■ Analysis of Corn Margarine

Fig. 7 and Fig. 8 show the results of analysis of actual margarine samples that were subjected to methyl esterification, in which analyses were conducted using the Rtx-2560 and BPX-90 columns, respectively. With either column, improved separation between the cis and trans isomers of C18:1 isomers was seen when the column temperature was raised. Fig. 9 shows an overlay graph of the column temperature dependency of the ECLs of corn margarine with those of the graphed standard samples shown for each peak in Fig. 4. The approximate slopes of the linear plots revealed closely matching behaviors between the respective ECLs of the peaks generated from the standard sample and the actual sample, and it was possible to distinguish the cis isomers from the trans isomers by examining the approximate slopes of the linear plots of the peaks of the actual sample.

Since commercially available standard samples containing fatty acid trans isomers are limited in composition, peak identification by GC is extremely difficult. In the analysis using the BPX-90 heated to temperatures equal to or greater than 180 ˚C, the peak following C18:1 trans-13 (labeled peak X) is separated from the C18:1 cis-9 peak. This unidentified peak can easily be assumed to be a trans isomer from the behavior of the ECLs.

This investigation demonstrated that cis- and trans-isomers could easily be distinguished, even without the use of specific analytical standards, by comparing the temperature dependencies of the ECL's.

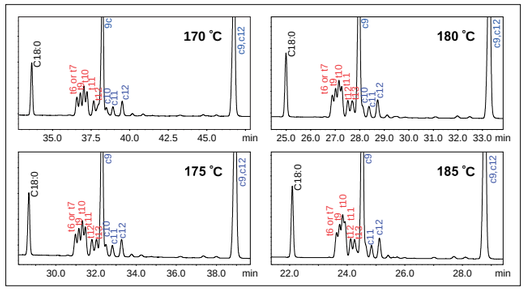

Fig. 7 Chromatograms of Corn Margarine Obtained Using Rtx-2560 (100 m)

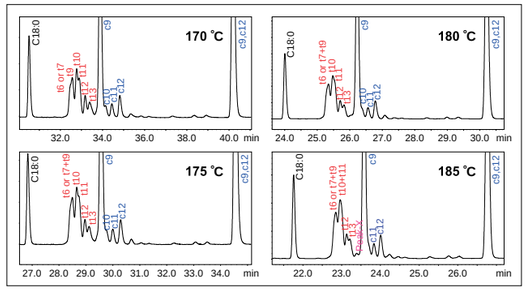

Fig. 8 Chromatograms of Corn Margarine Obtained Using BPX-90 (160 m)

