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Isomer identification and gas chromatographic retention studies of monomeric cyclic fatty acid methyl esters

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ABSTRACT

The structural isomers of saturated C_{18} cyclic fatty acid methyl esters were identified in a purified heated oil fraction by gas chromatography–mass spectrometry using high resolution capillary columns and selected ions abundance. Empirical mathematical models were developed based on the sequential pattern of elution of *trans*- and *cis*-1,2-disubstituted cyclohexyl fatty acid esters, in order to be able to predict their chromatographic retention characteristics. These models are proposed as an auxiliary technique for the fast identification of the different cyclic fatty acid isomers present in a used fat. The experimental design and the statistical significance of the results are discussed.

INTRODUCTION

The non-urea adducting fraction (NUA-fraction) isolated from heated fats and oils contains the most important toxic substances formed during thermal treatments [1,2]. Among these substances, monomeric C_{18} cyclic fatty acids (CFAs) deserve special consideration because of their proven digestive absorbability, hepatic toxicity and biological effects [3–5].

Several analytical approaches have been proposed to determine the amount of CFAs formed in fats and oils. Most are based on quantitative gas chromatography (GC) of the CFA as the corresponding hydrogenated methyl ester derivatives, which are then isolated and concentrated in the NUA fraction [6–9,10]. Concentration of CFA as low as 0.01% can be easily detected in fats and oils using this approach. So far, all the proposed analytical methods for CFA, face a number of limitations such as: uncertainty in CFA identification, lack of resolution of CFA from interfering substances, unavailability of standards (pure CFA), ambiguities in the analytical conditions used and long analysis time per sample. Furthermore, the statistical reliability of these methods has not been properly evaluated.

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Recently, there has been renewed interest in research on cyclic fatty acids. Several studies on heated fats and oils proved the formation of C_{18} CFA isomers with a disubstituted cyclopentyl nucleus in addition to the most widely known disubstituted cyclohexyl isomers [6,8,9,11–14]. Next, the chemical synthesis of the former isomers confirmed their proposed structures [15,16]. Concurrently, more selective and efficient analytical methods were developed by applying novel techniques [8,9,10–13]. Sebedio [12] reported a method based on the application of methoxy-bromomercuric acid fractionation for the simpler isolation and characterization of CFA in heated oils. GC coupled with a Fourier transform infrared spectrometer (FT-IR) was used to characterize the geometry of the ethylenic bonds of CFA fractions [13] prepared by a multi-step procedure including high-performance liquid chromatography (HPLC) [9]. GC–mass spectrometry (MS) using high-resolution capillary columns has been shown to be the best alternative for the analysis and structure characterization of CFA [8–12]. This technique offers the unique possibility of identifying and quantifying individual cyclohexyl and cyclopentyl C_{18} CFA isomers contained in NUA fractions. Although many improvements have been achieved [10], auxiliary techniques to facilitate isomer identification, when GC–MS facilities are not available, have not received much attention.

In this study we describe the use of empirical mathematical models to predict and/or confirm the chromatographic retention characteristics of individual isomeric C_{18} hydrogenated cyclic fatty acid methyl esters (HCFAM). The models were developed based on the sequential pattern of elution of the different cyclic isomers at several isothermal conditions. GC–MS was used for initial isomer identification. The statistical evaluation and significance of the final models are discussed.

EXPERIMENTAL

Materials

GC reference fatty acid methyl ester (FAME) mixture containing equal amounts of saturated esters for equivalent chain length (ECL) calculation were purchased from Nu-Check Prep. (Elysian, MN, U.S.A.). All solvents used were nanograde quality.

Isomeric mixtures of C_{18} cyclic fatty acids

Methyl esters of C_{18} HCFAM prepared by alkaline isomerization of linseed oil according to Eisenhauer *et al.* [17] and Friedrich *et al.* [18], were further purified by urea fractionation as described by Rojo and Perkins [10]. Purity of the isomeric mixtures determined by GC–MS was over 95%.

Capillary gas chromatography

The system used was a Hewlett-Packard 5790A capillary gas chromatograph (Avondale, PA, U.S.A.) equipped with an inlet splitter system fitted with a Jennings glass liner, flame ionization detector and electronic integrator (HP 3390A). Three fused-silica wall-coated open tubular capillary columns (Supelco, Bellefonte, PA, U.S.A.) of different relative 'polarity' were used with this system. In order of increasing polarity they are: column A: 30 m × 0.25 mm I.D. coated with SPB®-1 (dimethylpolysiloxane bonded phase), 0.25 μm film thickness; column B: 30 m × 0.25

mm I.D. coated with Supelcowax®-10 (polyethylene oxides bonded phase), 0.25 μm film thickness; and column C: 100 m × 0.25 mm I.D. coated with SP-2560 (blend of cyanoalkylpolysiloxanes), 0.20 μm film thickness. The columns were operated at isothermal and/or programmed temperature conditions in the range 140 to 250°C as specified. The carrier gas was hydrogen at a split ratio 1:100 and average linear velocities in the range 32–50 cm/s were set by adjusting accordingly the column head pressure for each column. The injector port and detector temperatures were 250°C and 270°C, respectively.

