Concerted Action No. 1

Food authentication by spectroscopic techniques (FAST)

A QUEST Action Group guideline document

Spectroscopic techniques (NIR, NMR, FTIR) for rapid direct food quality assessment

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1.6 - Application of high field high resolution nuclear magnetic resonance spectroscopy to the authentication of edible oils

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Three batches of edible oils that were circulated by the members of the Concerted Action were analysed in the Superconducting NMR Laboratory in Bucharest. A total of 42 oil samples were received and analysed. Six additional samples of edible oils commercially available in Romania were also analysed. Among these samples three were also analysed at various other field strengths in several laboratories from Belgium and the United Kingdom. For these latter three samples, composition was also determined by the classical method of saponification followed by HPLC analysis in CFDRA laboratories.

The work performed in Bucharest had two major objectives : (i) to use the NMR spectra as fingerprints in order to discriminate various type of edible oils, and (ii) to assign NMR signals that might provide useful information related to composition of the oils.

Procedure and results - objective (i)

The ^{1}H-NMR spectra at 300 MHz and ^{13}C-NMR spectra at 75 MHz were recorded on a Varian Gemini-300 spectrometer.

The ^{1}H-NMR spectra were recorded in 5% solution in deuterated chloroform using 16 transients, an acquisition time of 6 seconds, recovery delays of 10 seconds and tip angles of 65°. The spectral width was 10 ppm. These parameters ensured that quantitative spectra were obtained.

The ^{13}C-NMR were recorded in 30% solution in deuterated chloroform on four different spectral windows, using tip angles of 60° and 512 transients. The spectral widths of 248 ppm (-10 to 238 ppm) were recorded with acquisition times of 0.8 seconds and relaxation delays of 5 seconds, and those of 2 ppm (172 to 174 ppm), 4 ppm (127 to 131 ppm) and 13.11 (22 to 35.11 ppm) were recorded with acquisition times of 5 seconds and relaxation delays of 15 seconds. In order to establish these parameters, spectra were run with acquisition times ranging from 0.8 to 20 seconds, relaxation

times between 0 and 200 seconds and 256, 512 or 1024 transients. The spectra were gated hydrogen decoupled only during the acquisition time in order to avoid the NOE effects. Although the relaxation delay of 5 seconds proved to be efficient for the spectra recorded with short acquisition times, for spectra recorded with acquisition times of 5 seconds a longer relaxation delay was employed in order to allow the quenching of any NOE effect induced by the decoupler. The parameters finally established ensured quantitative spectra.

All spectra were recorded in 5 mm, 507 Norell grade sample tubes. The total recording time for the hydrogen spectra was 4.3 minutes. For full ^{13}C spectral width (248 ppm) the total recording time was 49.5 minutes and for each of the three narrow spectral widths the time was 2 hours and 50 minutes. The total recording time for each of the 42 oil samples was about 9 hours and 30 minutes. To this time an additional hour was spent carefully shimming the instrument.

The ^{1}H-NMR spectra (Figure 1.6.1) exhibited non overlapping signals for the CH_3 groups (0.84 - 0.94 ppm), $COCH_2$-CH_2 (1.54 - 1.68 ppm), CH_2-CH_2 -CH=CH (1. 95 - 2.10), CO-CH_2 (2.26 - 2.32 ppm), CH=CH-CH_2 -CH=CH (2.73 - 2.78 ppm) and the OCH_2 group belonging to the glyceride residue (4.09 - 4.34 ppm). Each hydrogen atom in the OCH_2 groups had a different chemical shift, the whole group exhibiting an AB pattern. ^{13}C-NMR spectra (Figure 1.6.2) recorded on full spectral width (248 ppm) exhibited splitting of the non overlapped signals which were mainly due to differences in chemical shifts between the 1/3 and 2 positions of the triglyceride in the ratio 2/1 respectively. No signals corresponding to individual fatty acids could be found in these spectra.

The two types of spectra described above provided enough information in order to unambiguously discriminate among the following 8 groups : Olive, Pomace, Rape, Sesame, Soya, Grape and Walnut oils. Another group which can be easily distinguished from the above includes Sunflower and Corn oils. Figure 1.6.3 presents some typical patterns of the CO and CH=CH groups for these oils.

The narrow spectral width ^{13}C-NMR spectra revealed further splitting of each signal due to various fatty acid moieties. These spectra provide a much more reliable method for discriminating among various types of edible oils. Thus, the 42 sample oils were readily split into 9 groups (one for each type of oil). Figure 1.6.4 presents each group of signals recorded both on full and narrow spectral width for the same oil.

