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Separation of *cis/trans* fatty acid isomers on gas chromatography compared to the Ag-TLC method

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RESUMEN

Separación de isómeros *cis*/*trans* de ácidos grasos mediante cromatografía de gases, comparación con el método Ag-TLC.

El presente estudio investiga la separación de isómeros *cis*/*trans* de ácidos grasos mediante cromatografía de gases (GC) utilizando una columna de SP2560 de 75 m y diferentes condiciones que incluyen un programa isotérmico y una programación temperatura-tiempo. La programación temperatura-tiempo mostró una mejor separación de isómeros cis / trans de los ácidos grasos $C_{14:1}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ y $C_{18:3}$ con los ácidos grasos de cadena corta en comparación con el programa isotermo. La separación de los isómeros trans/*trans* de los ácidos grasos $C_{18:1}$ incluyendo ácido elaídico ($C_{18:1}$ Δ9t) y ácido vaccénico ($C_{18:1}$Δ11t) fué difícil mediante programación temperatura-tiempo. La cromatografía en capa fina impregnada con nitrato de plata (Ag-TLC) se realizó para separar fracciones cis/trans y el análisis de la fracciones se llevó a cabo mediante GC. El análisis GC mostró co-elución de los isómeros *trans* del ácido graso $C_{18:1}$. Así, el estudio muestra que una programación temperatura-tiempo en GC con columna de cianopropilo altamente polar es suficiente para resolver los ácidos grasos *trans*, junto con los ácidos grasos de cadena corta cuando un gran número de muestras tiene que ser analizado.

PALABRAS CLAVE: Ácidos grasos trans – Cromatografía de gases – Fraccionamiento Ag-TLC – Resolución

SUMMARY

Separation of *cis/trans* fatty acids isomers on gas chromatography compared to the Ag-TLC method

The present study investigates the separation of the *cis/trans* isomers of fatty acids on the 75 m SP2560 column under different gas chromatographic (GC) conditions including an isothermal program and a time-temperature program. The time-temperature program showed improved separation of *cis/trans* isomers of $C_{14:1}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ fatty acids along with short chain fatty acids compared to the isothermal program. The separation of *trans/trans* isomers of $C_{18:1}$ fatty acids including elaidic acid ($C_{18:1}$ Δ9t) and vaccenic acid ($C_{18:1}$ Δ11t) was difficult with the time-temperature program. The thin layer chromatography impregnated with silver nitrate (Ag-TLC) method was performed to separate *cis/trans* fractions and GC analysis was carried out with the *trans* fraction. But GC analysis showed a co-elution of *trans* isomers of $C_{18:1}$ fatty acid. Thus the study shows that a time-temperature programmed GC method with the highly polar cyanopropyl column is sufficient to resolve *trans* fatty acids along with short chain fatty acids when a large number of samples has to be analyzed.

KEY WORDS: Ag-TLC fractionation – Gas chromatography – Resolution – Trans fatty acids.

1. INTRODUCTION

Trans fat comprises unsaturated fatty acids having one or more isolated double bonds in the *trans* geometric configuration (U.S.FDA, 2003). *Trans* fatty acids (TFA) in food originate from three major sources, 1) through partial hydrogenation of fats, 2) high-temperature processing of edible oils, 3) from the natural occurrence of TFA in ruminant meat and dairy products (Valenzuela *et al.,* 1995; Mjøs, 2005; Tsuzuki *et al.,* 2010). Due to the presence of TFA, partially hydrogenated oils have some commercial advantages over many non-hydrogenated oils, such as longer shelf life, solidity at room temperature and greater stability during high temperature commercial deep frying (Micha and Mozaffarian, 2008). Several epidemiological and clinical studies on the health effects of TFA published in the last few years indicate that TFA intake may be a risk factor for cardiovascular disease (Mozaffarian *et al.,* 2006; Valenzuela *et al.,* 2011). Analysis of *trans* isomers of fatty acids has taken on more importance, since the world health organization recommended that the intake of *trans* fat should be limited to < 1% of the overall energy intake (Fournier *et al.,* 2007). In 2003, the U.S. food and drug administration (FDA) also passed a labeling requirement for *trans* fat in packaged food products, effective January1, 2006, requiring it to be reported on the nutrition label if present at ≥0.5g/serving, but allowed for it to be declared zero if the product contained <0.5g/serving. In the context of food labeling and nutrition *trans* fat does not include fatty acids with conjugated *trans* double bonds. Several analytical methods are reported for the determination and quantification of TFA in food samples including gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), thin layer chromatography impregnated with silver nitrate (Ag-TLC), liquid chromatography impregnated with silver nitrate (Ag-LC) and Fourier

transformed infrared spectroscopy (FT-IR). Each of these methods exhibits advantages and drawbacks. GC can be accomplished with equipment available in many laboratories; it detects lower levels of TFA and allows for the quantification of individual fatty acids in the course of determining a full fatty acid profile (Phillips *et al*., 2010; Juanéda *et al.,* 2007; Molkentin and Precht, 1995; Ratnayake and Beare-Rogers, 1990; Ledoux *et al.,* 2000; Huang *et al.,* 2006; Aro *et al.,* 1998; Delmonte and Rader, 2007; Tasan *et al.,* 2011).

