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ANALYSIS OF TRACE ALDEHYDES IN OLIVE OIL UTILIZING QUANTITATIVE 1D AND 2D NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY.

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Introduction

Olive oil is known to contain a number of trace components possessing health promoting potential. Among these are hydroxytyrosol and tyrosol derivatives including the dialdehydes oleacein and oleocanthal and the monoaldeydes oleuropein aglycon and ligstroside aglycone. The European Union permits specific health claims for olive oils containing >250 mg/kg hydroxytyrosol and tyrosol derivatives. An official analytical method for these compounds involves extraction with methanol and/or water followed by chromatographic analysis.[1] A recent study has shown that the aldehydes react with these solvents to form acetals or hemiacetals and, in addition, chromatography under both normal and reversed phase conditions results in isomerization of these compounds.[2] This presents serious questions about the reliability of chromatographic methods. Nuclear magnetic resonance (NMR) spectroscopy has proven to be well suited for analyses of complex chemical mixtures. A robust quantitative NMR (qNMR) method has been validated for measurement of these aldehydes.[2,3] This involves a hexane/acetonitrile extraction/partition followed by solvent removal and dissolving the extract in $CDCl_3$. More recently, an ultra rapid direct screening method utilizing selective pulse qNMR has been developed which does not require extraction.[4] This method presents signal overlapping limitations in some cases which may lead to overestimation of analytes and thus is presently utilized as a screening tool to eliminate oils lower in concentration than the EU criteria from further evaluation. In addition, a simplified extraction/concentration method is currently being evaluated which removes the overlap limitation without requiring rotary evaporation.

Ultra rapid screening method for aldehydes in olive oil using SELective Double Pulsed Field Gradient Perfect Echo (SELDPFGPE) NMR.

Quantitation of key trace analytes in the presence of very strong signals from the bulk matrix can be problematic using typical NMR broad band excitation. This is due to dynamic range limitations of the analog to digital converter (ADC). These limitations can be overcome by the use of band selective shaped pulses to excite only the region containing the minor analytes while excluding the regions containing strong matrix signals. This method utilizes the SELDPFGPE sequence to acquire the selective NMR spectra of the aldehyde region from 8-11 ppm, excluding the much stronger upfield lipid resonances (Figure 1). 225 mg of each olive oil was mixed with $CDCl_3$ (500 uL) containing 50 ug/mL syringaldehyde internal standard. 600 uL was transferred to an NMR tube and NMR spectra obtained with 16 scans on a Bruker AV-III 600 with a TCI cryoprobe. NMR experimental time was approximately 3 minutes per sample.

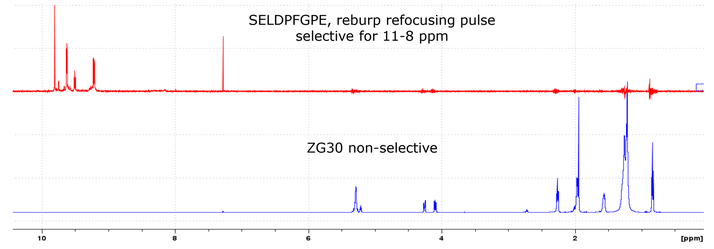
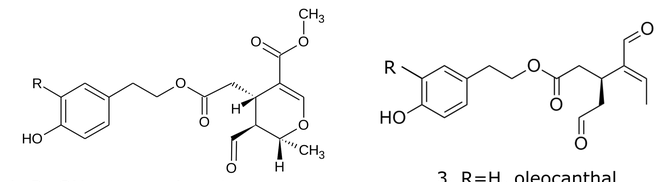


Figure 1. 1D ^1H spectrum of olive oil sample 1 using non-selective (blue) and SELDPFGPE (red) pulse sequences.

Automated screening of olive oil with Bruker Assure-RMS.

A series of 10 extra virgin olive oils (EVOOs) were screened with the ultra rapid method and quantitated automatically utilizing the Bruker Assure-RMS (raw material screening) package (Figure 2, Table 1). The pass/ fail criteria was set such that oils containing a total secoiridoid polyphenol content >250 mg/kg were designated "pass" for further evaluation via the extraction method.



