CONCLUSION European Federation for the Science and Technology of Lipids: 2001, l'EFL prend ses marques dans l'espace européen des lipides…

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Summary : One year ago, as President of the AFECG, I expressed in this tribune what was my personal feeling about the opportunity of building an European Federation with our partners of the DGF (Germany), the KNCV (the Netherlands) and the O & F Group of the SCI (UK). In this paper "Lipids in Europe, goals and ways" I discussed the way that was run from the Eurolipid in Angers, 1989, throughout the eclipse of the nineties, to deal with a new approach by presidents Spener and Vermeersch in the end of the year 1998.

Résumé : Il y a un an dans ces mêmes pages, je vous faisais part de mes réflexions sur la nécessité de créer une Association européenne des lipides à l'initiative conjointe de quatre organisations nationales : la DGF allemande, la KNCV hollandaise, le groupe O & F de la SCI britannique et l'AFECG française. Dans cette tribune intitulée « l'Europe des lipides, enjeux et méthodes », je retraçais le chemin parcouru depuis l'Eurolipid d'Angers, l'éclipse totale des années 90, puis la démarche relancée par les présidents Spener et Vermeersch fin 1998. Et, surtout, j'exprimais en quelques lignes les convictions qui guidaient mon action en qualité de président de l'AFECG pour les années charnières 1999 et 2000. Il est vrai que la plus grande part de mon activité dans l'AFECG a été consacrée à faire avancer, avec quelques collègues et amis européens, cette nouvelle approche commune, faite à la fois de pragmatisme, de négociation, de petits et de grands pas, mais avant tout de conviction et de foi dans un avenir partagé.

ARTICLE

Introduction

Conjugated linoleic acid (CLA) is a generic term to describe 18:2 isomers containing two conjugated double bonds [1-3]. CLA has been recently shown to have beneficial effects on health parameters [4-6]. CLA are available as commercial mixtures and they are present in natural products. For example, CLA is naturally present in dairy products [7-9], meat from ruminants [10, 11], where the 9*c*,11*t*-18:2 represents more than 80-85% of the total CLA isomers.

It can also be formed during partial hydrogenation of vegetable oil and is therefore present in margarine [12]. CLA has also been detected in sunflower oil heated under drastic conditions in the laboratory [13]. However the mixtures produced by processing, especially frying, seem to be of different composition compared to that naturally present in milk fat or obtained by chemical reaction [14].

The commercial CLA mixtures, produced by chemical reaction, contain-ed mainly the 9*c*,11*t*- and 10*t*,12*c*-18:2 [2, 3] accompanied by smaller quantities of 8*t*,10*c* and 11*c*,13*t*-18:2 and of 8,10- ; 9,11- ; 10,12- ; 11,13- *cis/cis* and *trans/trans* isomers.

In order to elucidate the structure of the CLA isomers produced during frying treatment, heat-treated oils were collected from restaurants. The CLA mixtures were analysed by gas chromatography (GC) and silver-ion high-performance liquid chromatography (HPLC). Their structures were studied by gas chromatography coupled with mass spectrometry (GC-MS).

Experimental

Chemicals and samples

All solvents were distilled before used. For HPLC, acetonitrile was of UV grade (SDS, Peypin, France). The chemical reagents were obtained from Sigma (L'Isle d'Abeau, France). Heated oil samples were collected from restaurants by the Laboratoire Interrégional de la Répression des Fraudes (Massy, France).

Oil analyses

The amount of polar compounds was determined according to the standardized method [15].

The samples were transesterified using sodium methoxide (2N) [16] followed by BF3/methanol (14%) at room temperature. The fatty acid methyl esters (FAME) were analysed by GC and silver-ion HPLC, either as total FAME or after Reversed-Phase-HPLC fractionation *(Figure 1)*.

Gas chromatography

FAMEs were analysed by GC using an HP5890 serie II chromatograph (Palo Alto, C.A. USA) fitted with a split-splitless injector (250°C) and a FID (250°C). The column was a CP Sil 88 (100m **x** 0,25mm ID, 0,2µm film thickness, Varian SA, Les Ulis, France). After injection in the splitless mode, the oven temperature was programmed from 60°C to 170°C at 20°C/min, and the final temperature was maintained during 50 minutes. Hydrogen was used as carrier gas (0.7ml/min at 60°C). Quantitative analyses were performed using a Borwin integrating system (JMBS, Grenoble, France).

