Comments on the Resolution of Individual *trans*-18:1 Isomers by Gas–Liquid Chromatography

Sir:

A major breakthrough in the analysis of individual *trans*-18:1 isomers (separation, identification, and quantitation) by gas–liquid chromatography (GLC) of their methyl or isopropyl esters was achieved independently in 1995 by Wolff and Bayard (1) and by Molkentin and Precht (2) through the use of a 100-m capillary column coated with a cyanopropyl polysiloxane stationary phase, i.e., a CPSil 88 column (Chrompack, Middelburg, The Netherlands). This simple methodology avoids the time-consuming ozonolysis procedure, followed by GLC of the resulting fragments (3), which has been used for decades and was the reference method. GLC is an even better procedure than ozonolysis because there is no risk of loss of the shortest fragments, and there is no need to establish and apply correction factors for unequal responses of the flame-ionization detector. Depending on the operating temperature of the column, the elution of all *trans*-18:1 isomers can be achieved between *ca.* 30 and 80 min (1,2), allowing routine analysis of these acids, extracted from any food or biological samples. Quantitation of such components with a 100-m CP Sil 88 column has already been achieved in hundreds of different milk fats, margarines, and shortenings (4,5).

However, owing to the unavoidable overlaps between *trans*- and *cis*-18:1 isomers during GLC on all known columns (1,2), a prerequisite to the analysis of these isomers is their fractionation by argentation chromatography (high-performance liquid chromatography or thin-layer chromatography) prior to GLC (also needed in the ozonolysis procedure). A minor drawback of the CPSil 88 column and of the use of methyl esters (or isopropyl esters) is that the Δ6 to Δ8 and the pair of Δ13-Δ14 isomers are not, or only partly, resolved, because of the almost identical equivalent chain-lengths (1) [or retention times (2)]. The combination argentation–chromatography/GLC on 100-m capillary columns coated with cyanopropyl polysiloxane should attract a following in the future because of its simplicity and the need for only basic equipment typically found in all lipid research laboratories.

In a recent issue of this journal, Mossoba *et al.* (6) used 4,4-dimethyloxazoline (DMOX) derivatives of isolated *trans*-18:1 acids and succeeded in partly resolving the *trans* Δ13 and Δ14 isomers on either 100-m CPSil 88 or 100-m SP-2560 (Supelco, Bellefonte, PA) capillary columns. However, on both columns, the *trans* Δ6 and Δ7 isomers co-eluted with the *trans* Δ8 and Δ9 isomers, respectively. Moreover, to achieve the resolution of the critical pair Δ13-Δ14, a low temperature (140°C) was necessary, which led to an exaggeratedly long time of elution of the last-emerging *trans* Δ16 isomer, *ca.* 150 min, which is not convenient for routine analyses. Thus, the superiority of DMOX derivatives over methyl esters is not evident. The mere interest of the study by Mossoba *et al.* (6) was the confirmation (highlighted in the title) by GLC–mass spectrometry of the successive step-by-step elution order of individual isomers from a low to a high Δ position, which was unequivocally demonstrated earlier (1,2) by using authentic individual octadecenoic acids [either as methyl (1,2) or isopropyl (1) esters] of both the *trans* and the *cis* configurations (2).

Moreover, it was claimed in this paper that this was the first time that the pair of *trans* isomers Δ13-Δ14 could be (partly) resolved as DMOX derivatives, but not as fatty acid methyl esters. This is not exact. Five years ago, in 1991, Wood (7) reported on the baseline resolution of these isomers (as methyl esters) in a 60-m SP-2100 column operated isothermally at 150°C, in about 160 min. This author could even obtain a better resolution of the corresponding *cis* isomers. On this column, the *cis*- and *trans*-18:1 isomers elute before stearic acid, in contrast to the CPSil 88 and SP-2560 capillary columns. More recently, Precht and Molkentin (8) also obtained a partial resolution of the critical pair of *trans* Δ13-Δ14 isomers (as methyl esters) on a 100-m CPSil 88 column by simply decreasing the operating temperature (Fig. 1). The lower the temperature, the better the resolution.

Lowering the temperature results in longer elution times and enhanced capacity ratios, and hence, in a better resolution. This is probably the reason why the DMOX derivatives of the Δ13 and Δ14 isomers are resolved, but not their methyl ester counterparts: the former elute *ca.* 1 h after the latter under the conditions (140°C) used by Mossoba *et al.* (6), with an elution time similar to that presented by methyl esters analyzed at 125°C (*ca.* 160 min) (Fig. 1). The selectivity of DMOX derivatives is probably not modified as compared to that of methyl esters, and consequently, their usefulness for routine analyses is questionable, even if resolution of the Δ13 and Δ14 *trans* isomers is required. Under the experimental conditions of Mossoba *et al.* (6), the Δ13 and Δ14 isomers were separated not because they were analyzed as DMOX derivatives but only because the elution time of these components was sufficiently long to allow the separation.

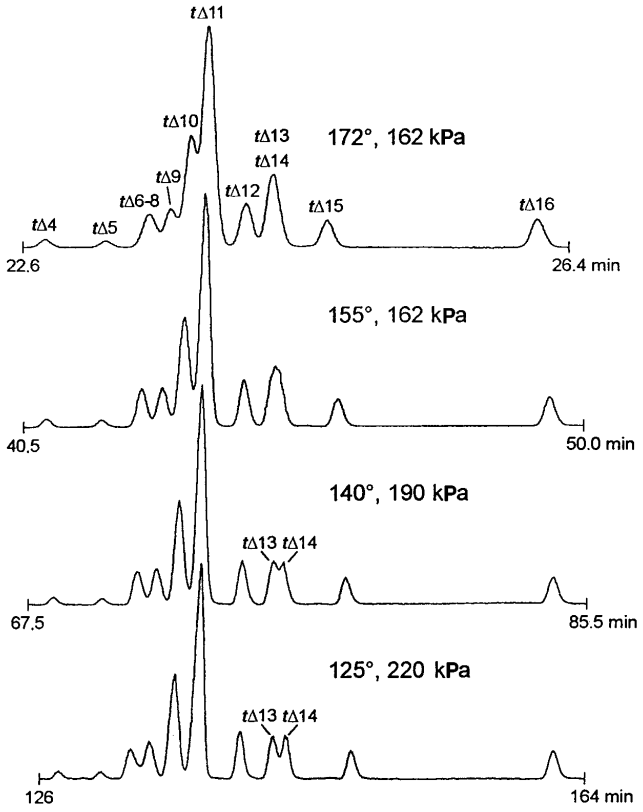

FIG. 1. Resolution of *trans*-18:1 acid positional isomers, prepared from milk fat by argentation thin-layer chromatography of the methyl esters, as a function of the oven temperature and the inlet pressure of the carrier gas (H_2). Separations were carried out on a 100-m CPSil 88 capillary column (Chrompack, Middelburg, The Netherlands) operated at the indicated isothermal temperatures and carrier gas pressures.

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