Ratnayake *et al.,* (2002) discussed the temperature sensitive resolution of *cis/trans* isomers of fatty acid isomers in PHF on 100 m SP2560 and CP-Sil88 capillary columns on GC, which eventually lead to the approval of AOCS Ce 1h-05 method for determination of *cis/trans* fatty acids vegetable or non-ruminant fats and oils (Ratnayake *et al.,* 2006). An analysis of ruminant fats including butter with shorter chain fatty acids cannot be separated by this method. Moreover, a single method cannot be applied to the same type of column with different lengths. In this context, the present study discusses the variations in resolution between *cis/trans* isomers of major fatty acids along with short chain fatty acids at different chromatographic conditions on the 75 m SP 2560 column. We have also compared the GC method with Ag-TLC coupled with the GC method and highlighted its advantages and disadvantages.

2. MATERIALS AND METHODS

2.1. Reagents and standards

Standards of fatty acids methyl esters (FAME) ($C_{4:0}$ Methyl butyrate, $C_{6:0}$ Methyl caproate, $C_{8:0}$ Methyl capryllate, $C_{10:0}$ Methyl caprate, $C_{12:0}$ Methyl laurate, $C_{14:0}$ Methyl myristate, $C_{14:1}$-9c Methyl myristoleate, $C_{14:1}$-9t Methyl myristelaidate, $C_{16:0}$ Methyl palmitate, $C_{16:1}$-11c Methyl palmitoleate, $C_{16:1}$-11t Methyl palmitelaidate, $C_{18:0}$ Methyl stearate, $C_{18:1}$-9c Methyl oleate, $C_{18:1}$-9t Methyl elaidate, $C_{18:1}$-11t Methyl vaccenate, $C_{18:2}$-9c, 12c linoleate (cis/*trans* isomer mixture), $C_{18:2}$-9c, 11c & 10c, 12c linoleate (CLA *cis*/*trans* isomer mixture), $C_{18:3}$-9c, 12c, 15c Methyl linolenate (*cis/trans* isomer mixture), $C_{20:0}$ Methyl arachidate, $C_{20:5}$ Methyl eicosapentaenoate, $C_{22:6}$ Methyl docosahexanoate), Supelco 37 component FAME standard mixture were purchased from Sigma (Steinheim, Germany). Silver nitrate ($AgNO_3$), Silica Gel 'G' for Thin layer chromatography (TLC) with particle size 10-40 microns and TLC Aluminum sheets (20×20 cm) with silica gel 60 F_{254} and High Performance Liquid Chromatography (HPLC) grade solvents were purchased from Merck (Darmstadt, Germany).

2.2. Samples for study

For the present study partially hydrogenated fat (PHF), butter, puff and cake samples were collected from local market and subjected to further analysis.

2.3. ANALYTICAL METHODS

2.3.1. Sample preparation

Fat extraction was carried out according to the Folch extraction method (Folch *et al*., 1957). This method was selected as the reference for fat extraction because of its mild working conditions, which avoid potential alterations of the extracted fat (Ruiz-Jiménez *et al*., 2004). Fatty acid methyl esters (FAME) were prepared by esterifying them with alcoholic sulfuric acid reagent according to the International Union of Pure and Applied Chemistry (IUPAC) procedure (IUPAC, 1987).

2.3.2. GC analysis

The FAME was analyzed using a Shimadzu GC 2010 fitted with a split injector (250 °C) and a flame ionization detector (FID 300 °C). A highly polar cyanopropyl capillary column, SP-2560 (Supelco column 75m, 0.18mm i.d. with 0.14 µm film thickness) was used and operated at a) isothermal program at 180 °C, b) time-temperature program which included an 80 °C hold for 2 min to 200 °C with a 10 °C min^{-1} raise, hold for 30 min. followed by a further increase to 220 °C with a 10 °C min^{-1} raise and hold for 20 min. This program was selected after checking the resolution between different *cis/trans* fatty acids at different time-temperature ramping modes. Nitrogen was used as carrier gas at a linear velocity of 13.0 cm s^{-1}. Another experiment was performed using helium as carrier gas to check the effect of the carrier gas on the resolution between fatty acids at the same time-temperature program mentioned above at a linear velocity of 13.0 cm s^{-1} and also at 17.0 cm s^{-1}. FAME were identified by comparing their retention time with authentic standards, and the peaks were quantified using digital integration according to the AOCS official method Ce 1-62 (AOCS, 2003). The fatty acid levels were reported as the relative proportions of the total composition.

2.3.3. Resolution

Resolution (R_s) is the measurement of separation between two eluted peaks. Resolution is the most important parameter to optimize a chromatographic method. The resolution, R_s, between two peaks in a chromatogram is usually calculated by,

$$R_s = 2(t_{R(2)} - t_{R(1)})/(w_{h(1)} + w_{h(2)}) \quad (1)$$

Where $t_{R(1)}$ and $t_{R(2)}$ are the retention times of the two peaks and $w_{h(1)}$ and $w_{h(2)}$ are the corresponding peak widths at half peak height (Ettre, 1993).

2.3.4. LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) are important factors in the optimization in

any chromatographic analysis, which depends on instrumental sensitivity. LOD and LOQ for TFA analysis in this study were calculated by signal to the noise ratio of individual FAME components (Ettre, 1993).

