^{13}C Nuclear Magnetic Resonance Spectroscopic Analysis of the Triacylglycerol Composition of Some Margarines

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ABSTRACT: The triacylglycerol fraction of three samples of margarine, namely "Flora" (Holland), "Kaliakra" (Bulgaria), and "Corona" (Holland), were studied by ^{13}C nuclear magnetic resonance spectroscopy. By examining the various carbon chemical shifts of the saturated and unsaturated carbon nuclei, "Flora" margarine was shown to contain a mixture of hydrogenated and unhydrogenated vegetable oils. This technique allowed all major acyl groups (saturated, oleate, linoleate, and linolenate) and minor acyl components [different positional isomers of long-chain (*E*)- and (*Z*)-monoenoic moieties, arising as by-products during catalytic hydrogenation] to be identified. The amount of each fatty acid present in the margarine was also estimated from the relative intensities of the corresponding signals. "Kaliakra" margarine consisted of a blend of unhydrogenated natural fats and oils that contained saturated fatty acids, oleate, and linoleate. There were no signs in the spectrum of "Kaliakra" of any (*E*)-isomers, nor signals associated with positional unsaturated acyl groups (other than oleate and linoleate). The sample of "Corona" margarine consisted of a mixture of hydrogenated and unhydrogenated vegetable oils and butter (1.3%). The presence of butter in this sample was identified by the characteristic carbon shifts of the C-1 to C-4 carbon atoms of butyrate. The distribution of the fatty acids on the glycerol "backbone" also was estimated by this technique.
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The triacylglycerol molecules in margarines contain a large amount of monounsaturated fatty acids, which have been derived from polyunsaturated vegetable oils by partial catalytic hydrogenation. During the hydrogenation process, double-bond migration and *cis/trans*-isomerization also occur. The fatty acid composition of margarines is a complex mixture of saturated, positional isomers of (*Z*)- and (*E*)-monounsaturated and some polyunsaturated fatty acids (1,2). The detection and quantitation of the positional isomers of fatty acids contained in such products remain difficult by chromatographic means (3–5). Gunstone (6,7) has successfully applied the technique of high-resolution ^{13}C nuclear magnetic resonance (NMR) spectroscopy to the analysis of hydrogenated fats and other fat products. From the results of the carbon chemical shifts of various critical carbon atoms in the triacylglycerol molecules, the position and configuration of the double bonds have been determined, but not their distribution on the glycerol "backbone" (6,7). We have recently described the application of ^{13}C NMR spectroscopy for determination of the triacylglycerol structure of whole oils from seeds (8). Much of this application relies on the extensive ^{13}C NMR shift data collected from over 70 synthetic triacylglycerol molecules (9–11). From the carbon shift results, we have demonstrated the ability not only to identify the position and configuration of the unsaturated centers, but also the distribution pattern of the various acyl chains on the glycerol "backbone." In this paper, we apply the same technique to margarine samples to study the composition of the acyl moieties present in the triacylglycerols.

EXPERIMENTAL PROCEDURES

"Flora" margarine (product of Holland) was purchased locally (Hong Kong) from food stores. "Kaliakra" margarine (product of Bulgaria) and "Corona" margarine (another product of Holland) were purchased from food stores in Sofia, Bulgaria. The triacylglycerol fraction was isolated from the margarine samples for ^{13}C NMR analysis by mixing margarine (1.0 g) with petroleum ether (b.p. 40–60°C) (20 mL). The organic extract was washed with water and dried over anhydrous sodium sulfate. The organic solution was passed through a silica gel (15 g) column and eluted with a mixture of petroleum ether (b.p. 40–60°C)/diethyl ether (95:5, vol/vol, 50 mL). The eluate was evaporated under reduced pressure to give a clear oil (.74 g). The triacylglycerols were interesterified by heating the triacylglycerols (50 mg) in a mixture of methanol/benzene/sulfuric acid (84:10:4, vol/vol/vol, 6 mL) at 80°C for 2 h. Water (15 mL) was added, and the reaction mixture was extracted with diethyl ether (3 × 10 mL). The extract was dried over Na_2SO_4 and filtered. The filtrate was evaporated under a stream of nitrogen to give methyl esters in quantitative yield. The stereospecific hydrolysis reaction of triacylglycerols from "Flora" margarine with pancreatic lipase was performed according to the method described by Luddy *et al.* (12). Gas–liquid chromatographic (GLC) analysis of the methyl esters was carried out on a Pye

