonly used in this capacity, can be beneficial given its simple sample preparation and rapid data collection. Once a calibration curve is prepared, adulteration rate can be easily determined in mere minutes.

References

[1] Baggio, G. *et. al. Am. J. Clin. Nutr.* **1988**, *47*(6) 960
[2] Ferrara, L. A. *et. al. Arch. Intern. Med.* **2000**, *160*(6), 837
[3] www.oliveoiltimes.com/olive-oil-basics/olive-oil-tops-fraud-list/36802 [Viewed April 9, 2014]
[4] www.internationaloliveoil.org/ [Viewed April 9, 2014]
[5] www.usda.gov/wps/portal/usda/usdahome [Viewed April 9, 2014]
[6] www.euro.who.int/_data/assets/pdf_file/005/98447/E84423.pdf [Viewed April 9, 2014]
[7] mestrelab.com/software/mnova/nmr/ [Viewed April 8, 2014]

The adulteration of olive oil is only one potential application for the NMReady-60. For more information on this, or for other ideas of how to include a benchtop NMR spectrometer into your daily workflow, please contact us directly or visit:

www.nanalysis.com/apps.html