2.3.5. Separation efficiency

In addition to the selectivity of a chromatographic method for the particular compound of interest, the efficiency should also be taken into consideration. To evaluate the efficiency of an isothermal method, separation factor (α) values are taken into account and are calculated by,

$$\alpha = t_{R(2)}/t_{R(1)} \quad (2)$$

While, efficiency in a temperature programmed GC is usually evaluated by the separation number, SN, which is the number of peaks that can be resolved between two members of a homologous series i.e two fatty acids differ by one methylene group (for example $C_{14:0}$, $C_{16:0,}$ $C_{18:0}$ or $C_{14:1}$, $C_{16:1,}$ $C_{18:1}$). SN is usually calculated by,

$$SN = (t_{R\,(z+1)} - t_{R\,(z)}/w_{h(z+1)}+w_{h(z)}) - 1 \quad (3)$$

Where, $t_{R(z\,+\,1)}$ and $t_{R(z)}$ are the retention times of the two homologous fatty acids and $w_{h(z\,+\,1)}$and $w_{h(z)}$ are the corresponding peak widths at half peak height (Ettre, 1993).

2.3.6. Ag-TLC method

Ag-TLC fractionation of FAME was performed to separate the *trans* fraction from the *cis* fraction. Preparative Ag-TLC plates were prepared by incorporating 10% silver nitrate into the silica slurry in distilled water and applied onto glass plates and activated by drying at 100 °C for 1 hour (Morris, 1966). The FAME of PHF, butter, vaccenic and elaidic acid were spotted onto Ag-TLC plates and developed using hexane and diethyl ether in the ratio 80:20. Saturated, *cis* and *trans* fractions were located under U.V light and scrapped out from the prepared TLC plate, further re-extracted using hexane and further injected into GC.

3. RESULTS AND DISCUSSION

3.1. Effect of column temperature on resolution

In the isothermal program, the $C_{4:0}$, $C_{6:0,}$ $C_{8:0}$ fatty acid peaks were eluted along with the solvent peak at the early retention time (Figure 1a). We have also observed less resolution between *cis/trans* fatty acids with partial overlapping in the isothermal program. But, in the time-temperature program, there was a distinct separation among all fatty acids including short chain fatty acids. High initial temperatures in the GC program causes overlapping of the solvent front and short chain fatty acid peaks. This fact is the main cause of error in fat analysis (Kramer *et al.,* 2008). In the isothermal program, resolution between *cis/trans* fatty acids was <1.0 (Figure 1b) while, in time-temperature program improved separation was observed. Resolution between *cis/trans* isomers of $C_{14:1}$ fatty acid was 2.9 and between *cis/trans* isomers of $C_{16:1}$ fatty acid was 1.8. Resolution between *cis/trans* isomers of $C_{18:1}$ is an important factor in GC analysis. It was 2.3 between $C_{18:1}$ 9t and $C_{18:1}$ 9c (Figure 2a), while it was 1.8 between $C_{18:1}$ 11t and $C_{18:1}$ 9c (Figure 2b). But the resolution between a mixture of *trans* isomers i.e. between $C_{18:1}$ 9t and $C_{18:1}$ 11t was 0.9 in the time-temperature program and no resolution was observed in the isothermal method. It has been found that a decrease in the operating temperature (180 °C) allows for better separation of the *trans* isomers of $C_{18:1}$ fatty acid (Ratnayake *et al.,* 2002). These results suggest that the time-temperature program is preferable to an isothermal program to separate *cis/trans* isomers of oleic acid, which is predominant in PHF. Resolution between $C_{18:2}$ *tt* and *ct* isomers was 3.6 and between *ct* and *tc* isomers it was 1.5. The resolution obtained between the $C_{18:3}$ isomers was >1. It was 3.9 between *ttt* and *ttc* isomers and 3.1 between *ctt* and ctc isomers. The resolution between *tct* and *ctt* isomers was 1.2. *ttc* and *tct* and *ctc* and *tct* isomers were completely co-eluted (Figure 2c). These results clearly indicate the higher efficiency of the cyanopropyl column to separate fatty acid mixtures in the time-temperature program.

The geometrical isomers of conjugated linoleic acids (CLA), which are major compositions of

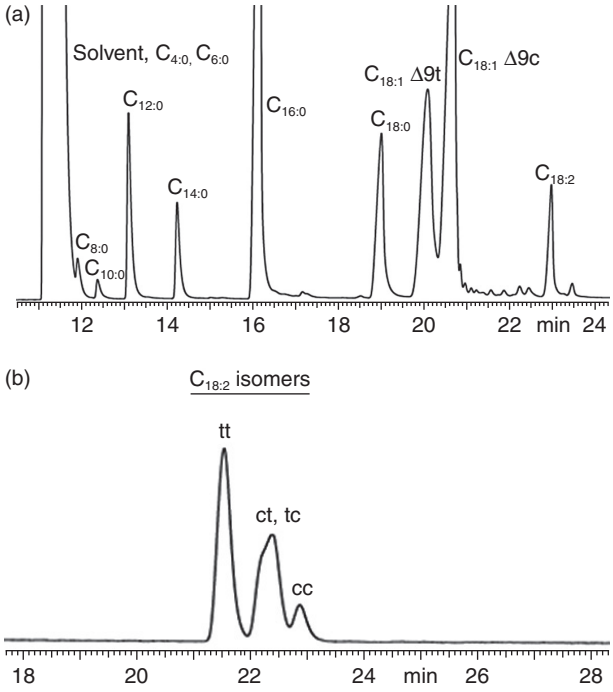

Figure 1
Overlapping between fatty acids in isothermal method (a) GC fatty acid profile of the puff sample (b) Separation of *cis/trans* isomers of linoleic acid methyl ester standard