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Unicam (Pye Unicam Ltd., Cambridge, United Kingdom) Series 304 chromatograph, fitted with a glass capillary (30 m × 0.2 mm i.d.), coated with Silar 10C stationary phase, and a flame-ionization detector. The column temperature was increased from 165 to 220°C at a rate of 20°C/min. ^{13}C NMR spectra were obtained by dissolving 0.3 g of the oil in deuterated chloroform on a JEOL GSX-270 spectrometer (270 MHz) (JEOL, Tokyo, Japan) as described elsewhere (9).

RESULTS AND DISCUSSION

By referring to the carbon shift values established for the saturated and unsaturated triacylglycerols reported elsewhere (9–11), the various signals in the spectrum can be readily assigned. The assignments of the signals are achieved by taking into account the (i) observed chemical shifts; (ii) shift differences between similarly positioned carbon atoms in the α- and β-acyl chains; (iii) differences of the shifts of the ethylenic carbon atoms in the same acyl chain; and (iv) the relative intensities of the signals. For convenience, the following abbreviations to describe the acyl groups are used: Sat for saturated acyl (16:0, 18:0) groups, O for oleate, L for linoleate, Ln for linolenate, and (*Z*) or (*E*) for unsaturated acyl groups containing a *cis*- or *trans*-ethylenic system, respectively. The results of the GLC analyses of the methyl esters of the three margarine samples are presented in Table 1.

"Flora" margarine. "Flora" margarine is most likely prepared from hydrogenated (hard stock) and unhydrogenated vegetable oils. During the hydrogenation of the vegetable oil, acyl groups containing 18:1 are converted to 18:0, while acyl groups containing 18:2(9Z,12Z) are either totally hydrogenated to give 18:0 or partially to give ideally 18:1(9Z) and 18:1(12Z) isomers. However, during catalytic hydrogenation, there is the possibility of (*Z*)/(*E*)-isomerization of the ethylenic system and double-bond migration along the chain. The hydrogenated vegetable oil is normally void of polyunsaturated acyl chains. Small amounts of conjugated diene fatty ester may be found at times. By blending unhydrogenated vegetable oils to the hard stock, the resulting margarine consists of a complex triacylglycerol mixture, with acyl groups consisting of saturated and a range of 18:1 positional and geometric fatty acid isomers in addition to O, L, and Ln groups. Thus, the fatty acid composition of margarines is rather complex. The ^{13}C NMR spectrum of the triacylglycerols of "Flora" margarine shows, without surprise, over 70 signals of varying intensities. The assignments of the various signals are presented in Tables 2 and 3.

TABLE 1
Fatty Acid Composition of Margarines by Gas–Liquid Chromatographic Analysis

Fatty acid	Margarine samples (%)		
	"Flora"	"Kaliakra"	"Corona"
14:0	0.5	1.3	5.4
16:0	22.2	17.7	22.9
16:1	1.6	—	5.5
18:0	3.9	8.8	9.0
18:1	22.5	22.5	35.3
18:2	44.8	49.7	20.5
18:3	4.5	—	1.4

The C-1 carbon shift region. There are three signals in this region, viz., 173.236, 173.194, and 172.792 ppm (Table 2). The last two signals can be paired because the difference of these shift values is 0.402 ppm, which is characteristic of the difference value found for the similarly positioned C-1 carbon atoms in the α- and β-acyl position of unsaturated triacylglycerols of type AAA (9). The remaining signal at 173.236 ppm corresponds to the shift of the C-1 carbon atom for Sat in the α-acyl positions. Because there is no signal corresponding to the shift of the C-1 carbon signal for the β-acyl chain in this region, we concluded that the saturated acyl groups are predominantly located in the α-acyl position of the glycerol "backbone." ^{13}C NMR spectroscopy cannot determine the chainlength of the acyl groups.