The attempt to split each group of oils into further smaller groups leads to the following result. For three groups (Rape, Grape and Sesame) only one sample was available . For the other groups, especially Olive, Sunflower, Soya and Pomace, enough samples were available in order to draw a conclusion. The careful examination of the ^{13}C narrow recorded spectra for each oil revealed a slight and continuous variation in the

relative ratio of some signals in each spectrum, indicating variations in oil composition. The differences were such that any attempt to define new groups within each group would be artificial. As an example the olive oils could be ordered in the increasing ratio of the group of signals from 129.58 - 129.66 to those from 128.06 - 129.00 ppm. The lowest ratios are found in samples no 35, 40, 36 and 14 and the highest ratios in 38, 2, 17 and 37. The only oils showing large differences in spectra within a series are the Pomace ones. In addition, the latter are suspected to contain both free acids and glycerin. No special work was carried out in order to confirm this hypothesis but the presence of additional signals in the range 173-179 and 64-69 ppm indicate the presence of these compounds. A partial hydrolysis process was suspected.

The influence of the field strength on the signal overlapping was also investigated. Thus, two commercially available oils were recorded at 200, 300, 400, 500 and 600 MHz. In the ^{1}H-NMR spectrum at 600 MHz (Figure 1.6.5) spreading of the signals in comparison with the spectrum at 300 MHz is noticeable. However, the only additional non-overlapped signal is the $^{-}$OCH group belonging to the position 2 of the glyceride moiety. For the authentication of edible oils this signal is not relevant, the group being present in all constituents of any oil. The ^{13}C-NMR spectra at 150 MHz, the highest available field strength in 1993 (equivalent of 600 MHz for proton NMR) does not exhibit additional useful splitting patterns. The conclusion of the variable field strength investigations is that 300 MHz is ideal for standardization of this type of analysis. The variations in spectra between 200 and 300 MHz or between 400 and 300 MHz are small enough for the case of edible oils to allow the use of the method at any of these fields without performing additional research.

Once both the potential and limitations of the method were established, further work was carried out in order to establish the minimum amount of time (and money) required for the authentication of edible oils by high resolution NMR. The following experimental conditions were found ideal as a compromise between time spent on analysis and quality of information gained. Both ^{1}H- and ^{13}C-NMR spectra should be recorded using the same concentration of 50 % v/v oil in deuterated chloroform. This confers the advantage of performing only one shimming session for each sample and of reducing the number of required transients for a reasonable sensitivity to noise ratio in the ^{13}C-spectrum. The tip angle (pulse width) in the ^{1}H spectrum should be reduced to about 9 in order to acquire the spectrum. The ^{1}H spectra were recorded with 8 transients, 2 seconds acquisition times and 10 seconds relaxation delays. The ^{13}C - spectra were recorded with 256 transients and a relaxation delay of 5 seconds for both the full spectral width (an acquisition time of 0.8 seconds ensures quantitative conditions) and in the three narrow spectral windows (a small NOE effect was induced during the 5 seconds of acquisition time providing semiquantitative spectra). The times required for these fast analyses are 1.6 minutes for ^{1}H, 24.7 minutes for the full window ^{13}C and 42 minutes for narrow window ^{13}C spectra. The method was straightforward and ensured 100% discrimination of the samples belonging to the 9

groups described above, in less than a third of the previous experimental time. The shimming time can also be reduced from about one hour to less than 10 minutes if only ^{1}H and ^{13}C on full window are required (for the case when only discrimination between Olive, Pomace, Rape, Sesame, Soya, Grape and Walnut is desired). However, if the presence of both Corn and Sunflower is suspected, then the narrow window spectra should be performed with a very good shim. The time can be further reduced by recording only the ^{1}H spectrum if the discrimination only between Olive, Pomace, Soya, Rape and Walnut is desired. A logical approach chart is presented in Figure 1.6.6.

To summarize, the 42 samples of various edible oils were unambiguously split into 9 groups (Table 1.6.1). The value of the method for authentication purposes and its limitation are evident. The minimum time required for accurate results was also established. Further improvements in discriminating power might be possible (see below).

Results and procedure - objective (ii)

In order to assign various signals both in ^{1}H- and ^{13}C-NMR spectra the following additional 1D- and 2D spectra were performed : H-H COSY, Long Range H-C COSY, NOE, APT spectra recorded at 300 MHz and H-C COSY at 600 MHz.

Based on some signals assigned to various CH_2 groups in the ^{13}C-NMR spectrum, the composition of three edible oil samples was determined. The results are in good agreement with those achieved by classical analysis performed at CFDRA (Table 1.6.2).

