SHORT COMMUNICATION

Analysis of Alpha-Linolenic Acid Geometrical Isomers in Deodorized Oils by Capillary Gas-Liquid Chromatography on Cyanoalkyl Polysiloxane Stationary Phases: A Note of Caution

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Analysis of alpha-linolenic acid geometrical isomers in deodorized or heated oils by capillary gas–liquid chromatography (GLC) on polar cyanoalkyl polysiloxane stationary phases requires some care to avoid interferences with other fatty acids. Depending on the temperature of the column, the *cis*-11 20:1 acid may elute before, with or after the *cis*-9, *cis*-12,*cis*-15 18:3 acid during GLC. In some instances [temperature higher than 180°C with a CP Sil 88 column (Chrompack, Middelburg, The Netherlands)], the 20:1 acid coelutes with the *trans*-9,*cis*-12,*cis*-15 18:3 acid, leading to abnormally high levels of this last isomer. Consequently, the degree of isomerization of alpha-linolenic acid will be overestimated under such conditions. It is recommended that the behavior of *cis*-11 20:1 acid relative to temperature be checked carefully prior to the determination of alpha-linolenic acid geometrical isomers by GLC. Temperatures lower than 160°C seem appropriate to separate all of these components from each other and from *cis*-11 20:1 acid in a 50 m × 0.25 mm i.d. CP Sil 88 capillary column.

KEY WORDS: Alpha-linolenic acid, cyanoalkyl polysiloxane stationary phase, deodorized oils, equivalent chainlength, fatty acid methyl esters, gas–liquid chromatography, geometrical isomers, temperature effects, *trans* fatty acids.

Alpha-linolenic acid geometrical isomers (A-LAGIs) are present in almost all alpha-linolenic acid-containing oils that have been subjected to deodorization or to any other heat treatment, provided the processing temperature was higher than *ca.* 200°C (1–7). Detailed studies of the distribution of individual isomers have been performed with commercial rapeseed, soybean and walnut oils, and with linseed oil heated under vacuum, presenting degrees of isomerization (DI; ratio of *trans* isomers and total octadecatrienoic acids times 100) of alpha-linolenic acid that vary between a few percent and 69% (more than 40 samples) (4–7). They show that the level of the *cis*-9,*cis*-12,*trans*-15 18:3 isomer is always higher than that of the *trans*-9,*cis*-12,*cis*-15 18:3 isomer (4–7). The probabilities of formation of these two isomers, at the beginning of the isomerization reaction, are 0.53 and 0.42, respectively (7). However, a recent study claimed that the opposite situation invariably occurred in the fat extracted from liquid infant formulas that contained soybean oil (10 samples) (8). This last observation would imply that the reaction mechanisms of *cis–trans* isomerization of ethylenic bonds in alpha-linolenic acid through heat activation may be variable. Because this seems unlikely, we tried to understand the discrepancies between observations made with commercial oils or with heated linseed oil (4–7) and the fat from infant food formulas (8). Based on the assumption that the *cis–trans* isomerization mechanism of ethylenic bonds in alpha-linolenic acid is unique (R.L. Wolff, submitted for publication), it becomes clear that some differences in the analytical conditions are the reason for these differences. We thus studied the effects of some parameters on the gas–liquid chromatographic separation of A-LAGIs, with particular attention for potential interfering fatty acids.

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EXPERIMENTAL PROCEDURES

Samples. Five samples of rapeseed and soybean oils were purchased in supermarkets in St. Hyacinthe, Canada, and one item was selected because of its relatively high level of *cis*-11 20:1 acid for this study. The *cis*-11 20:1 acid authentic standard was from the Sigma Chemical Company (St. Louis, MO).

Preparation of fatty acid methyl esters (FAME). FAME were prepared essentially (7) as described by Morrison and Smith (9) with 10% BF_3 in methanol as a reagent.