The C-2, C-3, ω3, ω2, and ω1 carbon shift regions. Three signals appear at the C-2 carbon shift region (*ca.* 34 ppm). The signal at 34.070 ppm is characteristic of the shift of the C-2 of Sat in the α-acyl position. This shift result confirms the saturated acyl groups (Sat) in the α-acyl position of the triacylglycerol. The remaining two signals at 34.040/34.208 (with a shift difference of 0.168 ppm) can be paired and assigned to the shifts of the C-2 carbon atoms of the unsaturated acyl groups in the α- and β-acyl position, respectively.

TABLE 2
Chemical Shift (ppm) of C-1, C-2, C-3, allylic, ω3, ω2, and ω1 Carbon Signals of "Flora" Margarine[a]

Shift region	Shift (α/β)	Intensity	Assignment
C-1	173.236 (α)	21.735	Sat
	173.194/172.792	29.894/21.721	(*E*), O, L, and Ln
C-2	34.070 (α)	30.546	Sat
	34.040/34.208	44.666/28.096	(*E*), O, L, and Ln
C-3	24.910	50.940	O, L, Ln (β), and Sat (α)
	24.876	44.021	O, L, and Ln (α)
Allylic	32.665	22.591	(*E*)
	27.263	42.045	C-11 of O
	27.240	100.000	C-14 of L and Ln
	27.229	80.732	C-8 of L and Ln
	27.210	36.817	C-8 of O
	25.666	77.019	C-11 of L, Ln and C-14 of Ln
	20.494	12.324	C-17 of Ln
ω3	31.985	44.679	Sat
	31.966	50.561	(*E*), O
	31.576	85.866	L
	31.939	10.829	Others
ω2	22.744	58.387	Sat
	22.736	56.368	(*E*), O
	22.626	79.266	L
	22.706	10.685	Others
ω1	14.344	13.326	Ln
	14.145	77.049	(*E*), O, Sat
	14.103	67.774	L

[a]O, oleate; L, linoleate; Ln, linolenate; Sat, saturated acyl (16:0, 18:0) groups; (*Z*) or (*E*) for unsaturated acyl groups containing a *cis*- or *trans*-ethylenic system, respectively.

TABLE 3
Chemical Shift (ppm) of the Ethylenic Carbon Atoms in the Spectrum of "Flora" Margarine

Observed chemical shifts	Shift difference		Intensity	Assignment	
	Calc.	Ref.		Acyl chain	Carbon no.
127.143/127.136	0.007	0.006[a]	5.535/3.847	Ln	C-15
127.780/127.794	0.014	0.019[a]	4.239/2.545		C-10
128.243/128.231	0.012	0.013[a]	5.174/3.277		C-12
128.289/128.297	0.008	0.009[a]	5.053/3.220		C-13
130.163	—	—	10.352		C-9
131.921	—	—	5.442		C-16
127.927/127.916	0.011	0.012[a]	99.416/75.730	L	C-12
128.093/128.111	0.018	0.019[a]	81.709/53.058		C-10
129.980/129.955	0.025	0.026[a]	77.141/59.406		C-9
130.185/130.192	0.007	0.008[a]	100.000/65.239		C-13
129.709/129.684	0.025	0.026[b]	37.778/19.579	O	C-9
130.006/130.021	0.015	0.015[b]	41.223/18.516		C-10
129.581	—	—	2.203	8(*Z*)	C-8
129.784/129.762	0.022	0.023[b]	2.923/2.743	10(*Z*)	C-10
129.829/129.810	0.019	0.014[b]	4.520/2.587	11(*Z*)	C-11
129.857	—	—	3.573	7(*E*)	C-7
129.891	—	—	2.267	12(*Z*)	C-12
129.924	—	—	6.315	11(*Z*)	C-12
130.060	—	—	5.136	8(*E*)	C-8
130.163	—	—	10.352	9(*E*)	C-9
130.259/130.239	0.020	0.020[b]	6.514/4.639	10(*E*)	C-10
130.305/130.289	0.016	0.016[b]	5.050/3.660	11(*E*)	C-11
130.314/130.327	0.013	0.012[b]	2.588/2.306	12(*E*)	C-12
130.381/130.388	0.007	0.006[b]	3.183/3.020	12(*E*)	C-13
130.401/130.409	0.008	0.007[b]	5.032/3.845	11(*E*)	C-12
130.434/130.445	0.011	0.009[b]	6.860/4.620	10(*E*)	C-11
130.489/130.502	0.013	0.015[b]	8.031/5.471	9(*E*)	C-10
130.586/130.599	0.013	0.016[b]	5.545/3.073	8(*E*)	C-9
130.742	—	—	2.624	7(*E*)	C-8