ruminant derived foods, are difficult to separate by the same method. So, there is a need to follow a different method for the separation of CLA isomers, especially in the analysis of milk and meat products (Kramer *et al.,* 2008). *tt, ct* and *tc* forms of CLA ($C_{18:2}$ Δ8 Δ10, $C_{18:2}$ Δ9 Δ11 and $C_{18:2}$ Δ10 Δ12) are not included in the definition of *trans* fat given by the US FDA (U.S.FDA, 2003). So, CLA has no significance with respective to the labeling of *trans* fat contents. But, Negative effects of conjugated *tt* isomers similar to non-conjugated *tt* isomers are still making question marks regarding CLA and *trans* fat definitions.

3.2. EFFECT OF CARRIER GAS AND LINEAR VELOCITY ON RESOLUTION

Carrier gas is an important factor in chromatography i.e. the separation and the elution order can change when one carrier gas is substituted by another (Berezkin *et al.,* 2003). At the same linear velocity of nitrogen and helium (13.0 cm s^{-1}), resolution between *cis/trans* isomers of fatty acids was similar (Table 1). There was not much change in the resolution or the total run time when the linear velocity was increased. The linear velocity range is more restricted by the choice of length and inner diameter of the column relative to the inlet pressure that the instrument can provide. Linear velocity for GC column was optimized by the retention time of an unretained peak and its length (Ettre, 1993). In our study, we have observed that an increase in linear velocity affects the resolution between *cis/trans* isomers of fatty acids. Maintaining the medium linear velocities gives good resolution between fatty acids and total run time. Changing from helium to nitrogen and maintaining the same linear velocity would not change the elution pattern. Hydrogen can be used as a carrier gas and it provides very good resolution with a very short run time of analysis. Using hydrogen as a carrier gas is included with risk of explosions, through an electric spark igniting a buildup of gas caused by a leak within the instrument (Christe, 1989). So, nitrogen can be used as carrier gas instead of helium, because it is widely available, inexpensive and recommended for safety purposes.

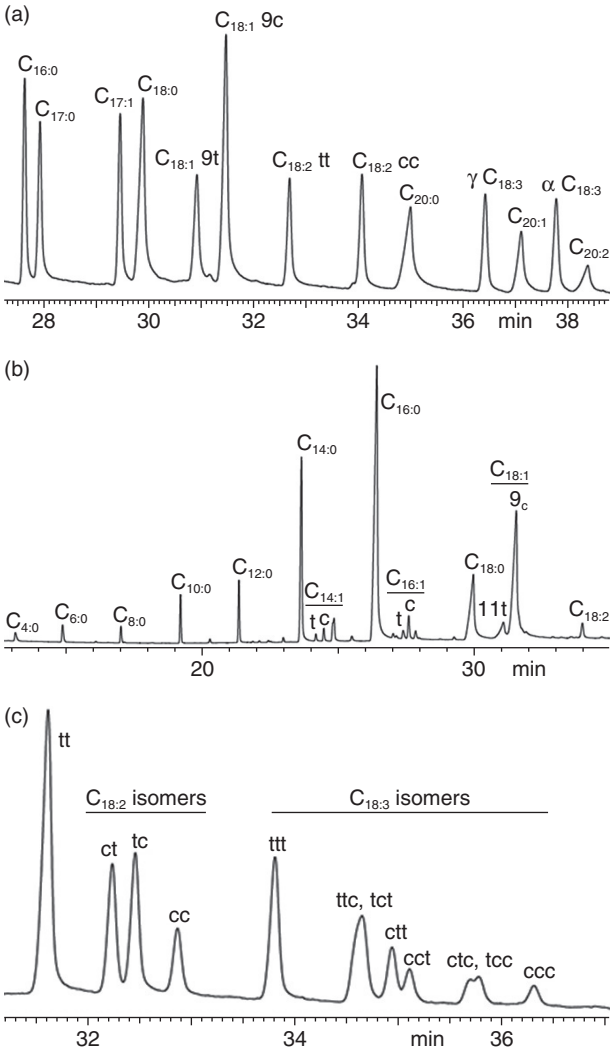

Figure 2
Separation of *cis/trans* isomers in time-temperature programmed method (a) Partial GC profile of 37 component fatty acid reference mixtures (b) Fatty acid profile of butter (c) Separation of standard $C_{18:2}$ and $C_{18:3}$ isomers.

3.3. LOD AND LOQ

LOD and LOQ in any analysis provide the detection limit and quantification limits of compounds of interest. In this study, the estimated LOD and LOQ for individual TFA components were 0.002 mg mL^{-1} and 0.006 mg mL^{-1} respectively. Lower detection limits and quantification limits indicate higher instrumental sensitivity.