GC–MS

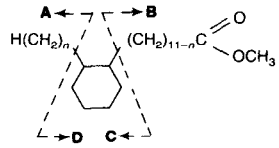
A Hewlett-Packard 5985B GC–MS system was used with the chemical ionization-electron impact (CI-EI) source set at 70 eV and 200°C. The capillary columns specified above were also used with this system. Each column was interfaced directly to the mass spectrometer and operated at the specified temperature, using helium as the carrier gas, with a split ratio of 1:20 using column pressures below 10 p.s.i. in order to compensate for the vacuum existent at the column end connected to the mass spectrometer system. The injector port temperature was 250°C. For CI mode, methane was used as the reactant gas.

Model fitting methodology for retention data of HCFAM

The isomeric mixture of HCFAMs was used to obtain retention data at selected temperatures of individual C_{18} methyl ω-(2-*n*-alkylcyclohexyl) alkanoate structural isomers (mol.wt. 296). The isomers were identified by their individual mass fragmentation pattern as previously described [8,14]. A series of seven structural isomers ($1 \leqslant n \leqslant 7$), each one separated as a pair of *trans*- and *cis*-ring isomer peaks, were detected during GC–MS. The retention data were obtained in an experiment designed to evaluate the effect of temperature on the retention profile of each group of isomeric HCFAM (either *cis* or *trans*). The effect was measured at two levels of constant column head pressure, 10 and 15 p.s.i. and every 10°C in the range of 160 to 210°C. The capillary column selected for this experiment was a new fused-silica Supelcowax®-10 with the specifications outlined above and operated under split conditions using hydrogen as a carrier gas. The data expressed as ECL [19] were fitted by the linear least squares method into polynomial equations with two independent variables, namely, the structural parameter *n* in the general formula and temperature. Several linear combinations of the independent variables were tested for statistical significance in order to build up the model. Statgraphics® software for microcomputers (STSC, Rockville, MD, U.S.A.) was used for this purpose.

RESULTS AND DISCUSSION

A combination of GC–MS and high-resolution gas chromatography (HRGC) was used to study the GC-retention profile of individual C_{18} HCFAMs. EI GC–MS of compounds eluted from capillary columns of different selectivity and "polarities", was carried out to identify all the HCFAM structural isomers present in the mixture. The procedure used for the identification is illustrated in Fig. 1. The expected MS fragmentation pattern of the cyclohexyl isomers has been already described in the literature [6] and is characterized by the formation of four important ions:



The presence of molecular ion at *m/e* 296 (confirmed by CI GC–MS) combined with relatively selected ion abundance of the most characteristic ion fragment of each isomer enables its conclusive identification in the mixture. For each structural isomer, characterized by the parameter *n*, one pair of two ring isomers, *cis* and *trans*, was detected as shown in Fig. 1.

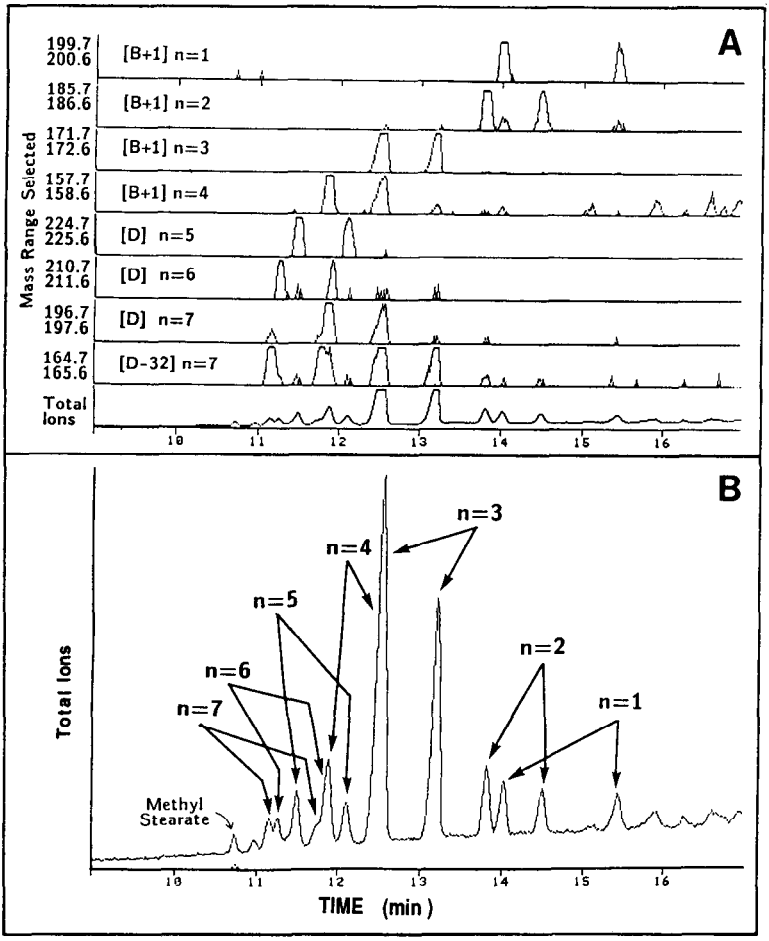


Fig. 1. GC–MS identification of HCFAM isomers in NUA-fractions prepared from linseed oil. (A) Relatively selected ion abundance of most specific ion fragments of HCFAM isomers. (B) Total ion chromatogram showing final peak identification. Conditions: initial temperature 190°C, raised up to 210°C at 1.5°C/min; column B (Supelcowax-10); carrier gas helium. EI GC–MS, source at 70 eV and 200°C.