High-Performance Liquid Chromatography

The HPLC analyses were performed using a solvent pump (Varian model 9010, Les Ulis, France), equipped with a Valco compressed air injector fitted with a 100mul loop.

The FAME were fractionated by RP-HPLC [17] using a Nucleosil-C18 column and a refractometer detector [18].

A diode array detector (Jasco model MD 1510, Nantes, France) was used for silver ion -HPLC. Two ChromSpher 5 Lipids columns (250mm **x** 4.6mm ID, 5mum, Varian, Les Ulis, France) were used in series [19]. The mobile phase was a mixture of hexane/acetonitrile (99.9/0.1, v/v) at a flow rate of 1ml/min. UV spectroscopy detection from 190mm to 300mm was applied to detect the conjugated fatty acids (234nm) and the non-conjugated fatty acids (200nm). The qualitative analyses were performed using a Jasco-PDA integrating system (JMBS, Grenoble, France).

Identification and structure determination of the conjugated fatty acids by GC-MS

CLA were converted into 4-methyl-1,2,4-triazoline-3,5-dione adducts (MTAD) as previously described [20], for identification by GC-MS, as already reported [21].

Results and discussion

Sunflower oils were selected for their high level of 18:2 n-6 (60-70% of total fatty acids), the peanut and rapeseed oils for their low ones (approximately 20-30%) , moreover rapeseed oil contained more 18:3 n-3 (about 8%) than sunflower or peanut oils (0.1%). Sunflower, peanut and rapeseed oil samples were selected according to their levels of polar compounds.

The quantities of the polar compounds recovered in these used frying oils, are presented in the *Table 1*. The five oil samples contained between 33 to 53% of polar compounds. Considering that fats and oils contain-ing more than 25% of total polar compounds are unfit for human consumption [22], these oils should have been discarded.

Table 1 showed the fatty acid composition of the used oils. For the sunflower oils, the level of 18:2 (42.9, 47.3 and 53.2%) was lower than what was observed in untreated oils (62-70%) [23, 24]. Frying treatment using rapeseed oil results in a loss of linolenic acid (2.6% *versus* about 8% in untreated oil).

In the five oils, small quantities of trans isomers of linoleic acids (0.6-1.3%) have also been detected. Furthermore, trans isomers of linolenic acid appeared in used rapeseed oil, essentially cct and tcc structures and tct and ctt isomers have been detected. These isomers are known to be formed during frying or desodorization of vegetable oils [25, 26]. The presence of di-trans isomers indicates that frying treatment have been carried out at temperature probably above 200°C [27]. Similar quantities of geometrical isomers have already been found in frying oils collect-ed from restaurants [13, 28].

The percentage of total CLA was about 0.3-0.5% of the total fatty acids *(Table 1)*. Neither the type of oil nor the degree of alteration seems to have any effect on the amount of CLA found in the used frying oils. However, only a few samples were analysed. It would be necessary to screen a larger number of samples in order to confirm this hypothesis.

Figure 2A shows the separation of CLA methyl esters prepared from sunflower oil, on a CP Sil88 column. The GC order of elution of the different CLA isomers is now well documented [3, 29]. The GC analysis revealed that the 9,11- and the 10,12-18:2 were the major isomers. They were accompanied by smaller quantities of 8,10- and 11,13-18:2. Both the *cis-trans* and *trans-cis* forms were present for 9,11- and 10,12-18:2 in the sample analysed. However, the *trans-trans* isomers represented more than 50% in sunflower and peanut oils and more than 40% of the total CLA in rapeseed oil. GC analyses did not allow separation of the different *trans-trans* isomers and utilisation of silver-ion HPLC was a mandatory step.

After separation of the CLA fraction by RP-HPLC, chromatography using two silver-ion columns in series *(Figure 2B)* enhanced the separation of CLA isomers as described by Sehat *et al*. [19]. The *trans-trans* CLAs eluted first, followed by the *cis-trans* and *trans-cis* ones, while the *cis-cis* isomers were eluted with a larger retention volume. Each peak was collected and MTAD adducts were made, in order to confirm the structure of the different isomers. The MS characteristic ions of the MTAD adducts of the di-*trans* isomers are presented in *Table 2*. All the peaks have a molecular ion at m/z = 407. Characteristic M^+-R_1 and M^+-R_2 ions were detected for the different CLA isomers and confirmed the order of elution previously reported with silver-ion HPLC columns [19, 30, 31]. The same characteristic ions were obtained for the *cis-trans/trans-cis* and *cis-cis* isomers.