3.4. SEPARATION EFFICIENCY

The separation factor for the all fatty acids was > 1 in isothermal conditions. In the time-temperature programmed method, the greater the separation number, the greater the separation will be of compounds in the column. Programmed methods with SN <10 will show poor performance in GC analysis. In the time-temperature programmed method SN between myristic acid methyl ester and palmitic acid methyl ester was 15, between palmitic acid methyl ester and stearic acid methyl ester it was 12. SN between myristoleic acid methyl ester and palmitoleic acid methyl ester was 18. While, it was 19 between palmitoleic acid methyl ester and oleic acid methyl ester, this was reasonably good separation efficiency.

3.5. EFFECT OF AG–TLC FRACTIONATION

TLC plate is generally used for confirmation of trans-esterification reaction completion (Figure 3a). TLC plates with silica gel 60 F_{254} produce yellow-green emissions, upon irradiating with short wave UV

Table 1
Resolution between major fatty acids with different carrier gasses

Fatty acid	Nitrogen as Carrier gas		Helium as Carrier gas	
	t_R [a]	R_s [b]	t_R [a]	R_s [b]
$C_{4:0}$	13.1	–	12.0	–
$C_{6:0}$	14.8	14.8	13.3	11.2
$C_{8:0}$	17.0	22.1	14.0	24.5
$C_{10:0}$	19.2	26.4	16.0	12.9
$C_{12:0}$	21.3	27.2	18.0	13.1
$C_{14:0}$	23.6	24.3	19.9	21.7
$C_{14:1}$ Δ9t	23.8	8.3	20.8	9.6
$C_{14:1}$ Δ9c	24.2	2.9	20.9	1.0
$C_{16:0}$	26.4	8.6	22.1	9.5
$C_{16:1}$ Δ9t	27.3	5.8	22.9	5.3
$C_{16:1}$ Δ9c	27.5	1.8	23.0	1.6
$C_{18:0}$	29.9	3.8	24.9	3.5
$C_{18:1}$ Δ9t	30.9	4.7 (PHF)	25.5	4.7 (PHF)
$C_{18:1}$ Δ11t	31.0	4.2 (Butter)	25.7	3.4 (Butter)
$C_{18:1}$ Δ9c	31.5	2.3 (Δ9t) 1.8(Δ11t)	26.0	2.2 (Δ9t) 1.6 (Δ11t)
$C_{18:2}$ Δ9t Δ12t	33.1	3.4	26.8	3.6
$C_{18:2}$ Δ9c Δ12t	33.6	3.6	27.3	3.6
$C_{18:2}$ Δ9t Δ12c	33.9	1.5	27.5	1.5
$C_{18:2}$ Δ9c Δ12c	34.3	2.6	27.8	2.6
$C_{18:3}$ Δ9t Δ12t Δ15t	35.0	5.3	28.5	5.4
$C_{18:3}$ Δ9t Δ12t Δ15c	35.9	3.9	29.1	4.3
$C_{18:3}$ Δ9t Δ12c Δ15t	36.2	–	29.4	–
$C_{18:3}$ Δ9c Δ12t Δ15t	36.3	1.2	29.5	1.6
$C_{18:3}$ Δ9c Δ12t Δ15c	36.9	3.1	30.0	1.2
$C_{18:3}$ Δ9t Δ12c Δ15c	37.3	–	30.2	–
$C_{18:3}$ Δ9c Δ12c Δ15c	37.6	2.3	30.4	3.0

[a] Retention time; [b] Resolution

light (λ = 254nm). It is due to an additive, a so-called "fluorescence indicator", e.g. manganese-activated zinc silicate, that is usually mixed with the sorbent (Elke Hahn-Deinstrop, 2006). Usually, Fatty acids do not separate on a normal TLC plate (Figure 3a). But, on an Ag-TLC plate, *cis* fatty acids form a complex with silver ions on TLC, more strongly than *trans* fatty acids. It is due to the steric hindrance of the *trans* bond. It causes the *trans* fraction to separate from the *cis* fraction, which elutes on the Ag-TLC plate. Due to the lack of double bonds, mobility of saturated fatty acids is higher, which elutes near the solvent front. The order of separation of fatty acids is *cis, trans* and saturated forms (Figure 3b). The Ag-TLC method was found to be a good alternative for TFA analysis in samples having interferences that cause inaccurate results in the analysis of some complex food matrices in cyanopropyl columns (Mjøs and Haugsgjerd, 2011). Common interferences for TFA analysis in a cyanopropyl column are presented in Table 2. Results have been published based on the separation of linoleic and linolenic acid isomers through Ag-TLC (Elke Hahn-Deinstrop, 2006). But, the GC method could also successfully resolve *cis/trans* isomers and also *trans/trans* isomers of $C_{18:2}$ and $C_{18:3}$ fatty acids. But, the separation of the mixture of *trans/trans* isomers of oleic acid i.e. $C_{18:1}$ Δ9t and $C_{18:1}$ Δ11t by the Ag-TLC method is still limited. Figure 4 shows the co-elution of elaidic acid and vaccenic acid even after separation on an Ag-TLC plate. Plate lengths, activating time, the polarity of the solvent system are other critical parameters that affect the separation of *cis/trans* fatty acids on the Ag-TLC plate. Usually, impregnated plates are activated at 110 °C for 30 min for thin layers or for 60 min for thicker layers (1 mm). One serious difficulty in preparing plates is