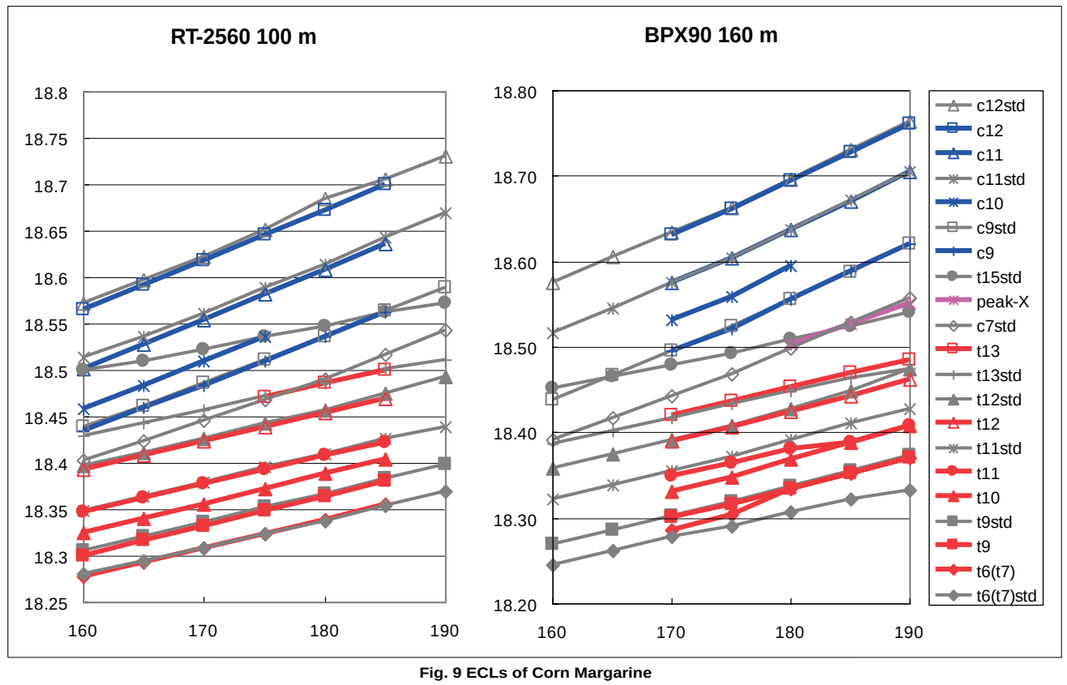

Fig. 9 ECLs of Corn Margarine

NOTES:
*This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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Application News

Gas Chromatography No.G263A

Chromatography of FAMEs Using Cyanopropyl Capillary Columns

Vegetable oils typically contain many unsaturated fatty acids, which have relatively low melting points, and are liquids at ambient temperature. When products like margarine and shortening are manufactured, hydrogen is added to the unsaturated fatty acids, converting them to the corresponding saturated fatty acids. This process results in products that are solids at ambient temperature. However, some of the unsaturated fatty acid isomers are not completely saturated in this process, and residual trans-unsaturated fats (triglycerides of trans-unsaturated fatty acids) remain in the hydrogenated product; these trans-unsaturated fats are associated with adverse health effects. For this reason, analysis of fatty acid isomer compositions has become important. Separation of the isomers of fatty acid methyl esters (FAMEs) by gas chromatography, and specifically the cis- and trans-isomers, is conducted by gas chromatography using strongly polar cyanopropyl capillary columns. Here we present our investigation of the chromatography of C18 fatty acid isomers using two types of columns, the BPX-90 and Rtx-2560. The structures of these two liquid phases are shown in Fig. 1.

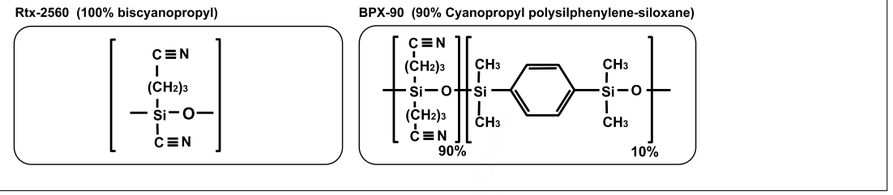


Fig. 1 Structure of Liquid Phases

■ Separation of C18 Isomers

In the separation of the isomers of C-18 FAMEs (fatty acid methyl esters with 18 carbons) using the 90 % cyanopropyl polysilphenylene-siloxane (BPX-90) and 100 % biscyanopropyl (Rtx-2560) columns, there was not a great discrepancy seen in the separation of the C18 single-double bond (C18 : 1) and two-double bond (C18 : 2) isomers, but the separation was quite different among the three-double bond (C18 : 3) isomers. Chromatograms showing the separation of C-18 unsaturated fatty acids on the two phases are shown in Figs. 2 and 3. The analytical conditions are summarized in Table 1 (note that the analyses are conducted isothermally). Table 2 indicates the C18 isomer abbreviations used in the chromatograms.

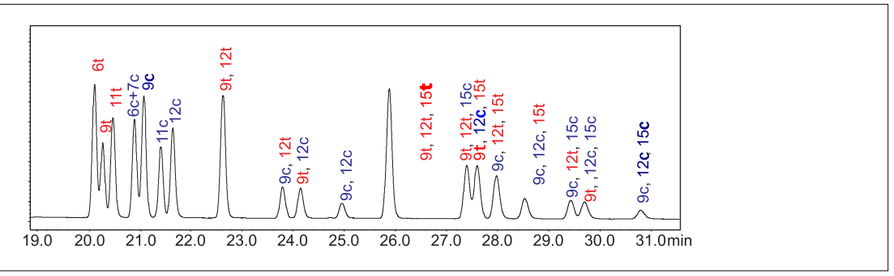


Fig. 2 Chromatogram Obtained Using BPX-90 (100 m)