[a]Reference 11.
[b]Reference 10. See Table 2 for abbreviations.

There are only two signals in the C-3 carbon shift region. No further useful information can be drawn from this region. The signal at 24.910 ppm arises from the shifts of the C-3 of the unsaturated acyl groups in the β-acyl position plus that of the C-3 of the saturated acyl chain in the α-positions. The remaining signal at 24.876 ppm is due to the shifts of the C-3 of unsaturated acyl group in the α-acyl position. The carbon chemical shifts of the ω1 (methyl), ω2, and ω3 carbon atoms appear as individual signals and are not resolved into pairs. From the results obtained for a mixed triacylglycerol [SLO, 18:0/18:1(9Z)/18:2(9Z,12Z)], triacylglycerol molecules are described by using either a combination of letters (e.g., LLL for trilinolein, or SLO for glycerol 1-stearate-2-linoleate-3-oleate) or by using the abbreviated structure of the fatty acid components—i.e., $[18:1(9Z)]_3$, for triolein or [18:0/18:2(9Z, 12Z)/18:1(9Z)] for glycerol 1-stearate-2-linoleate-3-oleate], we have noted the trend of the shifts of the ω3 carbon nuclei in the following deshielding order: Sat (31.976 ppm) > O (31.954 ppm) > L (31.567 ppm) (10). This trend is also true for the carbon shifts of the ω2 carbon atoms of Sat, O, and L acyl groups. Furthermore, from the established shift data for the (*Z*)- and (*E*)-ethylenic triacylglycerols, we also have found that the shifts of the ω3 carbon atoms of O (at 31.956 ppm) and that of 18:1(9*E*) (at 31.937 ppm) are too close to allow the signals to be resolved. Thus, in assigning the signals in the ω3 carbon shift region, the signal at 31.985 ppm is due to the shift of the ω3 of Sat, while the signal at 31.966 ppm is due to the shift of ω3 of O and the (*Z*)-isomers. The remaining ω3 signal at 31.576 ppm is characteristic of the shift of ω3 of L. There is a weak signal at 31.939 ppm, which is due to the shifts of the ω3 carbon atoms of (8*E*)- and (9*E*)-ethylenic fatty acid isomers. The assignments of the shifts of the ω2 and ω1 carbon atoms are similarly achieved by referring to established shift values for these carbon atoms (10) (Table 2).

The allylic carbon shift region. In the spectrum of "Flora" margarine, the signal at 32.665 ppm confirms the presence of (*E*)-ethylenic bonds. There are six signals in the allylic carbon region (*ca.* 27 ppm) for the (*Z*)-ethylenic isomers (O, L, and Ln). The signals at 27.263 and 27.210 ppm are due to the shifts of C-11 and C-8 of O, respectively. The signals at 27.240 and 27.229 ppm are due to the shifts of the C-14 and C-8 carbon atoms, respectively, of L and Ln. The presence of L and Ln is confirmed by the signal at 25.666 ppm, which arises from the shifts of the C-11 of L and Ln, and from the C-14 of Ln. The remaining signal at 20.494 ppm is characteristic of the shift of C-17 of Ln.

