Gas chromatographic separation of the FAME prepared from dairy products, PHO, and refined vegetable oils applying a negative temperature gradient.

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Abstract

To date no single gas chromatographic method can simultaneously measure all fatty acids (FA), including trans FA (TFA), that are contained in dairy products, partially hydrogenated oils (PHO), and refined vegetable oils. Using 100% poly(biscyanopropyl siloxane) capillary columns, ruminant and dairy fats are preferentially analyzed by applying temperature programs that separate short chain FA, but not trans-18:3 from 20:1. Refined vegetable oils and PHO are preferentially analyzed by applying isothermal elutions that provide quantification of all 18 carbon TFA including trans-18:3 FA, but not of all short chain FA. In this short communication, we propose a temperature program method capable of simultaneously measuring short chain FA and all 18 carbon TFA including trans-18:3 by applying a negative temperature gradient after the elution of trans-18:1. A simplified version of the method is also described for equipment not able to perform negative temperature gradients.

Introduction

About two decades ago, food labeling regulations requiring the declaration of the trans-fat content on food packaging were introduced in several countries [1]. Available gas chromatographic (GC) methods for the quantification of fatty acids (FA) (as methyl esters, FAME) were redesigned to privilege the accurate quantification of the *trans*FA (TFA) contained in partially hydrogenated oils (PHO). All revised methods adopted 100% poly(biscyanopropyl siloxane) (BCS) capillary columns in the format 100 x 0.25 mm, the commercial columns providing the highest resolving capabilities for unsaturated fatty acids based on the number and geometry of double bonds (DB). More polar columns coated with ionic liquids, which provide even stronger selectivity, were introduced only a decade later [2,3]. The isothermal elution at 180°C emerged as the preferred compromise for the resolution of 18:1, 18:2 and 18:3 TFA from the other FA present in PHO [4] and was selected by the American Oil Chemists' Society (AOCS) for its Official Method Ce 1H-05 [5]. AOCS later introduced the sibling Official Method Ce 1J-07 [6], adding a temperature ramp after the elution of 18:3n-3 to shorten the retention time of remaining FAME.

Ruminant fats and dairy products, rich in short chain FA (SCFA), are generally analyzed applying a temperature gradient. Golay *et al.*developed a method capable of simultaneously measuring the SCFA,*trans* -18:1 and *trans* -18:2 present in ruminant fats by applying a rapid temperature ramp [7]. This method was validated for the quantification of TFA in milk products, infant formula, and adult/pediatric nutritional formula [8]. The application of the temperature program, however, resulted in the coelution of*trans* -18:3 with 20:1 FA, which affects the measurement of TFA when applying this method to the analysis of refined vegetable oils (RVO) and PHO.

Although each method proved very effective for the analysis of the lipid extracts it was developed for, no single method may be applied to all extracts. To address this gap, this short communication proposes

a modification of the method of Golay *et al.* [7,8]. This modified approach can be used to measure the SCFA, *trans* -18:1 and*trans* -18:2 present in ruminant fats while simultaneously achieving more accurate quantification of *trans* -18:3 present fat in PHO and RVO.

Material and Methods

Supelco 37 Component FAME Mix, Canola oil analytical standard, boron trifluoride (14% in methanol) and sodium methoxide (0.5M in methanol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Triheneicosanoin and individual FAME used for identification purposes were purchased from Nu-Chek Prep (Elysian, MN, USA). BCR 632A (anhydrous butter) was purchased from the EU Joint Research Centre (Geel, Belgium). Partially hydrogenated oils and other food oils were collected during previous studies. Samples were converted to FAME at the concentration of 20 mg/mL applying AOCS Official Method Ce 2-66 [9] after the addition of triheneicosanoin as internal standard (5% w/w).

Gas chromatographic analyses were performed with an Agilent 8890 GC equipped with a split/splitless injection port, FID detector, and Supelco SP2560 capillary column (0.25 mm x 100 m, 0.20 μm thickness, Supelco, Bellefonte, PA, USA). Hydrogen was the carrier at 1 mL/min constant flow and 1 μL of sample was injected with 100:1 split ratio. Temperature program method 1: 60°C for 5 min, ramp 15°C/min to 165°C, hold 1 min, ramp 2°C/min to 184°C, hold 25 min, ramp 4°C/min to 225°C, hold 20 min. Temperature program method 2: 60°C for 5 min, ramp 15°C/min to 165°C, hold 1 min, ramp 2°C/min to 190°C, hold 8 min., ramp 4°C/min to 175°C, hold 11 min, ramp 4°C/min to 225°C, hold 20 min.