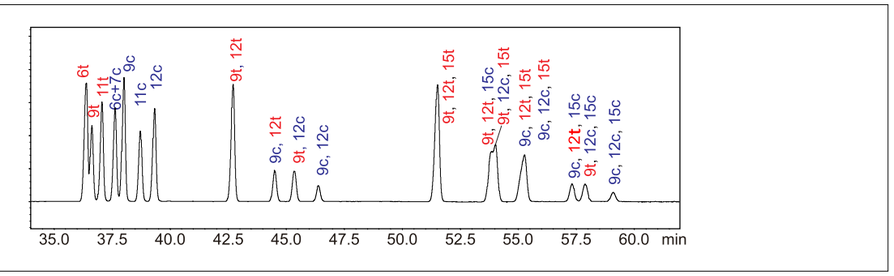


Fig. 3 Chromatogram Obtained Using Rtx-2560 (100 m)

No.G263A

Table 1 Analytical Conditions

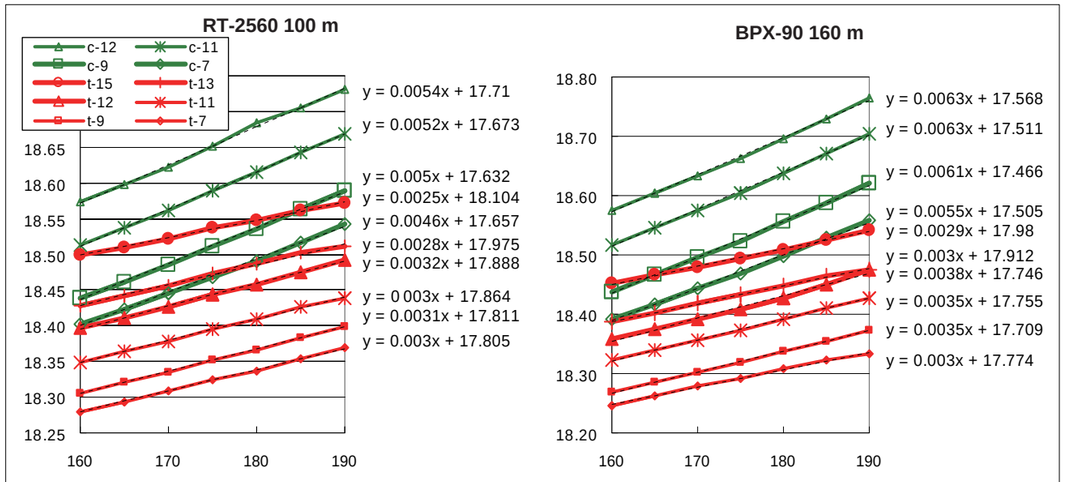
Instrument	: GC-2010
Column	: BPX-90 100 m or 160 m × 0.25 mm I.D. df = 0.25 µm RT-2560 100 m × 0.25 mm I.D. df = 0.20 µm
Column Temp.	: 170 ˚C
Carrier Gas	: He 20 cm/sec
Injection Port	: 280 ˚C
Injection Method	: Split
Split Ratio	: 1:30
Detector	: 280 ˚C FID-2010
H_2	: 40 mL/min
Air	: 400 mL/min