The ion fragment B + 1, which results after the rearrangement and addition of a hydrogen atom, was found to be very specific for the identification of the first four isomers of the series. As the size of the lateral alkyl chain, $H(CH_2)_n$-, increases ($n > 4$), fragment D, produced by α-cleavage next to the ring, and the ion D − 32 resulting from loss of methanol from D, both become more specific for the identification of the other members of the series. The identification of isomers with $n > 7$, is more difficult because of lack of resolution from other isomers and due to their relatively small concentration in the isomeric mixture.

It has been generally assumed that, under GC conditions for separation of FAME in polar capillary columns, the retention region for HCFAM lies between methyl stearate (18:0) and methyl arachidate (20:0). However, the possibility that some cyclic monomers may have retention times shorter or equal to methyl stearate with certain phases has been suggested [20], but no conclusive evidence was shown.

The polarity of the stationary phase is the most important parameter in determining the range of HCFAM elution (Fig. 2). The curves shown in Fig. 2 were obtained by connecting the ECL of all the HCFAM isomers identified by GC–MS for each column. Separate curves for *trans*- and *cis*-isomers are shown. In the least polar column (column A, dimethyl polysiloxane) it was clearly demonstrated that many of the cyclohexyl isomers eluted before 18:0 at 190°C. In addition, the resolution of many cyclic isomers is impaired in these conditions, as shown by the relatively smaller slope of the *cis* and *trans* ECL *vs.* *n* curves for column A in Fig. 2. On the other hand, for the most polar column (column C), two of the *cis*-isomers have ECL greater than 20. Similarly, the relatively greater separation between the *cis*- and *trans*-curves for column C illustrates the improved *cis*-/*trans*-isomer separation possible with this column.

Using column C, it was possible to obtain base-line separation, even under isothermal conditions, of the generally co-eluting pair of methyl *trans*-9-(2′-*n*-propylcyclohexyl) nonanoate ($n = 3$) and methyl *cis*-8-(2′-*n*-butylcyclohexyl) octanoate (n =

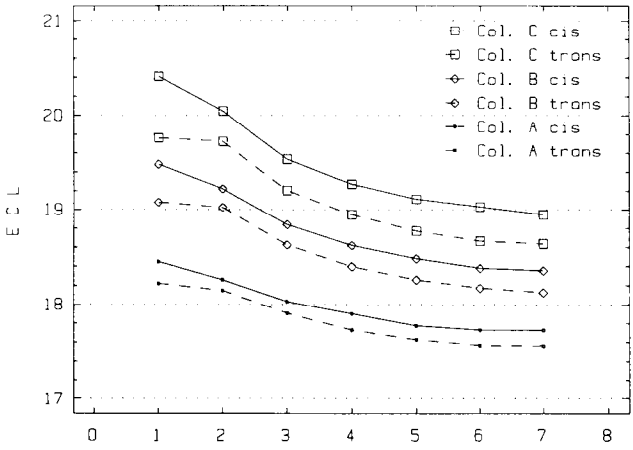


Fig. 2. Equivalent chain length (ECL) of HCFAM isomers in various capillary columns. Oven temperature constant = 190°C; column A: 30 m × 0.25 mm; SPB-1; column B: 30 m × 0.25 mm, Supelcowax-10; column C: 100 m × 0.25 mm, SP-2560.

4). The improved resolution observed for column C, however, should be attributed to the combined effect of stationary phase polarity and column length, when compared to columns A and B. Therefore, better isomer resolution may be achieved by increasing column length in non-polar columns. Finally, the trends observed (Fig. 2) suggest that an interesting alternative to improve HCFAM resolution should be obtained by coupling in series two capillary columns of different selectivity in increasing