Ethylenic carbon shift region. The ethylenic carbon shift

region is the most informative part of the ^{13}C NMR spectrum, which allows the positions of the ethylenic bonds to be determined and also permits an insight into the distribution of the various acyl chains on the glycerol "backbone." Pairs of signals are normally observed for most ethylenic carbon atoms, which result from the slight differences in shift values of similarly positioned ethylenic carbon atoms in the α- and β-acyl groups. The GLC results (Table 1) of the fatty acid composition of "Flora" margarine shows high levels of O and L, but a low level of Ln. The acyl groups are readily identified by referring to the carbon shift values established for the triacylglycerols OOO, LLL, and LnLnLn (9,10). The signals arising from the shifts of the ethylenic carbon atoms for O, L, and Ln in the margarine sample are readily identified and are presented in Table 3.

There are eight pairs and three single signals of low intensities around the region of 130 ppm that need special attention. These signals are characteristic of the shifts of the (*E*)-ethylenic carbon atoms. Referring to the carbon shift data established for the (*E*)-ethylenic triacylglycerols of type AAA (10), these signals are readily assigned by considering (i) the actual shift values; (ii) the chemical shift difference between similarly positioned carbon atoms in the α- and β-acyl chains; and (iii) the shift difference between the two ethylenic carbon atoms in the same acyl chain. By this means, we are able to identify the presence of the (*E*)-ethylenic bond at the Δ^8–Δ^{12} position of the alkyl chain. For example, the pair of signals at 130.586/130.599 ppm (shift difference of 0.013 ppm) is due to the shift of the C-9 carbon atoms in the α- and β-acyl chain of (8*E*)-acyl groups, because the shift difference matches closely with the shift difference value (0.016 ppm) obtained for the C-9 ethylenic carbon atoms of the triacylglycerol $[17:1(8E)]_3$ (10). The corresponding pair of signals for the C-8 carbon atoms is not obvious because this signal for the shift of the C-8 carbon atom of the (8*E*)-isomer in the β-acyl position coalesces with the intense signals of the shifts of the C-10 carbon atom of O in the α/β-acyl positions, which appear at 130.006/130.021 ppm. As a result, only the signal due to the shift of the C-8 carbon atom of the (8*E*)-isomer in the α-acyl chain is noticeable, as it appears at 130.060 ppm. The position of the ethylenic bond in this positional isomer is confirmed from the shift difference of the shifts of the two ethylenic carbon atoms (C-8 and C-9) in the α-acyl chains (130.060 and 130.586 = 0.526 ppm). This value agrees with that (0.527 ppm) obtained for the difference of the shifts of the C-8 and C-9 carbon atoms of $[17:1(8E)]_3$. The remaining (*E*)-ethylenic isomers likewise have been identified, and the assignments are given in Table 3.

The single signals at 130.060 and 130.163 ppm match the shifts of the C-8 and C-9 carbon atoms of the (8*E*)- and (9*E*)-isomers, respectively. The single signals at 129.857 and 130.742 ppm (shift difference of 0.885 ppm) are characteristic of the shifts of the C-7 and C-8 ethylenic carbon atoms, respectively, of a (7*E*)-isomer in the α-acyl position. This claim is supported by the shifts observed for the ethylenic carbon atoms of $[16:1(7E)]_3$. In the ^{13}C NMR spectrum of the latter triacylglycerol, the shifts of the ethylenic carbon atoms in the α-acyl chain appear at 129.864 (C-7) and 130.745 (C-8) ppm with a shift difference value of 0.881 ppm. Signals arising from the shifts of the ethylenic carbon atoms 18:1(8*Z*), 18:1(10*Z*), and 18:1(11*Z*) also have been identified from the signals in the spectrum (Table 3).