Results and Discussion

The dependence of 100% BCS phase selectivity on elution temperature may be exploited to precisely tailor the separation of all FAME that need to be quantified for nutritional labeling purposes [10]. Raising the elution temperature increases the relative retention of FAME with more DB relative to those with fewer DB. Also, with a lower magnitude, it increases the retention of FAME with DB in *cis* configuration relative to their analogs with same DB but in *trans*configuration. PHO and RVO are preferentially analyzed by isothermal elution at 180°C because it provides suitable resolution of*t* 13/*t* 14-18:1 from *c* 9-18:1, and the elution of*c* 11-20:1 in between *t* 9,*c* 12,*c* 15-18:3 and 18:3n-3 [4], but the separation of SCFA is sacrificed. Lowering the elution temperature to separate SCFA would cause a progressively stronger overlap between the *trans* -18:1 and *cis* -18:1 and increase retention of *c* 11-20:1 with respect to 18:3. Golay*et al.* [7,8] developed a temperature gradient method capable of simultaneously separating SCFA and 18:1 TFA contained in milk products, infant formula, and adult/pediatric nutritional formula. In this study, the applicability of this method to the analysis of PHO and RVO was tested using a 2:1 blend of PHO and soybean oil. Starting the temperature program at 60°C provided the separation of SCFA, then the rapid temperature ramp to 165°C followed by a single ramp to 225°C provided the elution of *trans* -18:1 ahead of *c* 9-18:1 (Fig. 1A). However, the elution of all C18 FAME with a single ramp to 225°C as prescribed in the original method caused the overlap of*t* 9,*c* 12,*c* 15-18:3 with *c* 11-20:1. This inconvenience may be resolved by tailoring the temperature program more precisely. The addition of a temperature plateau at 184°C (method 1) rephased the elution of *c* 11-20:1 in exchange for a minor loss of separation of *t* 13/*t* 14-18:1 from *c* 9-18:1 (Fig. 1B). The temperature of this plateau (184°C) may be finely tuned to account for minor column to column variability: the elution of *c* 11-20:1 may be moved toward *t* 9,*c* 12,*c* 15-18:3 by increasing this temperature, or toward 18:3n-3 by lowering it.

If a greater separation of *t* 13/*t* 14- and c9-18:1 is necessary, the temperature of the plateau may be increased 190°C (Fig 1C). This change improved the separation of *trans* -18:1, but it caused the partial overlap of *t* 9,*c* 12,*c* 15-18:3 with*c* 11-20:1. This co-elution may be resolved by adding a negative temperature gradient to 175°C after the elution of *trans* -18:1 (Fig. 1D, method 2). If necessary, the time that the column is maintained at 190°C may be slightly adjusted to optimize the separation of *c* 11-20:1 in between *t* 9,*c* 12,*c* 15-18:3 and 18:3n-3 (i.e., *c* 11-20:1 may be eluted closer to*t* 9,*c* 12,*c* 15-18:3 by increasing the time at 190°C). After the elution of 18:3n-3 the elution temperature is increased to 225°C to elute the remaining analytes. The retention time of 21:0 was used as reference point to start the final temperature gradient to 225°C,

which provided the elution of all FAME contained in the Supelco 37 mix in 71 minutes. To increase the reproducibility of separations the samples were injected back-to-back with the column re-equilibration time set to 5 min, and the column was conditioned 25 min at 225°C prior to the first injection.

The negative temperature program modification (method 2) was applied to the analysis of the Supelco 37 FAME mix, a canola oil, a PHO, a shortening blend (containing PHO and palm oil), and butter (BCR 632A) (Fig 2, 3, 4). The proposed modifications provided the separation of all PUFA contained in butter (Fig 4); however, the final ramp may be more precisely tuned if the resolution of other specific PUFA eluting after 18:3n-3 is necessary. While this adaptation of the Golay *et al.*method adds some complexity to the temperature program, the resolution of the overlap between *c* 11-20:1 and*t* 9,*c* 12,*c* 15-18:3 provides a remarkable gain in method versatility by providing accurate measurement of all TFA occurring in PHO and RVO. If the GC is not capable of negative temperature gradients, or more simplicity is desired, the introduction of the 184°C plateau is a suitable compromise.