Future trends

Additional signal splitting corresponding to various fatty acid moieties in the ^{13}C-NMR spectra allowing easy composition determinations might be obtained by recording very high field spectra on narrow spectral widths and with long acquisition times. Due to limited resources, it has not been possible to perform these experiments as part of the Action Group. It is hoped that this work will be carried out in the near future.

Further assignments of the other CH_2 groups in the ^{13}C-NMR spectrum are hoped to be obtained using additional types of NMR experiments.

Table 1.6.1 Edible oils circulated by the QUEST/FLAIR Concerted Action No. 1 and the discrimination performed by NMR

Sample code	Description	NMR assigned group
27	Olive, unripe, Greece	1
28	Olive, normal, Greece	1
29	Olive, over-ripe, Greece	1
30	Olive, normal, Greece	1
31	Olive, normal, greece	1
32	Olive, unripe, Italy	1
33	Olive, normal, Italy	1
34	Olive, over-ripe, Italy	1
35	Olive, normal, Italy	1
36	Olive, normal, Italy	1
37	Olive, unripe, Spain	1
38	Olive, normal, Spain	1
39	Olive, over-ripe, Spain	1
40	Olive, unripe, Spain	1
41	Olive, normal, Spain	1
42	Olive, over-ripe, Spain	1
1	Olive, extra virgin	1
2	Olive, extra virgin	1
8	Sesame seed	2
3	Other olive oil	1
4	Other olive oil	1
5	Other olive oil	1
12	Sunflower	3
9	Soya	4
10	Walnut	5
11	Walnut	5
6	Grapeseed	6
13	Corn	7
7	Rapeseed	8
14	Olive, extra virgin, Greece	1
15	Olive, extra virgin, Greece	1
16	Olive, virgin, Greece	1
17	Olive, virgin, Greece	1
18	Olive, virgin, Greece	1
19	Pomace, Greece	9
20	Pomace, Greece	9
21	Pomace, Greece	9
22	Pomace, Greece	9
23	Crude corn	7
24	Crude sunflower	3
25	Crude soya	4
26	Crude soya	4

Table 1.6.2 Comparison of the composition determination results using NMR and classical method of saponification followed by HPLC. The sample was commercial Romanian sunflower oil.

	Inst. for Org. Chem., Bucharest, Romania	CFDRA Chipping Campden, UK
	Composition ^{13}C-NMR, 75 MHz	**Analytical method** Saponification and GLC
Saturated fatty acids	9%	12%
Monounsaturated fatty acids	27%	27%
Polyunsaturated fatty acids	64%	61%

Figure 1.6.1. ^1H-NMR spectrum of a typical sunflower oil. The same groups of signals are present in all edible oils in various relative ratios and patterns

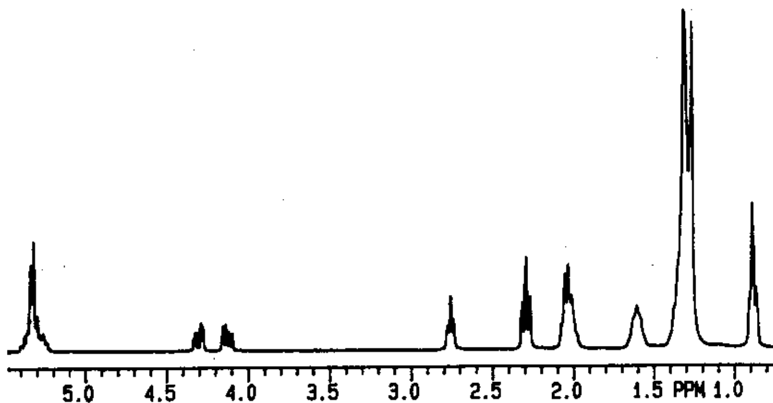
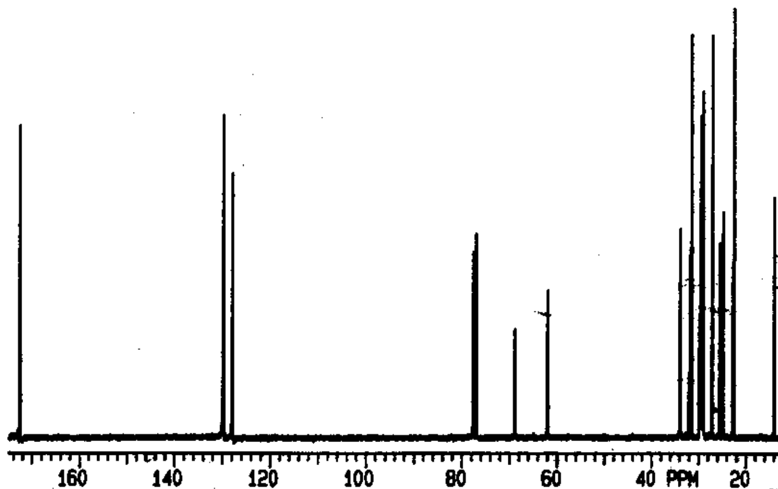