Gas–liquid chromatography (GLC). Analyses of FAME were carried out on a Carlo Erba 4130 chromatograph equipped with a flame-ionization detector and a split injector (Carlo Erba, Milano, Italy). A fused-silica capillary column (CP Sil 88, 50 m × 0.25 mm i.d., 0.20 μm film; Chrompack, Middelburg, The Netherlands) was used with helium as carrier gas (inlet pressure: 120 kPa). It was operated isothermally at temperatures comprised between 155 and 185°C. The injection port and the detector were maintained at 250°C.

Equivalent chainlengths (ECLs) were determined essentially as described by Ackman (10), with 16:0, 18:0 and 20:0 acid methyl esters as standards. A-LAGIs were identified according to Wolff (11).

RESULTS AND DISCUSSION

The effect of temperature on the relative order of elution of *cis*-11 20:1 acid and A-LAGIs is illustrated in Figure 1. As the temperature increases from 155 to 185°C, the *cis*-11 20:1 acid elutes after, with or before the *cis*-9,*cis*-12, *cis*-15 18:3 acid. Similar behaviors of these two acids with temperature were previously described for a 100 m × 0.25 mm capillary column wall-coated with OV 275 (12) and for a 30 m × 0.32 mm SP-2380 capillary column (13). At the highest temperatures, the 20:1 acid coelutes with the *trans*-9,*cis*-12,*cis*-15 18:3 acid. Plots displayed in Figure 2 show that the ECLs of *cis*-11 20:1, *cis*-9,*cis*-12,*cis*-15 18:3 and *trans*-9,*cis*-12,*cis*-15 18:3 acids increase linearly with the temperature of the column. The equations for these three acids are:

$$ECL_{20:1} = 0.005 \times t + 19.72 \text{ (corr. = 0.999)} \quad [1]$$

$$ECL_{c,c,c} = 0.012 \times t + 18.57 \text{ (corr. = 0.996)} \quad [2]$$

$$ECL_{t,c,c} = 0.010 \times t + 18.80 \text{ (corr. = 0.995)} \quad [3]$$

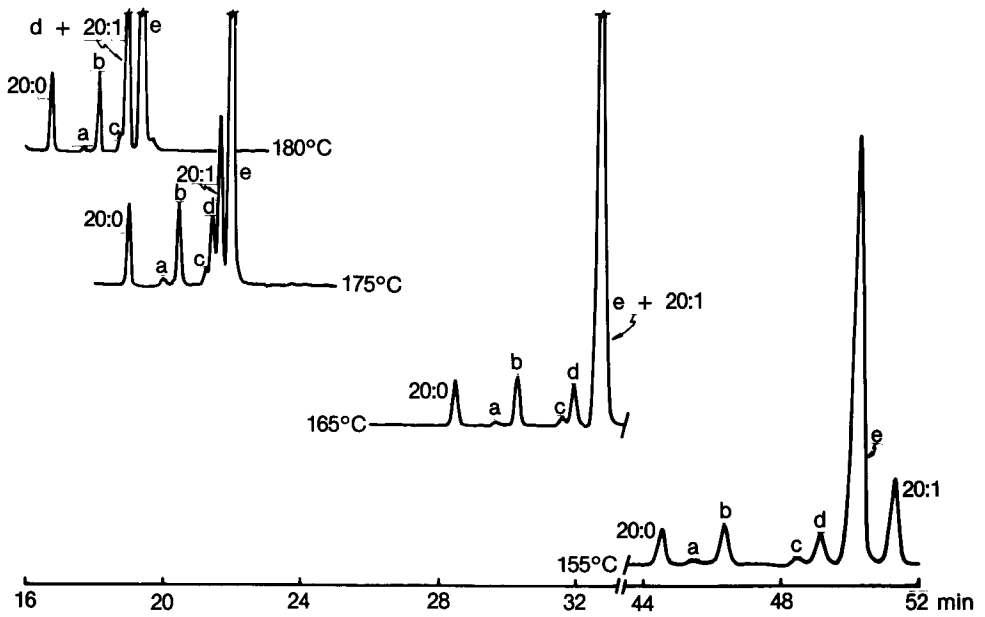

FIG. 1. Influence of temperature on the elution order of *cis*-11 20:1 acid and alpha-linolenic acid geometrical isomers. Selected chromatograms obtained with a CP Sil 88 capillary column (50 m × 0.25 mm i.d., 0.20 μm film; Chrompack, Middelburg, The Netherlands) of fatty acid methyl esters prepared with a sample of commercial deodorized rapeseed oil from St. Hyacinthe, Canada. Inlet pressure of the carrier gas (helium): 120 kPa. Temperature of the column as indicated on chromatograms. All injections at the same load. Identification of peaks: (a), *trans*-9,*cis*-12,*trans*-15 18:3 acid; (b), *cis*-9,*cis*-12,*trans*-15 18:3 acid; (c), *cis*-9,*trans*-12,*cis*-15 18:3 acid; (d), *trans*-9,*cis*-12,*cis*-15 18:3 acid; (e), *cis*-9,*cis*-12,*cis*-15 18:3 acid. 20:1 acid is the *cis*-11 isomer.

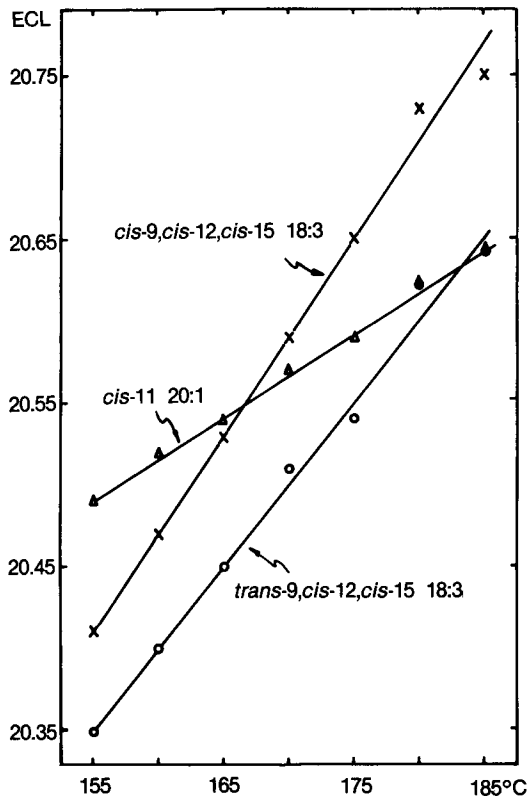

FIG. 2. Plots of equivalent chainlengths (ECL) of *cis*-11 20:1, *trans*-9,*cis*-12,*cis*-15 18:3 and *cis*-9,*cis*-12,*cis*-15 18:3 acid methyl esters as a function of temperature. Analyses on a CP Sil 88 capillary column. (see Fig. 1 for company source, characteristics and operating conditions of the column). ECL were determined with 16:0, 18:0 and 20:0 acid methyl esters as standards.

where t is the temperature of the column (in °C). Note that the ECLs of the *trans*-9,*cis*-12,*cis*-15 18:3 and *cis*-9,*cis*-12, *cis*-15 18:3 acid methyl esters increase by *ca.* 0.01 carbon atom/°C, which is twice the ECL increase rate of *cis*-11 20:1 acid. Because the ECL of *cis*-11 20:1 acid evolves less rapidly with temperature than the ECLs of the two octadecatrienoic acids, there are two critical zones where 20:1 acid coelutes with one of these acids. At about 167°C, the 20:1 acid has the same ECL as the all-*cis* 18:3n-3 acid. Between 180 and 185°C, it is not distinguishable from the *trans*-9,*cis*-12,*cis*-15 18:3 acid. To avoid interferences between 20:1 acid and A-LAGIs, the column temperature should be equal to, or better, less than 160°C. These are the conditions we use in our work (11). In the study on lipids extracted from infant food formulas (8), a CP Sil 88 capillary column similar to ours (same length, i.d. and film thickness) was used, and analyses were performed at 185°C. Our data clearly show that when the CP Sil 88 column is operated under such conditions, the *cis*-11 20:1 acid is mixed with the *trans*-9,*cis*-12,*cis*-15 18:3 acid, thus leading to an overestimate of this last isomer. In a study on A-LAGIs in margarines and hydrogenated soybean oil (14), similar coelution of *cis*-11 20:1 acid and one mono-*trans* isomer of alpha-linolenic acid (presumably the *trans*-9,*cis*-12,*cis*-15 18:3 acid) was observed on a CP Sil 88 capillary column operated with temperature programming. The level of *cis*-11 20:1 acid in soybean oil, used to increase the level of 18:3n-3 in infant food formulas, is about 0.2–0.3% of total fatty acids. This value is in the same order of magnitude as those of A-LAGIs.