Fatty acid content and distribution. The relative amount (in percentage) of Sat can be estimated from the intensity of the corresponding C-1 carbon signal in the ^{13}C NMR spectrum. The percentage of the Sat in the "Flora" sample is 29.6%. From the intensities of the ethylenic carbon signals, the relative amounts of the (7*E*)- to (12*E*)-positional isomers are in the ratio of 1:3:5:4:3:2, which indicates that the 18:1(9*E*) isomer is the most abundant (*E*)- isomer present. The amount of all (*E*)-isomers relative to the total amount of unsaturated fatty acids is 10.6%, which represents 7.4% of all fatty acids in the margarine.

We conclude from this part of the study that the various fatty acids in the triacylglycerols of the "Flora" margarine can be identified and semiquantified. The results show that the saturated acyl chains are predominantly located in the α-position of the glycerol "backbone," while the unsaturated acyl chains are distributed between the α- and β-acyl positions. To support our observation about the distribution of the various types of acyl groups on the glycerol "backbone," we have carried out a stereospecific hydrolysis with pancreatic lipase. The isolated 2-*sn*-monoglyceride fraction shows the composition of the main fatty acids as: 16:0 (1.63%), 18:0 (1.49%), 18:1 (35.92%), 18:2(48.24%), 18:3 (3.0%), and several unidentified components (9.72%). It is apparent from these results that the amount of saturated fatty acid in the 2-position of the glycerol "backbone" is small. However, their presence cannot be identified by ^{13}C NMR analysis.

"Kaliakra" and "Corona" margarine. "Kaliakra" margarine is manufactured in Bulgaria, while "Corona" is produced in Holland. The results of the gas chromatographic analysis of the methyl esters of the isolated fat of these two samples are given in Table 1. The results of the ^{13}C NMR spectroscopic analysis of the triacylglycerol fractions of "Kaliakra" and "Corona" margarine are presented in Tables 4 and 5, respectively.

The C-1 carbon shift region. The spectrum of "Kaliakra" margarine shows three readily identifiable pairs of signals in the C-1 carbon shift region. The first pair of signals at 173.232/172.829 is due to the shifts of C-1 of Sat in the α- and β-acyl chains. The second pair of signals at 173.199/172.797 is due to the shift of C-1 of O in the α- and β-acyl chains, while the third pair of signals at 173.191/172.787 ppm is due to the shifts of the C-1 carbon atoms of L in the α- and β-acyl chains. In the spectrum of "Corona" margarine, three similar pairs of signals have been identified. However, we also note an additional pair of signals of low intensity at 173.123/172.728 ppm (with a shift difference of 0.395 ppm). This pair of signals is characteristic of the chemical shifts of the C-1 carbon atoms of short-chain butanoic acid, which indicates the presence of butter fat in the "Corona" margarine.

TABLE 4
Chemical Shift (ppm) of C-1, C-2, C-3, allylic, ω3, ω2, and ω1 Carbon Signals of "Kaliakra" and "Corona" Margarines[a]

Shift region	Acyl chains	"Kaliakra" shift (α/β)	Intensity	"Corona" shift (α/β)	Intensity
C-1	Sat	173.232/172.829	32.606/12.058	173.221/172.818	50.001/39.292
	O and *E*	173.199/172.797	23.982/13.701	173.198/172.787	40.321/20.780
	L, Ln	173.191/172.787	44.806/30.858	173.190/172.779	36.583/23.526
	4:0	—	—	173.123/172.728	4.731/3.243
C-2	Sat	34.069/34.237	27.764/9.040	34.062/34.229	29.066/11.622
	O, L, Ln and *E*	34.038/34.206	39.656/22.790	34.043/34.206	17.620/10.316
	4:0	—	—	36.105	2.623
C-3	Sat (β)	24.947	10.683	24.939	13.533
	L, Ln O and *E* (β) and Sat (α)	24.909	46.658	24.899	38.632
	L, Ln O and *E* (α)	24.875	43.360	24.852	6.607
	4:0	—	—	18.374	1.792
Allylic	*E*	—	—	32.652, 32.541, 32.323	28.140, 3.794, 4.040
	C-11 of O	27.258	37.340	27.254	20.318
	C-14 of L, Ln	27.239	100.000	27.235	36.427
	C-8, of L, Ln	27.224	74.087	27.220	23.176
	C-8 of O	27.205	34.396	27.201	14.579
	C-11 of L, Ln; C-14 of Ln	25.665	77.441	25.658	24.612
	C-17 of Ln	20.489	8.105	20.486	3.481
ω3	Sat	31.979	45.298	31.976	65.634
	O and *E*	31.960	42.165	31.953	28.963
	L	31.571	84.814	31.567	30.062
	other	31.643	5.548	31.926, 31.879, 31.834	10.318, 3.446, 5.583
ω2	Sat	22.739	55.121	22.734	80.268
	O and *E*	22.732	49.108	22.732	39.955
	L	22.621	85.517	22.617	13.525
	other	22.640	24.895	22.693, 22.636	10.927, 8.642
ω1	Ln	14.340	8.676	14.340	3.855
	O, Sat and *E*	14.141	68.174	14.137	82.932
	L	14.099	69.906	14.095	22.520
	4:0	—	—	13.667	1.566