Conclusions

The addition of one (or two) intermediate temperature program steps to the method developed by Golay *et al* [7] for the analysis of FA in dairy products and infant formula extends the method to the analysis of PHO and RVO. While the addition of an isothermal plateau at 184°C (method 1) achieves the separation of *trans* -18:3 from*c* 11-20:1, the second proposed modification including a negative temperature gradient also provided greater separation between*t* 13/*t* 14-18:1 and *c* 9-18:1 (method 2).

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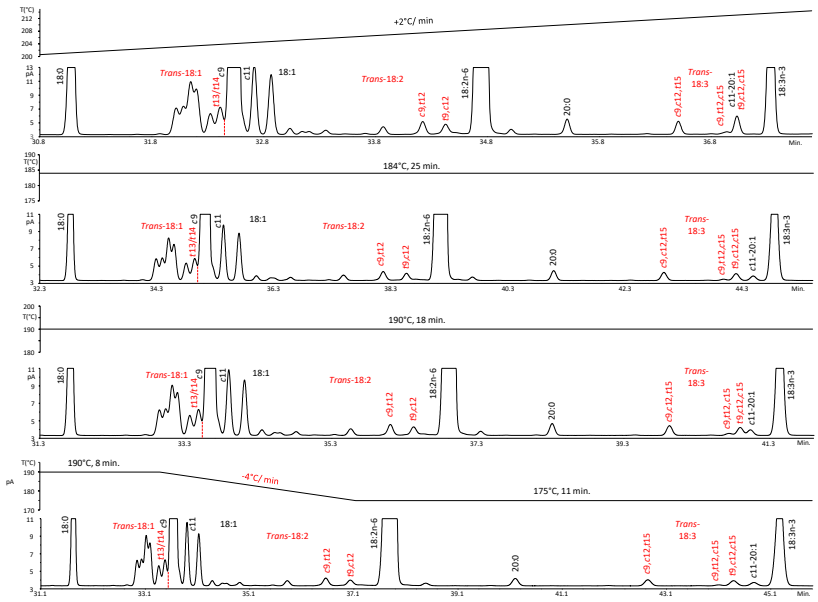
Figures

Figure 1. Partial separation from 18:0 to 18:3n-3 of a 2:1 blend of PHO and soybean oil. Temperature program: 60°C for 5 min, 15°C/min to 165°C, hold 1 min, added ramp, 2°C/min to 225°C, hold 20 min. Added ramp, from the top: A) none; B) 2°C/min to 184°C, hold 25 min; C) 2°C/min to 190°C, hold 18 min; D) 2°C/min to 190°C, hold 8 min, ramp -4°C/min to 175°C, hold 11 min. For panels B-D, final ramp to 225°C was increased to 4°C/min.

Figure 2. Partial separation from solvent front to 18:0 of Supelco 37 FAME mix and selected edible fats. Supelco SP2560 capillary column (100 m x 0.25 mm), hydrogen carrier at 1 mL/min. Elution temperature: 60°C for 5 min, ramp 15°C/min to 165°C, hold 1 min, ramp 2°C/min to 190°C, hold 8 min., ramp -4°C/min to 175°C, hold 11 min, ramp 4°C/min to 225°C, hold 15 min.

Figure 3. Partial separation from 18:0 to 18:3n-3 of Supelco 37 FAME mix and selected edible fats. Separation conditions as for figure 2.

Figure 4. Partial separation from 18:3n-3 to 22:6n-3 of Supelco 37 FAME mix and selected edible fats. Separation conditions as for figure 2.