Table 2 Abbreviations Used for C18 Unsaturated Fatty Acid Isomers

C18 : 1	
6t	trans-6-Octadecenoic (Petroselaidic) (C18:1, trans-6)
7t	trans-7-Octadecenoic (C18:1, trans-7)
9t	trans-9-Octadecenoic (Elaidic) (C18:1, trans-9)
11t	trans-11-Octadecenoic (trans-Vaccenic) (C18:1, trans-11)
12t	trans-12-Octadecenoic (C18:1, trans-12)
13t	trans-13-Octadecenoic (C18:1, trans-13)
15t	trans-15-Octadecenoic (C18:1, trans-15)
6c	cis-6-Octadecenoic (Petroselinic) (C18:1, cis-6)
7c	cis-7-Octadecenoic (C18:1, cis-7)
9c	cis-9-Octadecenoic (Oleic) (C18:1, cis-9)
11c	cis-11-Octadecenoic (cis-Vaccenic) (C18:1, cis-11)
12c	cis-12-Octadecenoic (C18:1, cis-12)
C18 : 2	
9t,12t	trans-9,trans-12-Octadecadienoic
9c,12t	cis-9,trans-12-Octadecadienoic
9t,12c	trans-9,cis-12-Octadecadienoic
9c,12c	cis-9,cis-12-Octadecadienoic
C18 : 3	
9t,12t,15t	trans-9,trans-12,trans-15-Octadecatrienoic
9t,12t,15c	trans-9,trans-12,cis-15-Octadecatrienoic
9t,12c,15t	trans-9,cis-12,trans-15-Octadecatrienoic
9c,12t,15t	cis-9,trans 12 trans 15 Octadecatrienoic
9c,12c,15t	cis-9,cis-12,trans-15-Octadecatrienoic
9c,12t,15c	cis-9,trans-12,cis-15-Octadecatrienoic
9t,12c,15c	trans-9,cis-12,cis-15-Octadecatrienoic
9c,12c,15c	cis-9,cis-12,cis-15-Octadecatrienoic

■ Cis and Trans Isomer Differentiation According to Equivalent Chain Length (ECL)

The equivalent chain length (equivalent chain length relative to saturated, straight chain fatty acid methyl esters, ECLs) is based on the relative retention time among saturated fatty acids. The retention time of a saturated fatty acid with a carbon number N is assumed to be N (i.e., the elution time of C18 : 0 is taken as 18.0), thereby indicating its relative elution position. Here, the column temperature dependencies were compared by plotting changes in ECLs when the column temperature was changed. Fig. 4 shows the column temperature dependency of ECLs (relative carbon chain length) of C18:1 isomers. The approximate slopes of the linear plots with the Rtx-2560 column were 0.0046 – 0.0054 for the cis isomers, and 0.0025 – 0.0033 for the trans isomers, while with the BPX-90 column, they were 0.0055 - 0.0063 for the cis isomers and 0.0029 – 0.0038 for the trans isomers. It is clear that both columns show smaller slopes for the trans than the cis isomers. The cis isomers and trans isomers of fatty acids can therefore be distinguished by examining the temperature dependency of ECLs using the analysis results obtained at different column temperatures.



(Note) To facilitate comparison of the elution times of both columns, analysis using the BPX-90 was conducted by connecting two lengths of 100 m and 60 m.

Fig. 4 ECLs of C18 : 1 Isomers

Fig. 5 shows the column temperature dependency of ECLs of C18:2 isomers. The approximate slopes of the linear plots with the Rtx-2560 column were 0.0108 for the cis, cis isomer, 0.0083 and 0.0085 for the cis, trans isomer, and 0.0056 for the trans, trans isomer, while with the BPX-90 column, they were 0.0136 for the cis, cis isomer, 0.0103 and 0.0105 for the cis, trans isomer, and 0.0067 for the trans, trans isomer. It is clear that with both columns, the greater number of trans sites, the smaller the slope.