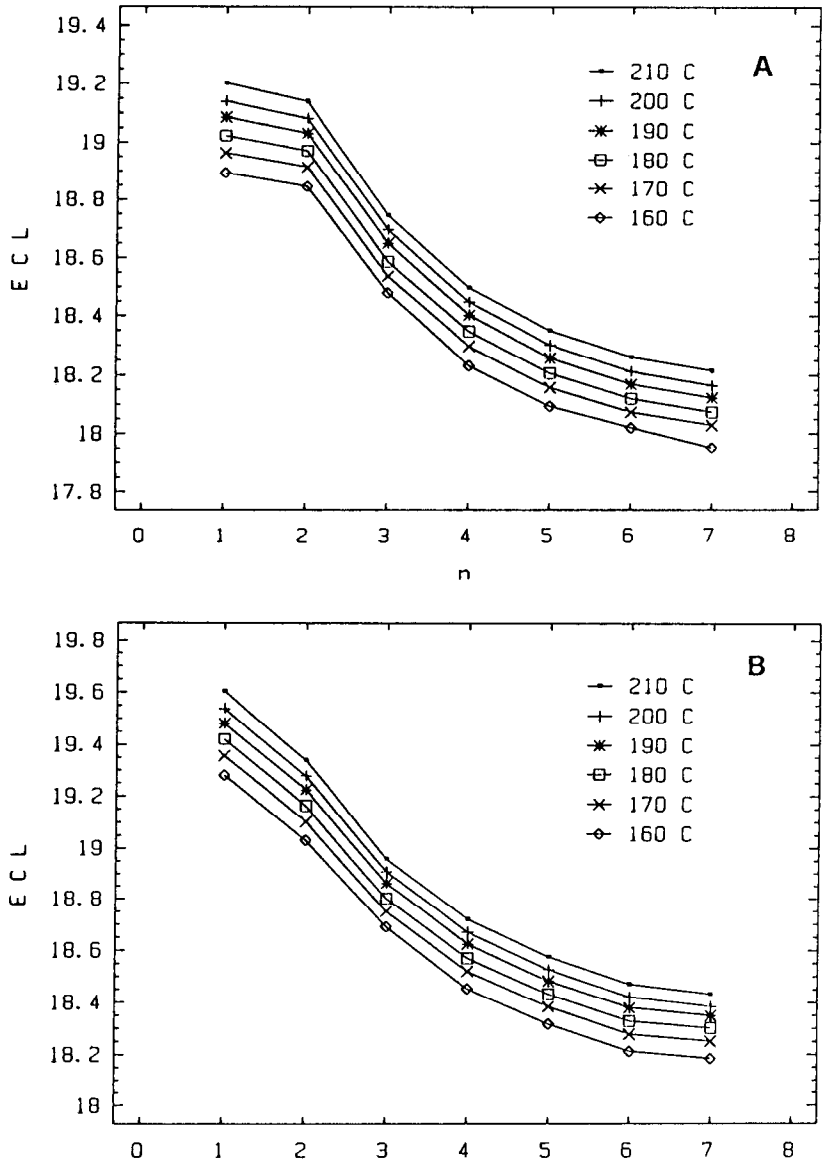


Fig. 3. Equivalent chain length of HCFAM at different temperature levels. (Column pressure 15 p.s.i.). (A) *Trans*-isomers. (B) *Cis*-isomers.

order of polarity. The expected effect is a higher slope and wider separation gap between the ECL *vs.* *n* curves.

For routine purposes, the capillary column B (polyethylene oxides bonded phase) offers good resolution in reasonable time for the most important isomers of HCFAM. Therefore, this column was selected to develop empirical mathematical models to predict the GC retention characteristics, expressed as ECL, of cyclic isomers under different oven temperature conditions.

The initial interest in developing retention models for HCFAM was to address the individual quantification of cyclopentyl as well as cyclohexyl isomers in fresh and heated oils. Furthermore, a more practical interest on these models lies in their application as an auxiliary technique to facilitate the identification of chromatographic peaks in complex mixtures when the availability of pure standards is limited. They become particularly useful in an industrial environment and in laboratories where GC–MS facilities are not readily available.

The results of the experiment performed at 15 p.s.i. constant column head pressure are shown in Fig. 3A and B for *trans*- and *cis*-isomers, respectively. The almost constant separation between ECL *vs.* *n* curves at different temperatures is equivalent to the change in ECL for each particular isomer. This constant change clearly shows the linear effect of oven temperature (T) on ECL increase (n = constant):

$$\mathrm{ECL} = a + bT$$

TABLE I

DIFFERENCES IN EQUIVALENT CHAIN LENGTH OF HCFAMs AT 15 AND 10 p.s.i. FOR TWO OVEN TEMPERATURES

Average carrier gas linear velocities (cm/s): column pressure 15 p.s.i.: 47.8 (170°C) 45.3 (210°C); column pressure 10 p.s.i.: 32.2 (170°C) 30.5 (210°C).

Oven temperature (°C)	*n*	*trans*-HCFAMs			*cis*-HCFAMs		
		A, 15 p.s.i.	B, 10 p.s.i.	|A − B|	A, 15 p.s.i.	B, 10 p.s.i.	|A − B|
170°C	1	18.962	18.964	0.002	19.355	19.361	0.006
	2	18.912	18.914	0.002	19.101	19.106	0.005
	3	18.537	18.526	0.011	18.749	18.743	0.006
	4	18.296	18.299	0.003	18.519	18.523	0.004
	5	18.156	18.163	0.007	18.382	18.388	0.006
	6	18.072	18.079	0.007	18.276	18.283	0.007
	7	18.028	18.032	0.004	18.249	18.262	0.013
		Average:		0.0051	Average:		0.0067
210°C	1	19.200	19.205	0.005	19.602	19.613	0.011
	2	19.138	19.142	0.004	19.337	19.345	0.008
	3	18.744	18.725	0.019	18.956	18.943	0.013
	4	18.497	18.499	0.002	18.719	18.722	0.003
	5	18.348	18.354	0.006	18.574	18.578	0.004
	6	18.260	18.269	0.009	18.469	18.476	0.007
	7	18.214	18.222	0.008	18.431	18.445	0.014
		Average:		0.0076	Average:		0.0086

The same experiment was conducted at 10 p.s.i. column pressure and very similar results were obtained (Table I). When, paired observations for the two pressure levels studied at two selected temperatures were compared, the deviations are smaller than 0.02 in all cases and may be attributed to the change in column efficiency with the change in gas linear velocity between pressure levels.

To broaden the scope of the model inference, the ECL values for each isomer obtained at both column pressure levels were used as independent replicates for statistical analysis and estimation of model parameters. In other words, the deviations were pooled with the overall experimental error and the ECL at both pressure levels were considered as estimates of the same means.

The pooled experimental data for 10 and 15 p.s.i. were used to fit a second degree polynomial illustrated by the equation:

$$y_i = b_0 + b_1x_{1i} + b_2x_{2i} + b_{11}x_{1i}^2 + b_{22}x_{2i}^2 + b_{12}x_{1i}x_{2i}$$

in which y_i represent the ECL, x_{1i} the oven temperature in °C, and x_{2i} is the reciprocal of the isomer structural parameter n ($1/n$) in the general formula of HCFAM given above. Other predictor variable and data transformations were attempted, but their coefficients of determination (R^2) were generally much lower than those for the former combination. The estimated regression coefficients and their 95% confidence interval were shown in Table II for *cis*- and *trans*-HCFAM.

