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^{13}C NMR Characterization of Triacylglycerides of Roodbar and Tarem Olive Oils of Iran and Comparison with Commercial Olive Oil of Olitalia (Italia) and Luna (Turkey)

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The ^{13}C NMR spectroscopy for study and characterization of crude olive oils of Roodbar and Tarem regions of Iran and comparing them with commercial samples of Olitalia from Italia and Luna from Turkey was utilized. The separation of oleic acid of these olive oils was subjected to detailed analysis by urea inclusion complex techniques.

Key Words: ^{13}C NMR, Olive oil, Triacylglycerids, Oleic acid.

INTRODUCTION

Nuclear magnetic resonance spectroscopy has played an increasing role in the study of properties of oils of vegetable origin during the last 10 years[1-7]. This work is aimed at the application of NMR spectroscopy as a versatile systematic tool for studying olive oil chemistry of two main olive oil supplier parts of Iran and analyzing them with two famous extra pure commercial samples from Italy and Turkey.

NMR spectroscopy of proton nuclei with nuclear spin quantum number I = 1/2, has the advantages of operating with a "condensed spin" given the high gyromagnetic ratio which is four times (26.75×10^7 rad T^{-1} s^{-1}) that of carbon-13 (6.73×10^7 rad T^{-1} s^{-1}) and the natural abundance of 99.985%[8]. The ^{13}C nucleus with I = 1/2 has become second in importance to the proton in spite of its low gyromagnetic ratio and its very low natural abundance (1.1%) which make this nucleus a "diluted spin". In the natural abundance spectrum, no ^{13}C–^{13}C couplings complicate the spectrum appearance and the ^{13}C–^{1}H couplings can be removed by proton decoupling thus making the ^{13}C spectra easily assignable.

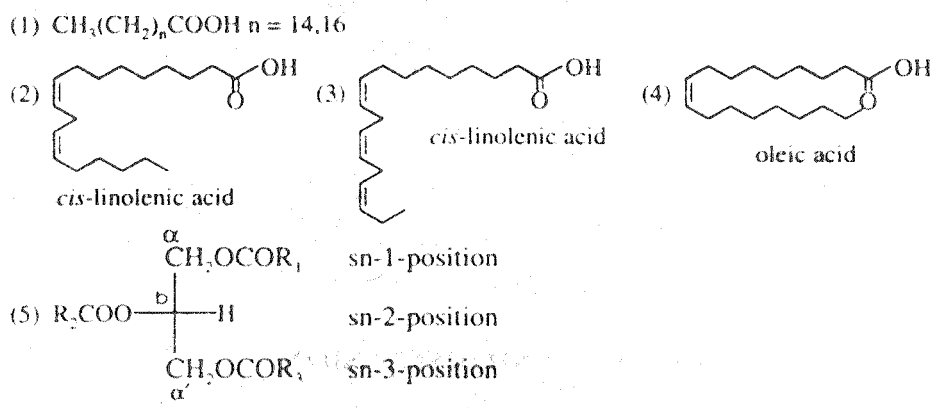

Fig. 1. Structure of the major fatty acids of olive oil: (1) saturated (2) linoleic (3) linolenic and (4) oleic. The basic structure of triacylglycerols by Fisher projection (5) is also shown. R_1CO, R_2CO and R_3CO are the acyl groups whose position on the glycerol backbone is designated either by the stereospecific numbering 1, 2, 3 or by the Greek symbols α, β, α′

EXPERIMENTAL

The oleic acid was separated from olive oils of two different parts of Iran: Roodbar Guilan and Tarem Zanjan, and two other commercial samples from Turkey (Commercial Tuna, extra virgin olive oil cold pressed) and Italy (Commercial Olitalia, extra virgin olive oil cold pressed, Forli, Italia). Yields refer to isolated pure centre cut from column chromatography or scratched from preparative TLC. Products were characterized by comparison with authentic sample (IR, NMR, GC, TLC and m.p.). Melting points are uncorrected and determined by Mettler FP5 melting point apparatus. IR spectra were obtained on a Shimadzu IR-470. All NMR data were recorded in $CDCl_3$ Brucker Avance 500-MHz spectrometer. Chemical shifts are reported in ppm (δ) using TMS as internal reference. Mass spectra were obtained from a GC-MS Agilent Technologies QP-5973N MSD instrument. For preparing the sample for ^{13}C NMR, 200 mg of oil was centrifuged and then was diluted with 0.5 mL $CDCl_3$.