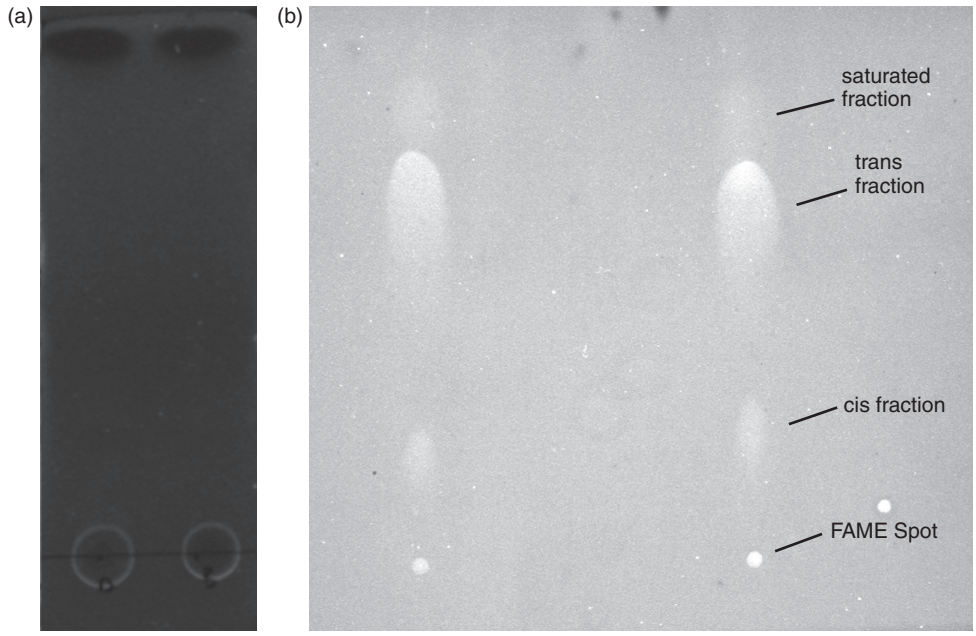

Figure 3
(a) Separation of PHF and butter FAME on pre-coated TLC plate
(b) Separation of PHF and butter FAME on Preparative Ag-TLC plate.

caused by the interaction of the silver nitrate solution with the metal of conventional plate spreaders. This interaction has two effects; metallic silver is precipitated onto the layers being prepared and the spreader itself becomes rapidly pitted and corroded until, eventually, it is useless (Morris, 1966). An Ag-HPLC method for the separation of *cis*/*trans* isomers has been reported (Momchilova *et al.,* 1998).

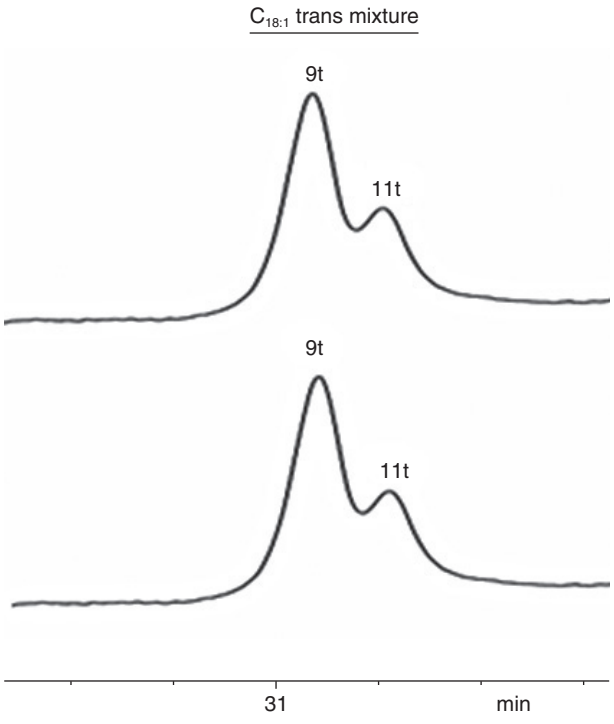

Figure 4
Separation of mixture of elaidic acid and vaccenic acid on GC, before and after Ag-TLC fractionation.

Fractionation by liquid chromatography prior to GC analysis can eliminate the co-elution of fatty acids belonging to different classes, but incorrect practices might lead to greater measurement errors than those caused by the co-elution that affect the direct GC analysis (Aro *et al.,* 1998). So, the GC method can be followed instead of Ag-TLC coupled with the GC method for the analysis of a large number of samples. But, in practice, it is difficult to separate all uncommon geometrical isomers of fatty acids in a GC column, which can be prepared chemically by *p*-toluene sulfonic acid isomerization or single step bromination and de-bromination. Recently, it has been found that SLB-IL-111, a highly polar ionic liquid coated capillary column can be used to separate *cis/trans* and *trans/trans* isomers of conjugated and non-conjugated fatty acids with less overlapping among fatty acids by simple GC analysis (Ragonese *et al.,* 2009; Delmonte *et al.,* 2011).