The statistical analysis of the model was carried out as described by Deming [21]. These results are included in Table III. The overall F-ratio associated with the complete second degree equation, that is $F = MS_{\text{factors}}/MS_{\text{residual}}$, is a measure of the goodness of fit of the model. For *trans*- and *cis*-HCFAM ECL models, the corresponding F-values are respectively 700 and 1400 times larger than the tabulated critical values at $p = 0.001$ significance level, implying that the fitted surface of the models is worthy of interpretation.

TABLE II

MODEL FITTING RESULTS

Regression coefficients and their 95% confidence intervals.

Model	Regression coefficient	Estimate	Standard error	95% Confidence interval	
				Lower limit	Upper limit
trans-	b_0	16.512077	0.399654	15.716250	17.307904
HCFAM	b_1	0.006020	0.004328	− 0.002599	0.014638
	b_2	4.033373	0.124699	3.785061	4.281685
	b_{11}	− 0.000004	0.000012	− 0.000028	0.000020
	b_{22}	− 2.744220	0.046186	− 2.836189	− 2.652250
	b_{12}	0.001445	0.000607	0.000236	0.002654
cis-	b_0	16.761162	0.306607	16.150618	17.371705
HCFAM	b_1	0.006363	0.003320	− 0.000248	0.012974
	b_2	3.578681	0.095667	3.388181	3.769182
	b_{11}	− 0.000005	0.000009	− 0.000023	0.000013
	b_{22}	− 2.238535	0.035433	− 2.309092	− 2.167977
	b_{12}	0.001837	0.000466	0.000909	0.002765

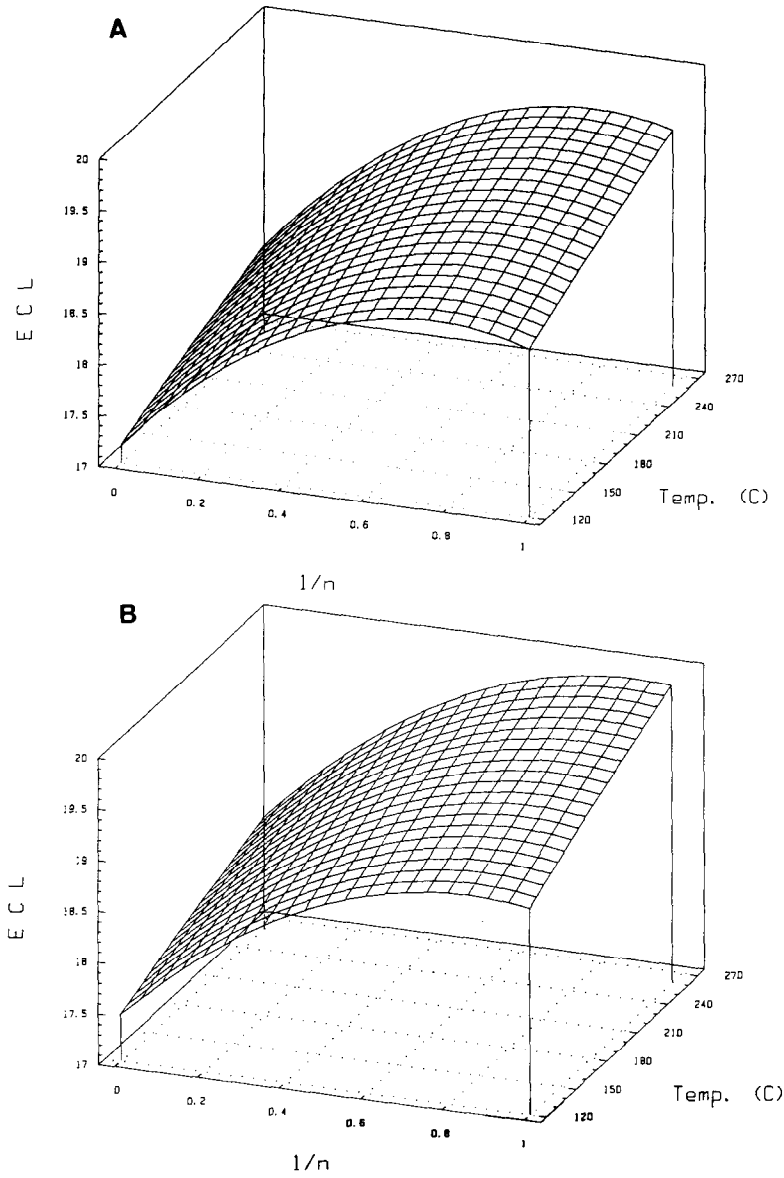


Fig. 4. Fitted polynomial response surfaces. (A) *Trans*-HCFAM isomers; (B) *cis*-HCFAM isomers.