[a]See Table 2 for abbreviations.

The C-2, C-3, allylic, ω3 to ω1 carbon shift regions. The spectrum of "Kaliakra" margarine shows no signals in the shift region of 32.6 ppm, which rules out the presence of (*E*)-isomers. This infers that "Kaliakra" margarine is not derived from partially hydrogenated polyunsaturated oils, unless a special catalyst has been employed that causes no isomerization whatsoever. This is unlikely, because such specialized catalysts are highly priced and not cost-effective for industrial processes. From these results it can be concluded that "Kaliakra" margarine is likely a blend of natural saturated and unsaturated vegetable oils, e.g., palm oil mixed with soybean or rapeseed oil in view of the fact that significant quantities of Ln are detected in the margarine.

The spectrum of "Corona" margarine shows three low-intensity signals at 36.105, 18.374, and 13.667 ppm, which are characteristic signals arising from the C-2, C-3, and C-4 of butyrate. The presence of a C-4 acyl chain in this sample of margarine is therefore reconfirmed (Table 4).

The ethylenic carbon shift region. The ethylenic region of the spectrum of the "Kaliakra" sample does not show any signals associated with the ethylenic carbon atoms of an (*E*)-unsaturated system. Only signals associated with O and L are found (Table 5). These results support our earlier conclusion that the "Kaliakra" margarine is not derived from partially hydrogenated fats or oils. In the ethylenic region of the spectrum, signals associated with the shifts of the ethylenic carbon atoms of O and L are readily identified. Low-intensity signals arising from the shifts of the ethylenic carbon atoms of (*Z*)- and (*E*)-unsaturated acyl chains show the presence of (8*Z*)- to (12*Z*)-isomers and (7*E*)- to (12*E*)-isomers (Table 5).

Fatty acid content and distribution. From the intensities of the signals of the C-1 carbon atoms, the total amount of Sat in "Kaliakra" margarine is estimated as 27.3%, which agrees closely with the result obtained by GLC (27.8%). For "Corona" margarine, the estimated total percentage of Sat is 40.9%, which is slightly higher than that obtained by GLC analysis (37.3%), which does not include the amount of butanoic acid in the sample. From the intensities of the signals at 173.123/172.728 ppm (C-1 of tributyrin), the estimated amount of C-4 acid present in the sample is 1.3% molar equivalent (supported by experiments on tributyrin-spiked samples of margarine fat, assuming the average molecular weight for triacylglycerols containing saturated and unsaturated long-chain fatty acids as 884 and that of tributyrin as 302). The total amount of (*E*)-isomers estimated in "Corona" margarine is 26.1% [derived from the signal intensity of the allylic carbon atoms (22.7%) and that of the unsaturated carbon atoms of the (*E*)-isomers (29.5%)].