1, R=OH, oleuropein aglycon
2, R=H, ligstroside aglycon

3, R=H, oleocanthal
4, R=OH, oleacein

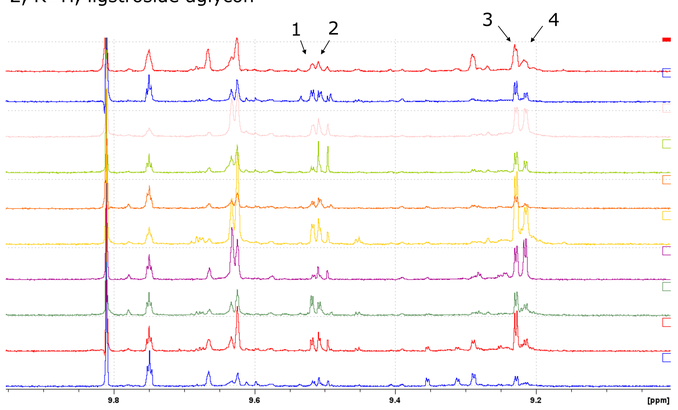


Figure 2. Aldehyde region of olive oil samples

EVOO	Aldehyde Concentration (mg/kg oil) oleuropein aglycon	ligstroside aglycon	oleocanthal	oleacein	total	pass/fail
1) Lapas	26.6	27.6	43.0	20.9	118.1	fail
2) Carapelli	51.0	65.6	140.4	57.5	314.5	pass
3) Botacelli	83.3	58.3	84.3	53.3	279.2	pass
4) Pons	22.1	36.9	135.0	154.9	348.9	pass
5) Colavito	101.0	97.5	307.9	198.3	704.7	pass
6) World Classic	38.7	50.8	51.4	22.4	163.3	fail
7) Olave	26.3	64.4	71.8	42.3	204.8	fail
8) Piccolo	65.7	66.0	144.8	132.9	409.4	pass
9) 365	53.4	49.8	69.4	37.3	209.9	fail
10) Paesano	47.6	40.7	134.8	64.0	287.1	pass

Table 1. Secoiridoid polyphenol aldehyde concentrations in extra virgin olive oils and pass/fail results as determined in automation using Assure-RMS.

Simplified extraction/concentration method for olive oil 1D and 2D NMR analysis.

The ultra rapid screening method presents some signal overlapping limitations. In particular, the aldehyde resonance of 2-hexenal, found in older oxidized oils, overlaps the ligstroside aglycon resonance at 9.51 ppm. In addition, although most EVOOs contain oleaocanthal and oleacein as the main dialdehyde compounds, some contain significant amounts of the dialdehydic forms of oleuropein and ligstroside which are not resolved in this method. To resolve the overlap issue, a simplified Acetonitrile (ACN) extraction procedure has been developed which does not require the rotary evaporation step in the current validated[2,3] qNMR method. In this procedure, 10 mL olive oil is weighed into a 15 mL plastic centrifuge tube. 3 mL ACN is added and the total weighed. This is mixed via inversion/vortexing followed by centrifugation to separate the lower oil and upper ACN phase. 1.0 mL of the upper ACN phase is then transferred to a 1 dram vial, carefully weighed, and the ACN removed under a stream of nitrogen. This is then dissolved in 600 uL $CDCl_3$ containing 50 ug/mL syringaldehyde as internal standard and analyzed via 1D and 2D NMR (Figure 3). Further validation of this method is underway.

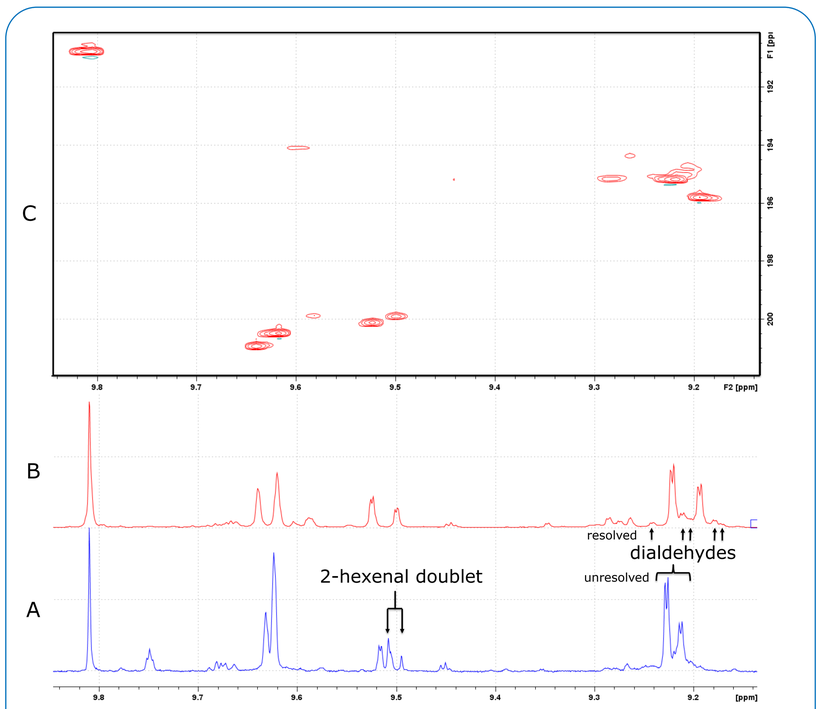


Figure 3. NMR of EVOO 5: A) unextracted, SELDPFGPE, B) extracted, SELDPFGPE and C) extracted, f2 region selective HSQC.

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