The fitted polynomial response surfaces are illustrated in Fig. 4A and B. Superimposing the surfaces demonstrates that the ECL separation gap between *cis*- and *trans*-isomers, (*i.e.* the space between surfaces) remains practically constant within the temperature range covered by the experiment. This means that isomer separation, in the particular GC column used, cannot be substantially improved by independent manipulation of the oven temperature within the range of column pressure used. In

TABLE III

ANALYSIS OF VARIANCE (ANOVA) OF LINEAR MODELS FOR ECL OF *trans*- AND *cis*-HCFAM

Source	d.f.[a]	ECL Models					
		trans-HCFAM			*cis*-HCFAM		
		SS[b]	*MS*[c]	*F*-ratio[d]	*SS*	*MS*	*F*-ratio
Total	84	28760.53	342.387		29516.56	351.388	
Due to the mean	1	28748.56	28748.556		29502.15	29502.150	
Correlation for the mean	83	11.9738	0.1443		14.4096	0.1736	
Due to the factors	5	11.9182	2.3836	3347.38**[e]	14.3769	2.8754	6860.60**
Residuals	78	0.055543	0.0007		0.032691	0.0004	
Lack of fit	36	0.053128	0.0015	25.68**	0.029186	0.0008	9.72**
Pure experimental uncertainty	42	0.002414	0.0001		0.003504	0.0001	
R^2			0.9954			0.9977	
R^2 (adjusted for d.f.)			0.9951			0.9976	
Standard error of estimate			0.0267			0.0205	

[a] Degrees of freedom.
[b] Sum of squares.
[c] Mean squares.
[d] $MS_{\text{factors}}/MS_{\text{residual}}$.
[e] ** = Significance at $p = 0.001$ level.

addition, the linear effect of temperature on ECL is shown by the constant slope and lack of curvature of the surfaces in the direction of temperature increase. This is also demonstrated by the extremely small and non-significant value of the regression coefficient for the quadratic temperature term (b_{11}) in Table II.

In addition, other conclusions arise by extrapolating the response surfaces to temperature regions not covered by the experiment and normally not used for the particular separation under study with Supelcowax-10 capillary columns. First, many *trans*-HCFAM isomers with high n values (small $1/n$) elute before methyl stearate (ECL = 18.0) at oven temperatures less than 160°C (Fig. 4A). The same was true for some *cis*-HCFAM isomers (Fig. 4B). Similarly, all of the HCFAM isomers elute before methyl arachidate (ECL = 20) even at temperatures as high as 270°C, close to the upper limit temperature for the stationary phase used. Finally, a word of caution should be outlined here as clearly stated by Cochran and Cox [22]: "The polynomial surface should be regarded only as an approximation to the true function within the region covered by the experiment". Therefore the above outlined predictions outside the region should be experimentally verified before putting reliance on them.

The lowest and highest oven temperature for GC analysis of HCFAM are limited by several analytical considerations. From a practical point of view, temperatures lower than 150°C require extremely high column head pressure to achieve acceptable peak efficiencies for C_{18} HCFAM in a reasonable elution time. Furthermore, there is a theoretical lowest limit related with the relative volatility of the compounds of interest. The upper temperature limit, however is mainly related with the thermal stability of the analytes and the stationary phase as well.

In order to check the models predictive ability, three chromatographic runs were performed at two isothermal oven temperatures not commonly used for this analysis. In Table IV the predicted and observed ECL values for *trans*- and *cis*-isomers are compared at the two selected temperatures (140 and 240°C) outside the experimental region under study. The relatively small differences, from a practical point of view, prove an acceptable extrapolation ability for the models presented here. Furthermore, the elution of two C_{18} *trans*-cyclohexyl isomers before ECL = 18 (methyl stearate), as postulated by the models, was confirmed experimentally. Again, the warning concerning extrapolation should be stressed here since the models represented by the regression surfaces are only an approximation to the true situations.

To make assertions about the adequacy of the function estimation, in a statistical sense, the F-test for lack of fit should be considered, that is $F = MS_{1of}/MS_{pe}$. In addition, the assumption that the residuals are normally distributed with constant variance needs also to be confirmed. In Table III, the F-ratios for lack of fit, calculated as described by Deming [21], exhibit highly significant values for both models. This observation obviously seems to be in conflict with the also highly significant goodness of fit previously oulined. This situation, however, often arises if we use a highly precise measurement process like those used in the area of instrumental analysis. In this case, the F-test for lack of fit was statistically significant because the estimated variance due to pure experimental uncertainty was relatively very small. Finally, in a practical sense, the residuals were small enough for our particular application and can be considered acceptable when using the described models as auxiliary techniques in the identification of HCFAM isomers.

When examining the distribution of residuals against the predicted values (Fig.

TABLE IV

COMPARISON BETWEEN PREDICTED AND OBSERVED ECL VALUES OUTSIDE THE TEMPERATURE REGION COVERED BY THE EXPERIMENT

Temperature (°C)		240			140			240		
Pressure (p.s.i.)		15			15			10		
Model	*n*	Observed	Predicted	Difference	Observed	Predicted	Difference	Observed	Predicted	Difference
trans-	1	19.379	19.382	0.004	18.777	18.775	0.002	19.380	19.382	0.003
HCFAM	2	19.305	19.250	−0.055	18.734	18.715	−0.019	19.305	19.250	−0.054
	3	18.862	18.901	0.039	18.371	18.390	0.019	18.862	18.901	0.039
	4	18.635	18.670	0.035	18.135	18.171	0.035	18.636	18.670	0.033
	5	18.488	18.512	0.024	18.000	18.021	0.021	18.491	18.512	0.021
	6	18.381	18.400	0.019	17.912	17.913	0.001	18.376	18.400	0.024
	7	18.381	18.316	−0.065	17.865	17.832	−0.033	18.376	18.316	−0.060
cis-	1	19.795	19.793	−0.002	19.162	19.155	−0.006	19.795	19.793	−0.002
HCFAM	2	19.514	19.462	−0.052	18.917	18.916	0.000	19.514	19.462	−0.052
	3	19.083	19.103	0.021	18.583	18.588	0.005	19.083	19.103	0.020
	4	18.858	18.877	0.019	18.357	18.377	0.020	18.860	18.877	0.017
	5	18.709	18.726	0.017	18.222	18.236	0.013	18.712	18.726	0.015
	6	18.588	18.620	0.032	18.103	18.135	0.032	18.595	18.620	0.025
	7	18.525	18.541	0.016	18.060	18.060	0.000	18.538	18.541	0.002