Figure 1.6.2. ^{13}C-NMR spectrum of a typical sunflower oil. The same groups of signals are present in all edible oils in various ratios and pattern



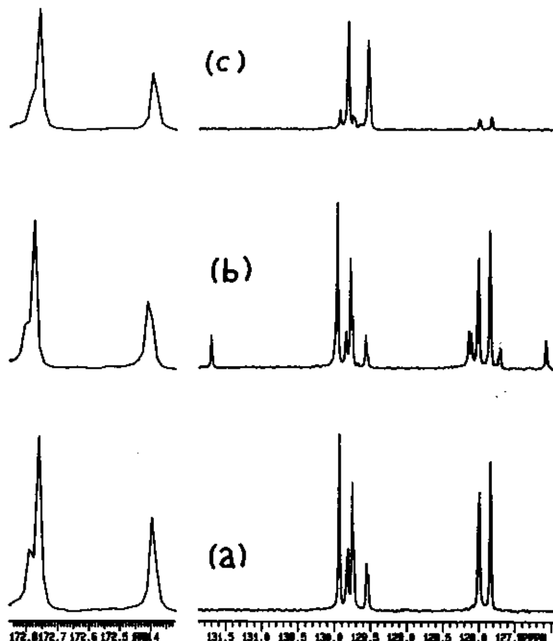


Figure 1.6.3 Typical patterns for the CO and CH=CH groups in the ^{13}C-NMR spectra of some edible oils recorded on full spectral width: (a) Sunflower, (b) Walnut and (c) Olive oils

Figure 1.6.4 The signals corresponding to -COO and CH=CH groups in the ^{13}C-NMR spectrum of a sunflower oil recorded (a) on the full spectral window and (b) on two narrow windows