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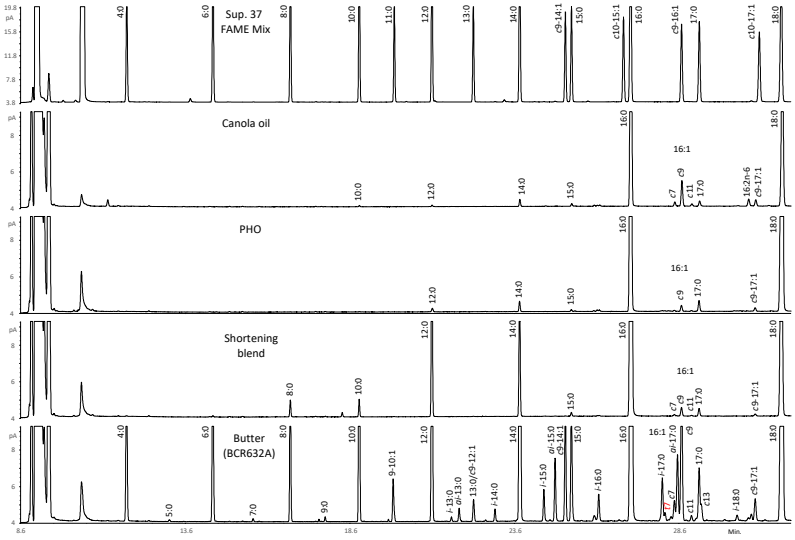
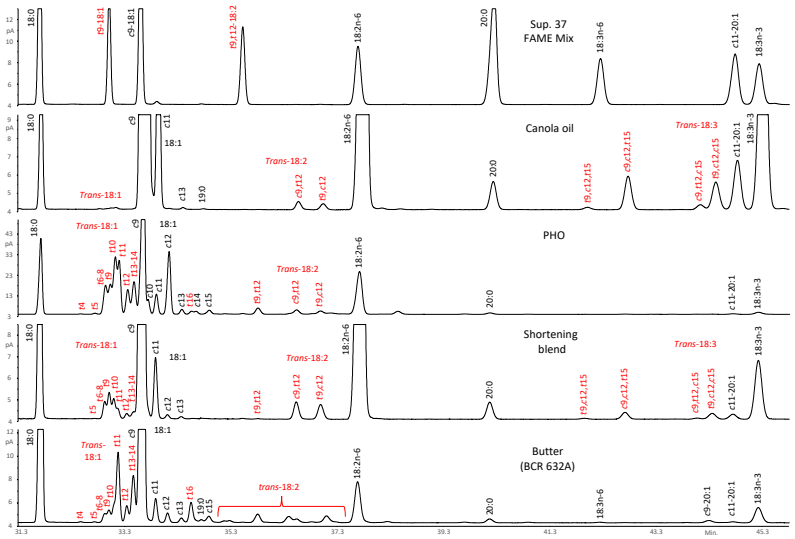


Figure 2: Partial separation from solvent front to 18:0 of Supelco 37 FAME mix and selected edible fats. Supelco SP2560 capillary column (100 m x 0.25 mm), hydrogen carrier at 1 mL/min. Elution temperature: 60°C for 5 min, ramp 15°C/min to 165°C, hold 1 min, ramp 2°C/min to 190°C, hold 8 min., ramp -4°C/min to 175°C, hold 11 min, ramp 4°C/min to 225°C, hold 15 min.



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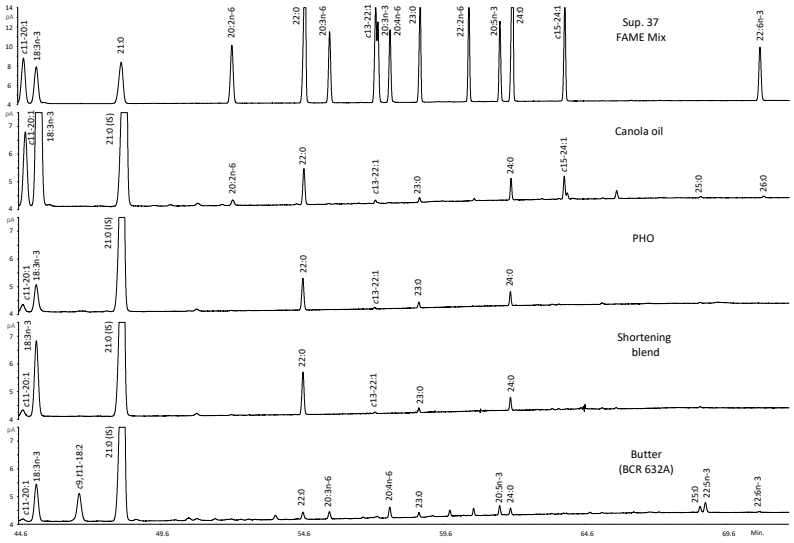

Figure 4: Partial separation from 18:3n-3 to 22:6n-3 of Supelco 37 FAME mix and selected edible fats. Separation conditions as for figure 2.