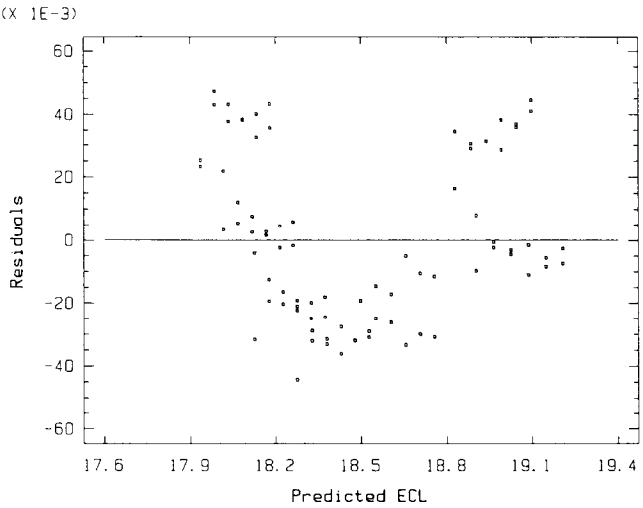


Fig. 5. Residual plot for predicted ECL of *trans*-HCFAM isomers using polynomial equation. (Regression coefficients in Table II; ANOVA in Table III).

5) it becomes apparent that the largest residuals are associated with small and/or large values for ECL (U-shaped distribution). In other words, this indicates that there was some kind of dependence of residuals from the data and the assumption of normal distribution of the error was not supported by the results.

This concern was addressed through examination of the data illustrated by Fig. 3A and B which demonstrate that the ECL values for isomers with $n = 1$ (*cis* or *trans*) are those that unexpectedly do not follow the apparent uniform exponential decrease trend in ECL observed by the other isomers (with $n > 1$). This suggests that the exclusion of the observations of ECL with $n = 1$ from the data should drastically

TABLE V

MODEL FITTING RESULTS USING REDUCED DATA (ISOMERS WITH $2 \leqslant n \leqslant 7$)

Regression coefficients and their 95% confidence intervals

Model	Regression coefficients	Estimate	Standard error	95% Confidence interval	
				Lower limit	Upper limit
trans-HCFAM	b_0	16.8422576	0.037506	16.767697	16.917456
	b_1	0.004436	0.000195	− 0.004047	0.004826
	b_2	2.600720	0.143695	2.313839	2.887601
	b_{22}	− 0.7388755	0.112359	− 0.963075	− 0.514435
	b_{12}	0.002386	0.000668	0.001052	0.003720
cis-HCFAM	b_0	17.083701	0.048204	16.987463	17.179938
	b_1	0.004252	0.000251	− 0.003751	0.004753
	b_2	2.575192	0.184682	2.206482	2.943902
	b_{22}	− 1.043228	0.144408	− 1.331532	− 0.754923
	b_{12}	0.003206	0.000858	0.001493	0.004919

TABLE VI

ANALYSIS OF VARIANCE (ANOVA) OF LINEAR MODELS FOR ECL OF *trans*- AND *cis*-HCFAM USING REDUCED DATA

Isomers with $2 \leqslant n \leqslant 7$.

Source	d.f.[a]	ECL Models					
		trans-HCFAM			*cis*-HCFAM		
		SS[b]	*MS*[c]	*F*-ratio[d]	*SS*	*MS*	*F*-ratio
Total	72	24404.74	338.955		24976.68	346.898	
Due to the mean	1	24397.16	24397.158		24969.46	24969.455	
Corr. for the mean	71	7.5824	0.1068		7.2247	0.1018	
Due to the factors	4	7.5731	1.8933	13632.95**[e]	7.2093	1.8023	7856.71**
Residuals	67	0.009305	0.0001		0.015370	0.0002	
Lack of fit	31	0.007112	0.0002	3.76**	0.012309	0.0004	4.67**
Pure experimental uncertainty	36	0.002193	0.0001		0.003061	0.0001	
R^2			0.9988			0.9979	
R^2 (Adjusted for d.f.)			0.9987			0.9977	
Standard error of estimate			0.0118			0.0151	

[a] Degrees of freedom.
[b] Sum of squares.
[c] Mean squares.
[d] $MS_{\text{factors}}/MS_{\text{residual}}$.
[e] ** = Significance at $p = 0.01$ level.