Separation of Oleic Acid from Olive Oil

To 125 mL Erlenmeyer flask was added 10 g of olive oil and 2 g KOH and 20 mL triethylene glycol. The reaction mixture was heated to 160°C for 20 min. The reaction mixture was cooled and to this was added a solution of 50 mL of water and 10 mL conc. HCl. In three portions each time with 10 mL diethyl ether the unsaturated acids were extracted from emulsion mixture. The extracted layer was washed with saturated NaCl and dried over $MgSO_4$. The solvent was evaporated and to the residue 75 mL acetone was added. The flask was cooled to 15°C in isopropyl alcohol dry ice bath for 15 min. The remainder saturated crystals were filtered. This procedure was repeated until all saturated acid was removed. The yield of a mixture of stearic acid and palmitic acid was obtained 4.8–6.9 g. The filtrate was evaporated and the solvent was removed. The residue was transferred to a 250 mL Erlenmeyer flask and to this were added 10 g of urea and 50 mL MeOH. The reaction mixture was heated and the solution was allowed to stand and then cooled thoroughly until needle crystals had separated; the product was collected and 11–13 g of colourless oleic acid-urea inclusion was collected. These crystals were transferred to a 250 mL flask and to this was added 50 mL water and extracted with 3 × 20 mL ether; the solvent was evaporated and the yield of pure oleic acid was *ca.* 2.65–2.79 g (Table-1).

RESULTS AND DISCUSSION

Oleic acid was separated from olive oils of two main supplier parts of Iran (Roodbar Guilan and Tarem Zanjan) and two other commercial extra pure samples from Turkey (Tuna) and Italy (Olitalia). The urea inclusion complex technique was applied to all samples. In this procedure saponification of olive oil was accomplished by the use of a solvent permitting operation at 162°C. Typical universal composition of the five acids found in olive oil is: oleic acid 64%,

linoleic acid 16%, linolenic acid 2%, stearic acid 4% and palmitic acid 14%. Three are unsaturated and two are saturated. Oleic acid and linoleic acid are considerably lower melting acids and more soluble in organic solvents than the saturated components. When the solution of the hydrolyzate in acetone is cooled to 15°C, about half of the material separates as a crystallizate have the two saturated compounds stearic acid and palmitic acid. The unsaturated acid fraction was next recovered from the filtrate and treated with urea to form the urea inclusion complex. The oleic acid separated from 10 g olive oil with this technique was recorded in Table-1.

TABLE-1

Olive oil (%)	Roodbar (Iran)	Tarem Zanjan, (Iran)	Commercial Olitalia (Italy)	Commercial Tuna (Turkey)
Oleic acid	2.79	2.68	2.67	2.65

NMR spectroscopy has played an increasing role in the study of properties of oils during the last 10 years. This study aimed at proposing the ^{13}C NMR spectroscopy as a recent analytical tool for consideration and characterization of triacyl glyceride of Roodbar and Tarem and comparing them with Olitalia and Luna. The chemical shift region of the ^{13}C nucleus is large compared to the proton range, *ca.* 200 *vs. ca.* 10 ppm, respectively. The ^{13}C NMR spectrum of an olive oil sample contains the resonances of carbon from the triglyceride fraction of olive oil, *i.e.*, the fatty acid resonances. The ^{13}C resonances are classified in four sets of signals: carbonyl carbons resonating from 172.1–178.8 ppm (area D), unsaturated carbons in the range from 127.4–129.6 ppm (area C), glycerol backbone carbons from 61.6–68.5 ppm (area B) and aliphatic carbons from 13.05–33.71 ppm (area A).

The D area of the ^{13}C of Roodbar sample points toward three carbonyl carbon resonance peaks: nos. 1, 2 and 3 (Table-2). The resonance at 178.8 ppm indicates the presence of small amount of free fatty acid in the sample. The saturated, oleoyl and linoleoyl chains at glycerol 1,3-positions appeared at 172.5–172.6 ppm and the 2-position oleoyl and linoleoyl chains were detected at 172.1–172.2 ppm, respectively. The 1,3 and 2-position saturated chains, which in high quality olive oil triglycerides are present under 1.5%, were not detected under the experimental conditions adopted, see peaks nos. 1, 2, 3 (Table-2).