Combination of GC and silver-ion-HPLC gives a complete CLA composition of the used frying oils *(Table 3)*. In the five oil samples, the *trans-trans* isomers represented about 50% of the total CLA. In sunflower, peanut and rapeseed oils, the 9*t*,11*t*- and 10*t*,12*t*-18:2 were the major remain-ing CLA isomers. The amounts of 8*t*,10*t* in peanut and rapeseed oils, respectively (6%) and (9%), were much higher compared to sunflower oil (1-2%). For the *cis/trans* and *trans/cis* forms, we confirmed the results was obtained by GC. The major isomers were the 9*c*,11*t*- and 10*t*,12*c*-18:2 in the five oils, while their *t,c* and *c,t* counterparts were present in smaller quantity. This particular composition of CLA, a high level of *trans-trans* isomers and the presence of 9,11- and 10,12-18:2 in each different geometrical configuration could be a characteristic of the CLA in unsaturated used frying oils.

So far, the physiological effects of two CLA isomers (9*c*,11*t* and 10*t*,12*c*) have been extensively studied. 9c,11*t* is usually found in dairy products [7-11] and the 10*t*,12*c* is found in synthetic commercial CLA mixtures [2, 3] used for animal feeding experiments and in nutritional interventions on human [32]. The present study shows that the corresponding di-*trans* isomers are created during heat treatment like frying. It would therefore be of interest to test their biological properties in order to compare with those of the well studied CLA isomers.