4. CONCLUSIONS

In this study, the separation of *cis/trans* isomers of fatty acids was performed in a 75m SP2560 column. Improved separation of *cis/trans* isomers of the $C_{14:1,}$ $C_{16:1,}$ $C_{18:1,}$ $C_{18:2,}$ $C_{18:3}$ fatty acids and short chain fatty acids was observed in the time-temperature program compared to the isothermal program. The separation of a mixture of *trans* isomers of oleic acid i.e. $C_{18:1}$ Δ9t and $C_{18:1}$ Δ11t with high resolution through simple GC technique is quite challenging. Ag-TLC fractionation prior to applying the GC method was proven to be a better option to analyze *cis/trans* isomers of fatty acids accurately. But, the separation of a mixture of *trans* isomers by further GC analysis is limited

Table 2
Possible interferences in the TFA analysis in cyanopropyl columns

***Trans* fatty acid**	**Possible interference**
$C_{14:1}$ Δ9t (Myristelaidic acid)	–
$C_{16:1}$ Δ9t (Palmitelaidc acid)	a) cis 16:1n9 b) cis 16:1n11 c) Iso 17:0 (15-methyl hexadecanoic acid) d) ante-iso 17:0 (15-methyl hexadecanoic acid) e) Phytanic acid (3,7,11,15-Tetramethyl hexadecanoic acid)
$C_{18:1}$ Δ6t (petroselaidic acid) $C_{18:1}$ Δ9t (elaidic acid) $C_{18:1}$ Δ11t (*trans*vaccenic acid)	a) cis 16:3n3 b) cis 16:3n3 c) cis 16:4n3 d) cis 18:1n11
$C_{18:2}$ Δ9t Δ12t (Linoleic acid *trans* forms) $C_{18:2}$ Δ9c Δ12t $C_{18:2}$ Δ9t Δ12c	a) cis 16:4 n1 b) cis 16:4n3 c) 19:0
$C_{18:2}$ Δ7c Δ9c, Δ9c Δ11c & Δ10c Δ12c *trans* forms (tt, ct & tc forms) i.e CLA (Not included in the definition of *trans* fat by USFDA)	21:0
$C_{18:3}$ Δ9t Δ12t Δ15t (Linolenic acid *trans* forms) $C_{18:3}$ Δ9t Δ12t Δ15t $C_{18:3}$ Δ9t Δ12t Δ15c $C_{18:3}$ Δ9t Δ12c Δ15t $C_{18:3}$ Δ9c Δ12t Δ15t $C_{18:3}$ Δ9c Δ12c Δ15t $C_{18:3}$ Δ9c Δ12t Δ15c $C_{18:3}$ Δ9t Δ12c Δ15c	a) cis 18:3 n4 b) cis 18:3 n6 c) cis 20:0 d) cis 20:1 n9 e) cis 20:1 n11
$C_{20:1}$ Δ13t (Brassidic acid)	–
$C_{20:5}$ Δ5 Δ8 Δ11 Δ14 Δ17 (EPA) trans forms	a) 20:4n3 b) 22:0 c) 22:1
$C_{22:6}$ Δ4 Δ7 Δ10 Δ13 Δ16 Δ19 (DHA) trans forms	–

especially for $C_{18:1}$ FAME isomers. Moreover, the Ag-TLC method is more expensive and time consuming. A highly polar cyanopropyl column can be used for the analysis of TFA with a simple GC technique for a large number of samples. This work also suggests that the chromatographers' need to optimize the methods for TFA analysis depends on the column length.

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REFERENCES

AOCS. 2003. *Official methods and recommended practices of the American Oil Chemist's Society*. In Firestone (Ed.), 5th edition, Champaign. IL.

Aro A, Kosmeijer-Schuil T, Bovenkamp P, Hulshof P, Zock P, Katan M. 1998. Analysis of C18:1 *cis* and *trans* fatty acid isomers by the combination of gas-liquid chromatography of 4,4-dimethyloxazoline derivatives and methyl esters. *J. Am. Oil Chem. Soc.* **75**, 977-985.

Berezkin VG, Zagainov VF, Ivanov PB. 2003. Carrier gas as a new factor influencing the selectivity of the gas–stationary liquid phase chromatographic system. *J. Chromatogr. A* **985**, 57-62.

Christe WW. 1989. *Gas chromatography and lipids: a practical guide*. Oily press limited. First edition.

Delmonte P, Rader J. 2007. Evaluation of gas chromatographic methods for the determination of *trans* fat. *Anal. Biochem.* **389**, 77-85.

Delmonte P, Fardin Kia A-R, Kramer JKG, Mossoba MM, Sidisky L, Rader JI. 2011. Separation characteristics of fatty acid methyl esters using SLB-IL111, a new ionic liquid coated capillary gas chromatographic column. *J. Chromatogr. A* **1218**, 545-554.

Elke Hahn-Deinstrop. 2006. *Applied Thin Layer Chromatography. Best practice and Avoidance of Mistakes.* WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Second Edition.

Ettre LS. 1993. Nomenclature for chromatography. *Pure Appl. Chem.* **65**, 819.

Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497-509.

Fournier V, Destaillats F, Hug B, Golay P-A, Joffre F, Juanéda P, Sémon E, Dionisi F, Lambelet P, Sébédio J-L, Berdeaux O. 2007. Quantification of eicosapentaenoic and docosahexaenoic acid geometrical isomers formed during fish oil deodorization by gas-liquid chromatography. *J. Chromatogr. A* **1154**, 353-359.