TABLE 5
Chemical Shift (ppm) of the Ethylenic Carbon Atoms of "Kaliakra" and "Corona" Margarines[a]

Acyl chains	Carbon number	"Kaliakra" Chemical shift	"Kaliakra" Difference Calc.	"Kaliakra" Difference Refs. (4,6)	"Kaliakra" Intensity	"Corona" Chemical shift	"Corona" Difference Calc.	"Corona" Difference Refs. (4,6)	"Corona" Intensity
Ln	C-15	—	—	—	—	127.129/127.123	0.006	0.006	6.617/3.617
	C-10	—	—	—	—	127.767	—	—	4.090
	C-12	—	—	—	—	128.229/128.216	0.013	0.013	6.829/4.292
	C-13	—	—	—	—	128.274/128.283	0.009	0.009	6.254/4.357
	C-9	—	—	—	—	130.148	—	—	14.074
	C-16	—	—	—	—	131.906	0.000	0.000	7.187
L	C-12	127.924/127.913	0.011	0.012	95.250/69.289	127.914/127.902	0.012	0.012	60.942/50.877
	C-10	128.090/128.108	0.018	0.019	76.301/45.134	128.079/128.097	0.018	0.019	49.705/35.547
	C-9	129.978/129.953	0.025	0.026	83.065/56.040	129.966/129.940	0.026	0.026	54.336/50.880
	C-13	130.182/130.190	0.008	0.008	100.00/62.130	130.170/130.177	0.007	0.008	84.858/61.233
O	C-9	129.706/129.681	0.025	0.026	34.771/18.263	129.695/129.669	0.026	0.026	42.369/22.427
	C-10	130.004/130.189	0.015	0.015	34.655/15.035	129.991/130.004	0.013	0.015	43.470/22.074
8(*Z*)	C-8	—	—	—	—	129.569	—	—	5.196
10(*Z*)	C-10	—	—	—	—	129.769/129.754	0.015	—	7.391/4.460
11(*Z*)	C-11	—	—	—	—	129.813	—	—	11.407
7(*E*)	C-7	—	—	—	—	129.844/129.824	0.020	0.021	10.041/8.234
12(*Z*)	C-12	—	—	—	—	—	—	—	—
12(*Z*)	C-11	—	—	—	—	—	—	—	—
11(*Z*)	C-10	—	—	—	—	129.908	—	—	12.441
8 (*E*)	C-8	—	—	—	—	130.045/130.019	0.026	0.026	14.852/9.154
9(*E*)	C-9	—	—	—	—	130.148	—	—	14.074
8(*E*)	C-9	—	—	—	—	130.076/130.092	0.016	0.020	8.178/5.526
10(*E*)	C-10	—	—	—	—	130.243/130.223	0.020	0.020	22.082/12.404
11(*E*)	C-11	—	—	—	—	130.288/130.273	0.015	0.016	18.018/10.505
12(*E*)	C-12	—	—	—	—	130.312/130.301	0.011	0.012	13.016/10.480
12(*E*)	C-13	—	—	—	—	130.366	—	—	12.547
11(*E*)	C-12	—	—	—	—	130.385/130.393	0.008	0.007	16.091/10.575
10(*E*)	C-11	—	—	—	—	130.419/130.430	0.011	0.009	22.560/12.798
9(*E*)	C-10	—	—	—	—	130.472/130.485	0.013	0.015	25.806/12.524
8(*E*)	C-9	—	—	—	—	130.472/130.485	0.013	0.015	25.806/12.524
7(*E*)	C-8	—	—	—	—	130.726	—	—	6.957

[a]See Table 2 for abbreviations.

It can be concluded from this study that ^{13}C NMR spectroscopic analyses of margarines provide detailed information regarding the composition and nature of the acyl chains present in the triacylglycerols. This technique identifies the presence of short-chain fatty acids (e.g., C-4) as in "Corona" margarine, which can be missed during GLC analysis due to the volatility of methyl butanoate. ^{13}C NMR also provides details of the distribution of the various acyl chains and permits a semiquantitation of the amount of fatty acids present in the mixture of triacylglycerols.

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