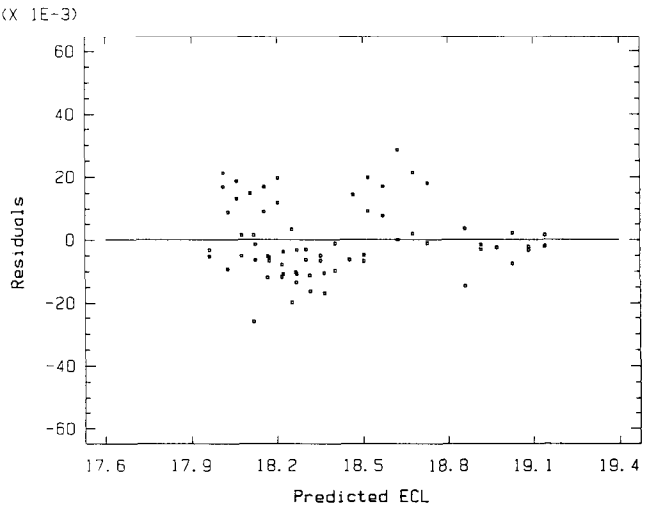


Fig. 6. Residual plot for predicted ECL for *trans*-HCFAM isomers using polynomial equation fitted with reduced data. (Regression coefficients in Table V; ANOVA in Table VI).

reduce the lack of fit and contribute to a more homogeneous distribution of residuals of the new "restricted" model.

The regression coefficients and the statistical analysis of the fitted models for the reduced experimental data ($2 \leqslant n \leqslant 7$) are shown in Tables V and VI, respectively. In Table V the quadratic term for oven temperature has not been included at 0.01% probability level for *trans*- and *cis*-HCFAM models. The results of the analysis of variance in Table VI show that the lack of fit for the new restricted models, even though still highly significant, has been substantially reduced when compared with the previous models. In addition, the residual plot for *trans*-HCFAM of the new restricted model, illustrated in Fig. 6, clearly shows a more homogeneous distribution of the errors which supports the validity of the statistical assumptions. A similar plot was obtained for the *cis*-HCFAM new model estimated with the reduced data.

Finally, this apparently arbitrary selection of data can be substantiated with two facts which support the exclusion of the HCFAM isomers with $n = 1$ from the data. First, these particular isomers have a unique MS fragmentation pattern when compared with the other isomers of the series, since they do not show the fragments D, D − 32 and D − 32 − 18 during EI mass spectrometry [6]. Secondly, these isomers do not occur at significant levels in fresh and heated edible fat and oils. Therefore, it seems that an additional unknown parameter is associated with cyclic isomers with $n = 1$. This parameter should account for the observed differences that justify an special treatment for the $n = 1$ cyclic isomers.

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REFERENCES

1 E. G. Perkins, *Rev. Fr. Corps Gras*, 23 (1976) 312.
2 B. Potteau and J. Causeret. *Rev. Fr. Corps Gras*, 18 (1971) 591.
3 N. Combe, M. J. Constantin and B. Entressangles, *Lipids*, 16 (1981) 8.
4 W. T. Iwaoka and E. G. Perkins, *Lipids*, 11 (1976) 349.
5 W. T. Iwaoka and E. G. Perkins, *J. Am. Oil Chem. Soc.*, 55 (1978) 734.
6 B. Potteau, P. Dubois and J. Rigaud, *Ann. Technol. Agric.*, 27 (1978) 655.
7 A. Gere, C. Gertz and O. Morin, *Rev. Fr. Corps Gras.*, 31 (1984) 341.
8 J. A. Rojo and E. G. Perkins, *J. Am. Oil Chem. Soc.*, 64 (1987) 414.
9 J. L. Sebedio, J. Prevost and A. Grandgirard, *J. Am. Oil Chem. Soc.*, 54 (1987) 1026.
10 J. A. Rojo and E. G. Perkins, *J. Am. Oil Chem. Soc.*, 66 (1989) 1593.
11 J. L. Sebedio, J. L. Le Quere, O. Morin, J. M. Vatele and A. Grandgirard, *J. Am. Oil Chem. Soc.*, 66 (1989) 704.
12 J. L. Sebedio, *Fette, Seifen, Anstrichm.*, 57 (1985) 267.
13 J. L. Sebedio, J. L. Le Quere, E. Semon, O. Morin, J. Prevost and A. Grandgirard, *J. Am. Oil Chem. Soc.*, 64 (1987) 1324.
14 J. A. Rojo, *MS Thesis*, University of Illinois, Urbana-Champaign, IL, 1985.
15 J. M. Vatele, J. L. Sebedio and J. L. Le Quere, *Chem. Phys. Lipids*, 48 (1988) 119.
16 J. A. Rojo and E. G. Perkins, *Lipids*, 24 (1989) 467.
17 R.A. Eisenhauer, R. E. Beal and E. L. Griffin, *J. Am. Oil Chem. Soc.*, 40 (1963) 129.
18 J. P. Friedrich, E. W. Bell and L. E. Gast, *J. Am. Oil Chem. Soc.*, 42 (1965) 643.
19 T. K. Miwa, K. L. Mikolajczak, F. R. Earle and I. A. Wolff, *Anal. Chem.*, 32 (1960) 1739.
20 A. Grandgirard and F. Julliard, *Rev. Fr. Corps Gras.*, 30 (1983) 123.
21 S. N. Deming, in B. R. Kowalski (Editor), *Chemometrics: Mathematics and Statistics in Chemistry* (*NATO ASI Series C*, Vol. 138), Reidel, Dordrecht, 1984, p. 267.
22 W. G. Cochran and G. M. Cox, Experimental Designs, Wiley, New York, 2nd ed., 1957, pp. 244–291.