Huang Z, Wang B, Crenshaw AA. 2006. A simple method for the analysis of *trans* fatty acid with GC–MS and AT™-Silar-90 capillary column. *Food Chem.* **98**, 593-598.

IUPAC. 1987. *Standard methods for the analysis of oils and fats and derivatives*. 7th Ed., London: Blackwell Scientific Publications.

Juanéda P, Ledoux M, Sébédio JL. 2007. Analytical methods for determination of *trans* fatty acid content in food. *Eur. J. Lipid Sci. Technol.* **109**, 901-917.

Kramer J, Hernandez M, Cruz-Hernandez C, Kraft J, Dugan M. 2008. Combining Results of Two GC Separations Partly Achieves Determination of All *cis* and *trans* 16:1, 18:1, 18:2 and 18:3 Except CLA Isomers of Milk Fat as Demonstrated Using Ag-Ion SPE Fractionation, *Lipids* **43**, 259-273.

Ledoux M, Laloux L, Wolff RL. 2000. Analytical methods for determination of *trans*-C18 fatty acid isomers in milk fat. A review. *Analysis* **28**, 402.

Micha R, Mozaffarian 2008. *Trans* fatty acids: effects on cardiometabolic health and implications for policy. *Prostag. Leukotr. Ess.* **79**, 147-152.

Morris LJ. 1966. Separations of lipids by silver ion chromatography. *J. Lipid. Res.* **7** (6):717-732

Mjøs SA. 2005. Properties of *trans* isomers of eicosapentaenoic acid and docosahexaenoic acid methyl esters on cyanopropyl stationary phases. *J. Chromatogr. A* **1100**, 185-192.

Mjøs SA, Haugsgjerd BO. 2011. Trans Fatty Acid Analyses in Samples of Marine Origin: The Risk of False Positives. *J. Agric. Food Chem.* **59**, 3520-3531.

Molkentin J, Precht D. 1995. Optimized analysis *trans*-octadecenoic acids in edible fats. *Chromatographia* **41**, 267-272.

Momchilova S, Nikolova-Damyanova B, Christie WW. 1998. Silver ion high-performance liquid chromatography of isomeric cis- and trans-octadecenoic acids: Effect of the ester moiety and mobile phase composition. *J. Chromatogr. A* **793**, 275-282.

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. 2006. *Trans* Fatty Acids and Cardiovascular Disease. *New Engl. J. Med.* **354**, 1601-1613.

Phillips K, Ruggio D, Amanna K. 2010. Optimization of Standard Gas Chromatographic Methodology for the Determination of *Trans* Fat in Unlabeled Bakery Products. *J. Food Anal. Methods* **3**, 277-294

Ragonese C, Tranchida PQ, Dugo P, Dugo G, Sidisky LM, Robillard MV, Mondello L. 2009. Evaluation of Use of a Dicationic Liquid Stationary Phase in the Fast and Conventional Gas Chromatographic Analysis of Health-Hazardous C18 Cis/Trans Fatty Acids. *Anal. Chem.* **81**, 5561-5568.

Ratnayake WMN, Beare-Rogers JL. 1990. Problems of Analyzing C18 Cis- and Trans-Fatty Acids of Margarine on the SP-2340 Capillary Column. *J. Chromatogr. Sci.* **28**, 633-639.

Ratnayake WMN, Plouffe JL, Pasquier E, Gagnon C. 2002. Temperature-sensitive resolution of *cis*- and *trans*-fatty acid isomers of partially hydrogenated vegetable oils on SP-2560 and CP-Sil 88 capillary columns. *J. Am. Oil Chem. Soc.* **85**, 1112-1118.

Ratnayake WMN, Hansen LS, Kennedy PM. 2006. Evaluation of the CP-Sil 88 and SP-2560 GC Columns Used in the Recently Approved AOCS Official Method Ce 1h-05: Determination of cis-, trans- , Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-ruminant Animal Oils and Fats by Capillary GLC Method. *J. Am. Oil Chem. Soc.* **83**, 475-488.

Ruiz-Jiménez J, Priego-Capote F, de Castro MD. 2004. Identification and quantification of *trans* fatty acids in bakery products by gas chromatography–mass spectrometry after dynamic ultrasound-assisted extraction. *J. Chromatogr. A* **1045,** 203-210.

Tasan M, Gecgel U, Demirci M. 2011. Comparison of geometrical isomerization of unsaturated fatty acids in selected commercially refined oils. *Grasas Aceites* **62**, 284-289.

Tsuzuki W, Matsuoka A, Ushida K. 2010. Formation of *trans* fatty acids in edible oils during the frying and heating process. *Food Chem.* **123**, 976-982.

U.S. Food and Drug Administration 2003. Food labeling: *trans* fatty acids in nutrition labeling, nutrient content claims, and health claims. 68 Federal Register 41433-41506 (codified at21 CFR §101).

Valenzuela A, King J, Nieto S. 1995. *Trans* fatty acid isomers from hydrogenated fats: The controversy about health implications. *Grasas Aceites* **46,** 357-365.

Valenzuela A, Delplanque B, Tavella M. 2011. Stearic acid: a possible substitute for *trans* fatty acids from industrial origin. *Grasas Aceites* **62,** 131-138.

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