Consequently, the observation of a level of *trans*-9,*cis*-12, *cis*-15 18:3 acid being systematically higher than that of *cis*-9,*cis*-12,*trans*-15 18:3 acid (8) is erroneous. From our

previous results on soybean, rapeseed and walnut oils commercialized in several European countries (4–6), together with results of linseed oil heated in ampoules sealed under vacuum (7), thus totalling about 35 samples with DIs between a few percent and 30%, the following relative proportions of A-LAGIs have been established: *trans*-9, *cis*-12,*trans*-15 18:3 acid, from traces to about 10% of total *trans* trienoic acids; *cis*-9,*cis*-12,*trans*-15 18:3 acid, 46 to 53%; *cis*-9,*trans*-12,*cis*-15 18:3 acid, 5.4 to 7.8%; *trans*-9, *cis*-12,*cis*-15 18:3 acid, 38 to 42%. A mean *trans*-9,*cis*-12, *cis*-15 18:3 acid/*cis*-9,*cis*-12,*trans*-15 18:3 acid ratio of 0.8 can be estimated for all samples. Values for A-LAGIs in soybean and rapeseed oils from Canada (5 samples) also fall in these narrow ranges and present the same ratio (results not shown). It is the author's opinion that any large deviation from these figures is indicative of an analytical problem, at least when the DI is equal to or less than 30%. A DI of 30% is the highest value we found in European and Canadian oils, although Ackman *et al.* (1) reported on a sample of Canadian oil with an exceptionally high DI of 53.8%. In the study on infant food formulas (8), two samples showed a value of 5–6 for the *trans*-9, *cis*-12,*cis*-15 18:3 acid/*cis*-9,*cis*-12,*trans*-15 18:3 acid ratio. This indicates that the peak identified as *trans*-9,*cis*-12, cis-15 18:3 acid corresponds almost exclusively to pure *cis*-11 20:1 acid.

Other chromatographic parameters may have an effect on the relative order of elution of *cis*-11 20:1 acid and A-LAGIs. In fact, any parameter that is able to modify the selectivity factor, such as the nature of the stationary phase or the phase ratio, will change this relative order. Aging of the column may also modify the elution order. On the other hand, the inlet pressure of the carrier gas, which is directly related to the linear gas velocity, will not modify the relative elution orders because it does not modify the ECLs. However, increasing the inlet pressure from 120 to 160 kPa diminishes the elution time of *cis*-11 20:1 acid from 52 min down to 39 min at 155°C, without affecting the resolution between the different peaks (results not shown). Thus, the use of cyanoalkyl polysiloxane stationary phases requires a careful examination of the apparent movement of the 20:1 acid relative to A-LAGIs with temperature when A-LAGIs have to be analyzed by GLC. Alternatively, a simple means to localize the *cis*-11 20:1 acid is to spike the sample with an authentic standard. Generally, the lowest temperatures are best suited to separate the *cis*-11 20:1 acid from the all-*cis* 18:3n-3 acid, the former being eluted after the latter with good resolution (11,12; this study).

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