In the C area each unsaturated carbon signal of oleoyl chain was split depending on the 1,3- and 2-positions 129.6 and 129.5 ppm for C-9 and C-10, respectively. The linolenyl chain double bond carbons were found to be determined from the oleoyl and linoleoyl carbons except for C-9. The signals of C-10, C-12 and C-13 were visible at 127.6, 129.1 or 129.2 and 127.4, respectively, see peaks nos. 4–8 (Table-2).

TABLE-2
125 MHz ^{13}C NMR CHEMICAL SHIFTS OF OLIVE OIL SAMPLES FROM TAREM ZANJAN AND ROODBAR GUILAN OF IRAN, OLITALIA (ITALY) AND LUNA (TURKEY) IN FOUR DIFFERENT REGIONS A

No. of peak	Tarem Zanjan (ppm)	Roodbar Guilan (ppm)	Olitalia Italy (ppm)	Luna, Turkey (ppm)	Region
1	172.6	178.8	172.7	172.5	D
2	172.2	172.6	172.2	172.1	D
3	—	172.2	—	—	D
4	129.6	129.6	129.6	129.6	C
5	129.5	129.5	129.5	129.5	C
6	129.2	129.1	129.2	129.1	C
7	127.6	127.6	127.6	127.6	C
8	127.4	127.4	127.4	127.4	C
9	61.6	68.5	68.5	68.5	B
10	68.4	64.5	61.6	61.6	B
11	—	61.6	—	—	B
12	13.05	13.61	13.61	13.61	A
13	22.19	13.62	13.63	13.63	A
14	24.36	22.18	22.23	22.21	A
15	25.14	22.22	24.39	24.37	A
16	26.68	24.24	25.18	25.16	A
17	26.73	24.37	26.77	26.70	A
18	28.61	25.16	28.64	28.62	A
19	28.69	28.62	28.84	28.83	A
20	28.84	28.83	28.87	28.86	A
21	28.99	28.87	29.05	29.04	A
22	29.05	29.04	29.08	29.07	A
23	29.14	29.08	29.22	29.21	A
24	29.22	29.22	29.25	31.03	A
25	29.28	37.07	31.08	31.45	A
26	31.04	31.46	31.46	33.51	A
27	31.43	33.52	33.55	33.65	A
28	33.51	33.68	33.71	—	A
29	33.67	—	—	—	A

In the B area the 1,3- and 2-glycerol carbons of triglyceride resonance at 61.6 and 68.5 or 68.4 ppm, respectively, without identifying the sn-1 and sn-3 positions of glycerol. However, the Roodbar and Luna samples contain two additional resonances at 64.5 ppm. These resonances could be attributed to the 1,3-diglycerides, see peaks nos. 9, 10, 11 (Table-2). The total content of diglycerides of olive oil [1,3/1,2-diglycerides] was accepted to be related considerably to olive fruit varieties and also with the 1,3/1,2-diglyceride ratio, to ripening process.

In the A area the signals of C-18 of different chains centred at 13.05 and 13.61 ppm, peaks nos. 12, 13 except for Tarem which contains only one peak C-17 at 22 ppm, peaks nos. 13 for Tarem and 14 for three other samples (Table-2) and C-16 at 31 ppm, are determined on the basis of chain double bond numbers. The C-2 resonances appearing at 33 ppm, oleoyl and linoleoyl as a signal but well resolved from saturated chains, are differentiated according to 1,3- and 2-position on glycerol backbone, the latter being shifted at higher frequency. The group of C-3 signals at 24 ppm. The allylic carbons of oleoyl and linoleoyl chains are displayed from C-11 (O), C-14 (L), C-8 (L), C-8 (O) towards lower frequencies, the chains are indicated in parentheses. They resonate at chemical shifts centred at 26 ppm, consequently assigning the *cis*-configuration of double bonds. The allylic carbons of *trans*-olefins are less compressed and resonate at higher frequency 31 ppm from the corresponding *cis*-olefin thus contributing to distinguish the *cis-trans* isomerism. The C-11 of linoleyl chain 25 ppm is further shifted by 2 ppm to lower frequency due to two γ-gauche interactions between C-14 and C-8 ppm.