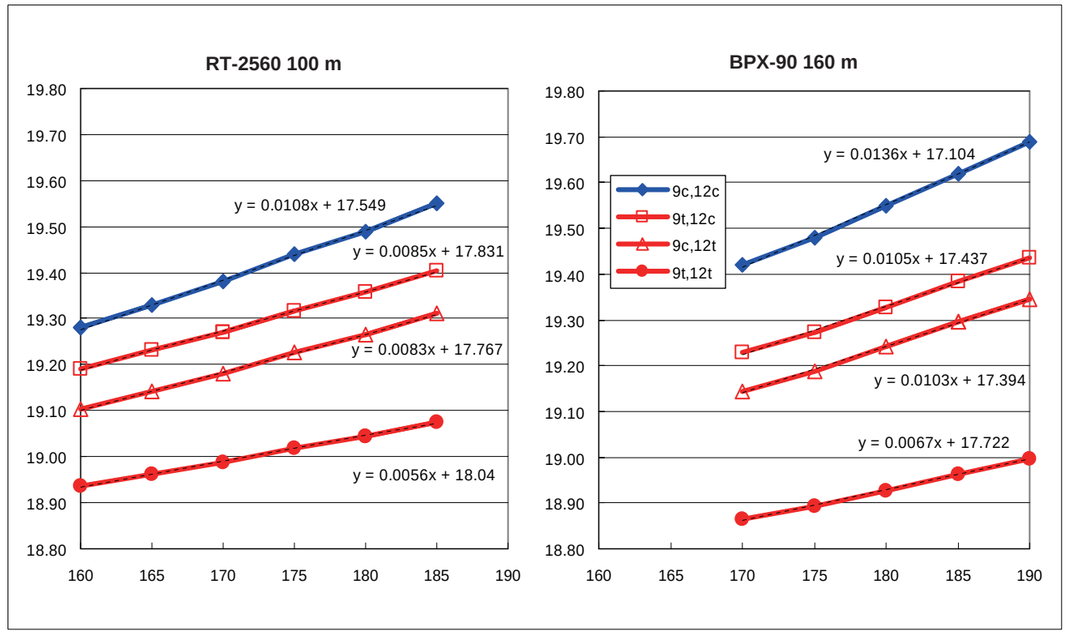


Fig. 5 ECLs of C18 : 2 Isomers

Fig. 6 shows the column temperature dependency of ECLs of C18:3 isomers. The approximate slopes of the linear plots with the Rtx-2560 column were cis × 3 isomers: 0.017, cis × 2, trans × 1 isomer: 0.0128 – 0.0144, cis × 1, trans × 2 isomers: 0.0107 – 0.0109, and trans × 3 isomers: 0.0073, while with the BPX-90 column, they were cis × 3 isomers: 0.0217, cis × 2, trans × 1 isomer: 0.0169 – 0.0181, cis × 1, trans × 2 isomers: 0.0131 – 0.0135, and trans × 3 isomers: 0.009, indicating that even in isomers having double bonds at three sites, the greater number of trans sites, the smaller the slope.

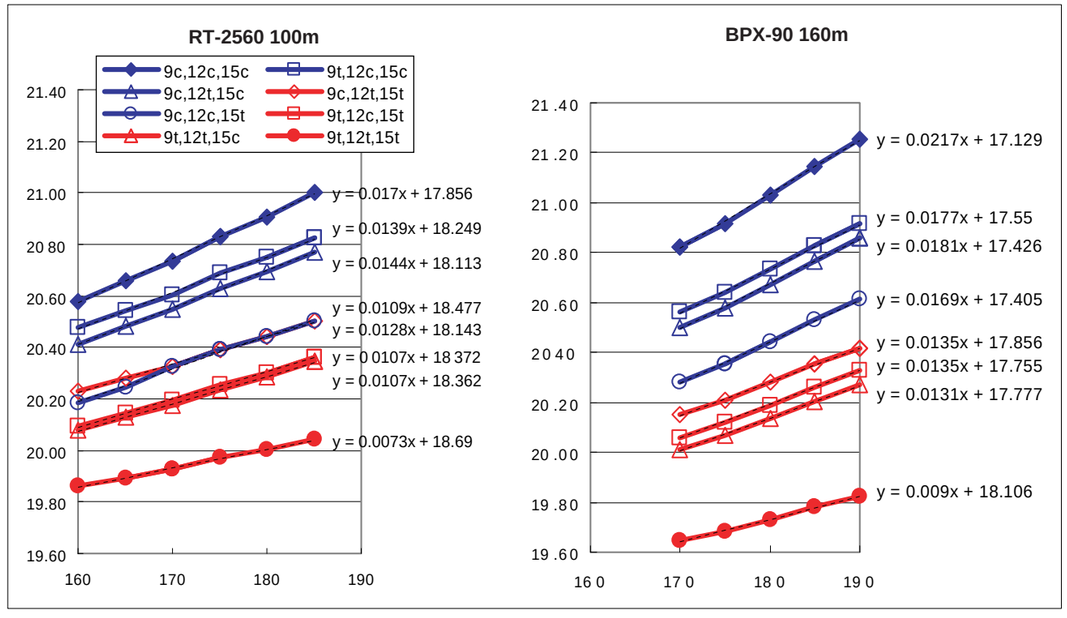


Fig. 6 ECLs of C18 : 3 Isomers