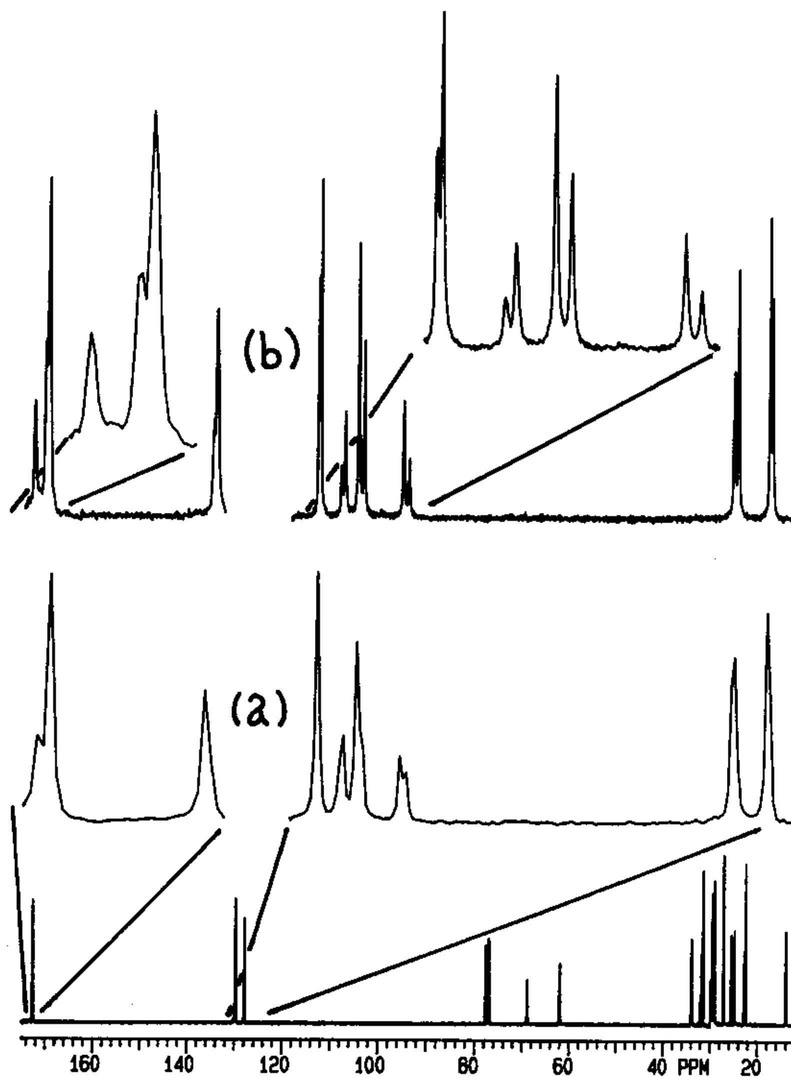


Figure 1.6.5 ^{1}H-NMR spectra of the same sample of olive oil recorded at (a) 300 MHz, (b) 400 MHz and (c) 600 MHz

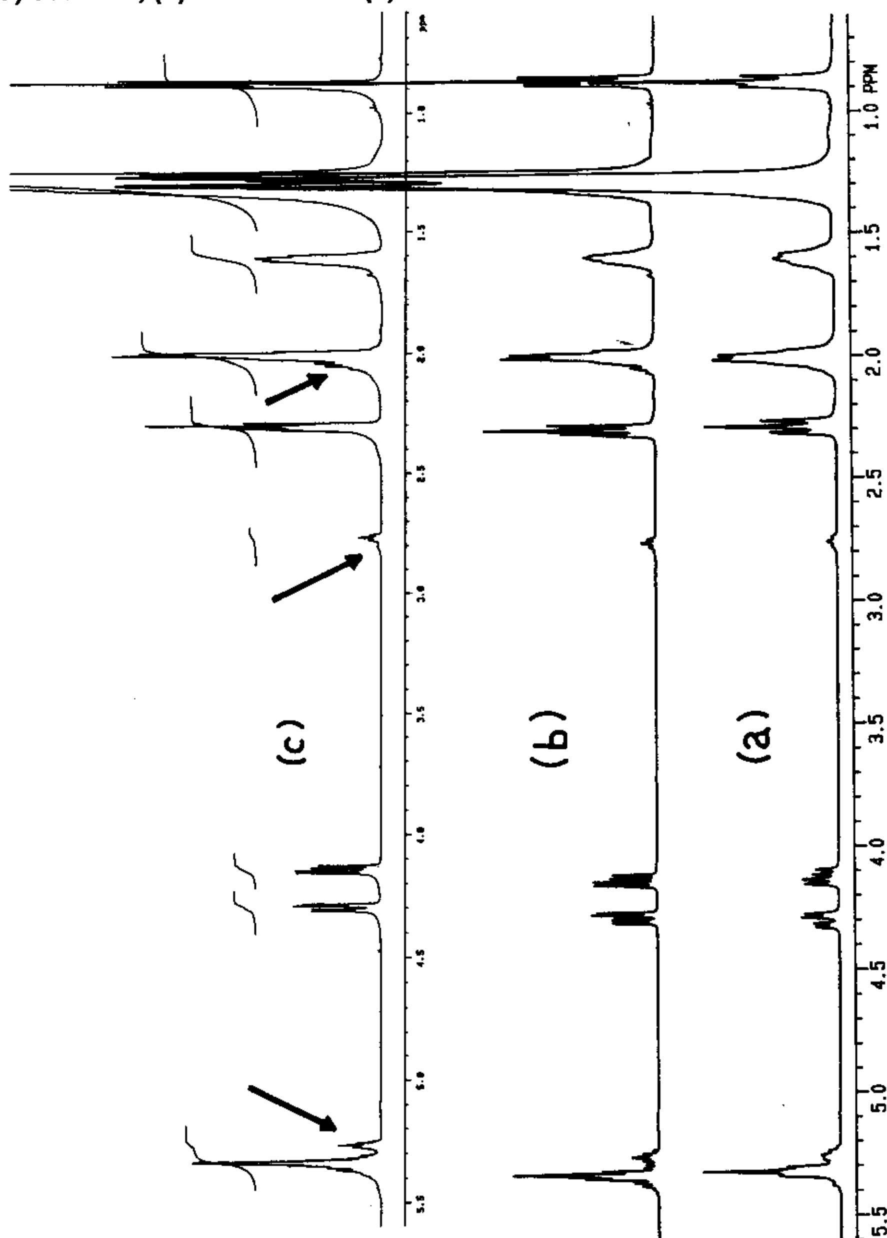


Figure 1.6.6. Flow chart for a logical approach to the authentication of edible oils using high resolution NMR spectroscopy