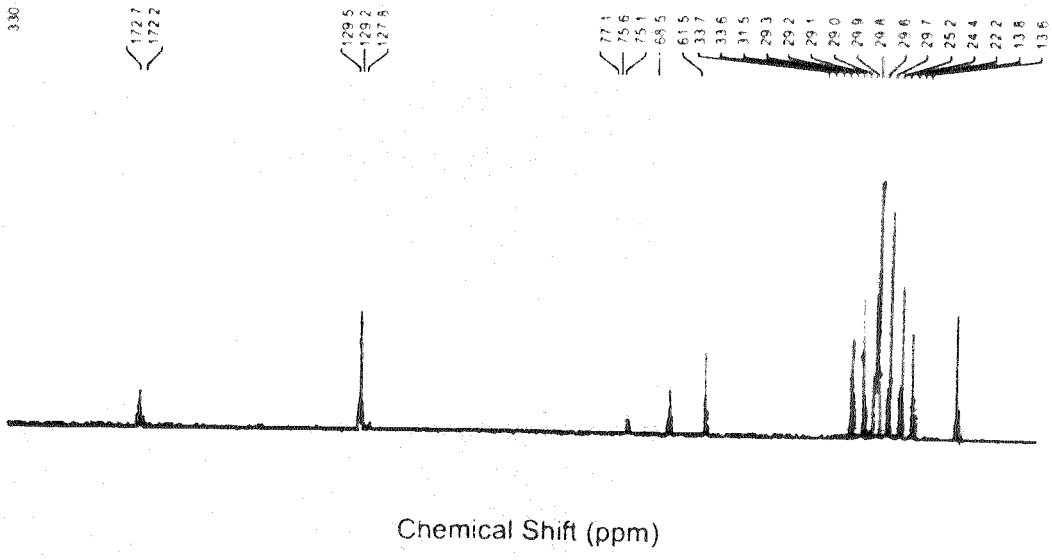

Fig. 2. ^{13}C NMR spectra of commercial olive oil (Italian oli sample); from left: D, C, B and A regions

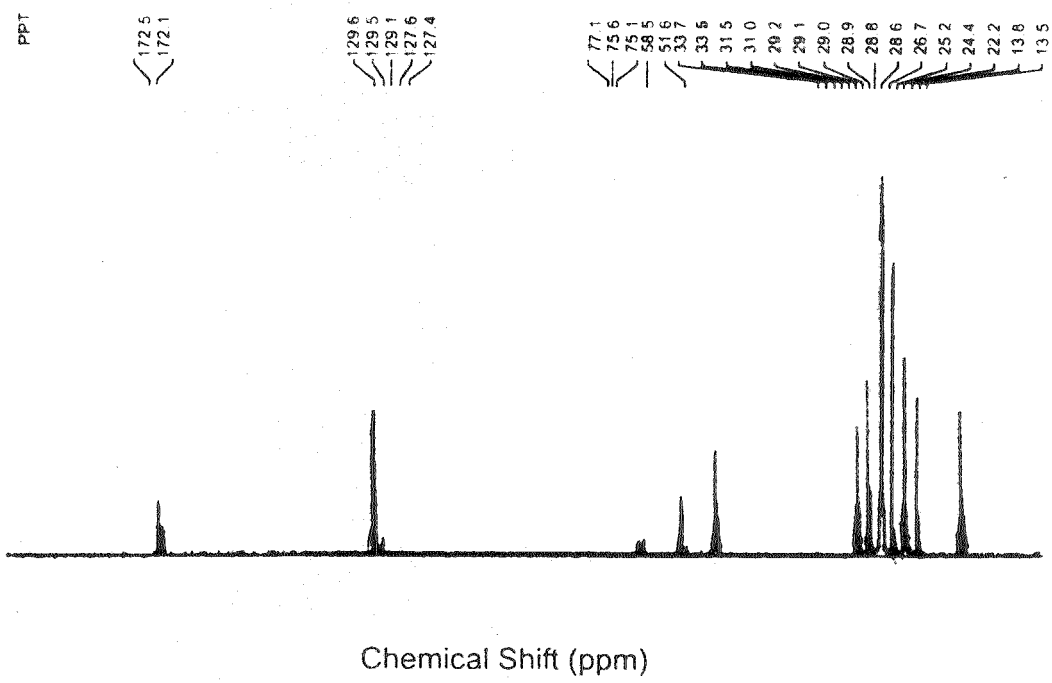

Fig. 3. ^{13}C NMR spectra of commercial olive oil (Luna, Turkey); from left: D, C, B and A regions

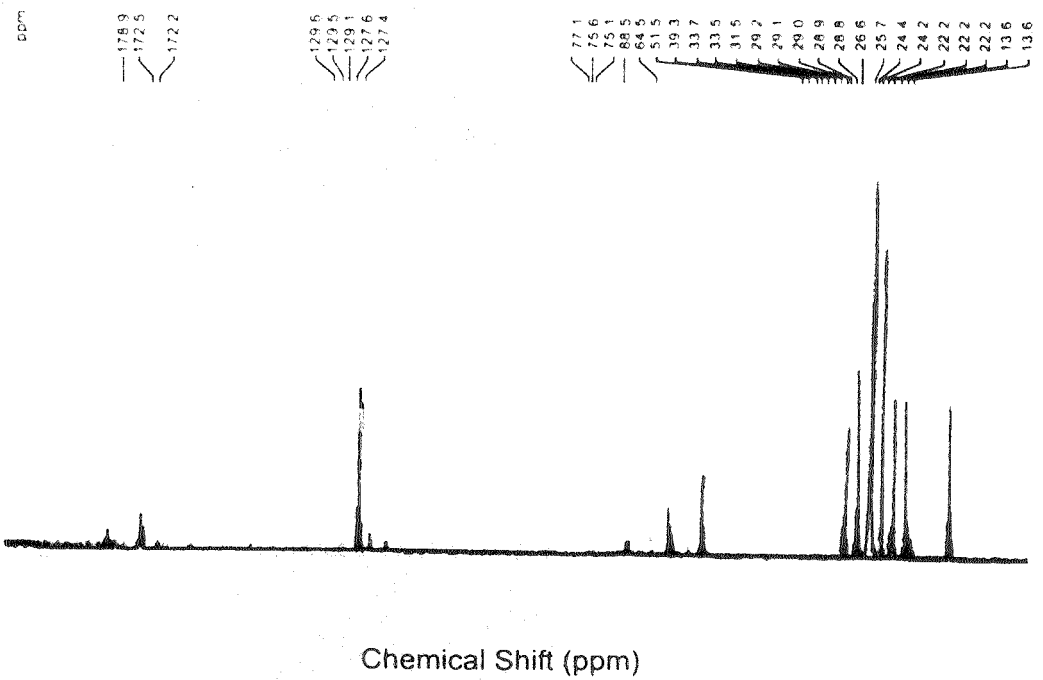

Fig. 4. ^{13}C NMR spectra of Roodbar olive oil (Roodbar, Iran); from left: D, C, B and A regions

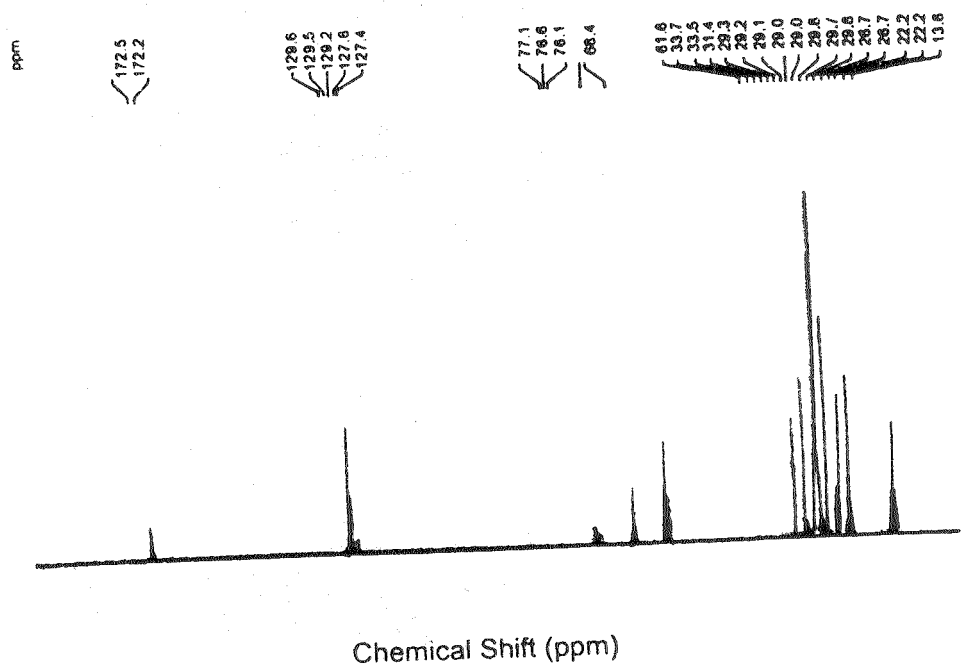

Fig. 5. ^{13}C NMR spectra of Tarem olive oil (Tarem, Iran); from left: D, C, B and A regions

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