CONCLUSION

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Illustrations

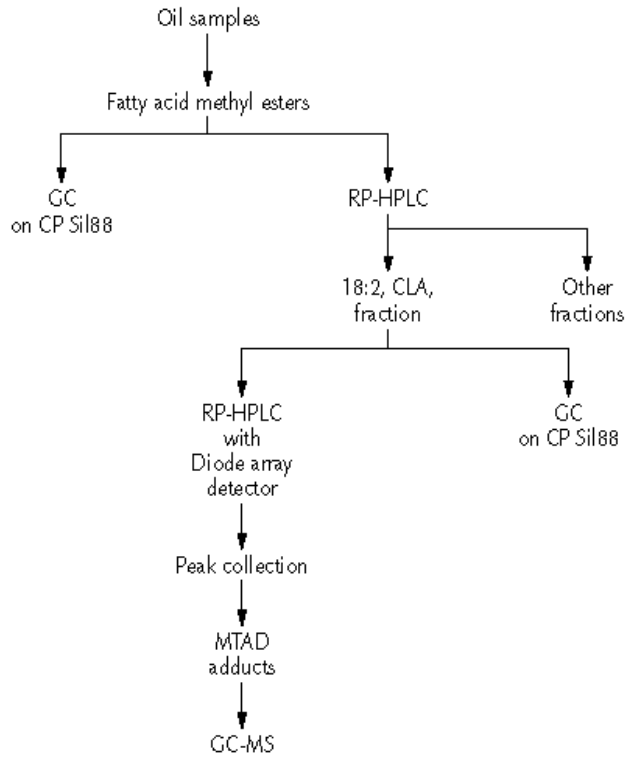

Figure 1. *Flow chart for CLA analyses in heated vegetable oils.*

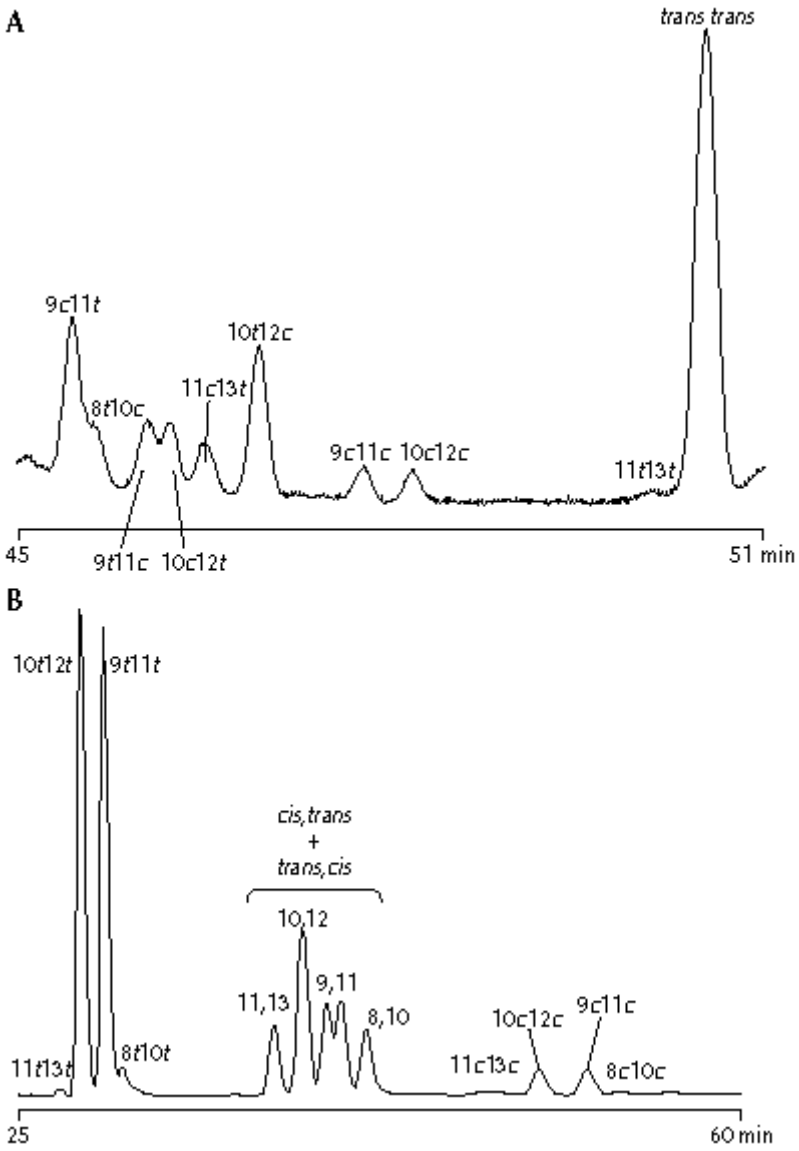

Figure 2. *Chromatograms of the CLA methyl esters fraction isolated from a used sunflower oil. A: GC analyses on CP-sil 88. B: silver-ion HPLC.*

Table 1. *Polar compounds and fatty acid composition (% of total fatty acids) of used vegetable oils.*

	Sunflower oil			Peanut oil	Rapeseed oil
	#1	#2	#3		
Polar compounds (%)	53	45	30	34	33
14:0	0.2	0.3	0.1	0.1	0.4
16:0	10.6	15.2	7.6	12.6	18.1
16:1 n-9	tr	tr	tr	0.1	tr
16:1 n-7	0.1	0.1	0.1	0.3	0.2
17:0	0.1	0.1	0.1	0.1	0.1
18:0	6.4	5.8	5.5	5.4	5.7
18:1 n-9	30.9	31.8	28.5	48.4	46.5
18:1 n-7	0.7	0.7	1.1	0.8	2.5
9 *trans*, 12 *trans*-18:2	0.1	0.1	0.1	0.1	0.1
9 *cis*, 12 *trans*-18:2	0.6	0.4	0.6	0.4	0.3
9 *trans*, 12 *cis*-18:2	0.5	0.4	0.6	0.3	0.2
18:2 n-6	48.5	43.6	54.4	26.9	20.1
18:3 n-3	tr	0.1	0.1	0.1	2.6
18:3 Isomers					1.0
Total CLA	**0.4**	**0.4**	**0.5**	**0.3**	**0.3**
20:0	0.3	0.3	0.3	1.2	0.5
20:1	0.1	0.1	0.1	0.9	0.8
22:0	0.4	0.5	0.3	1.9	0.3
22:1				tr	0.2

tr : < 0.1%

Table 2. *Characteristic ions in mass spectra of MTAD adducts of the* trans-trans *CLA isomers after silver-ion HPLC separation.*

	M+ m/z (intensity %)	M+-R1 m/z (intensity %)	M+-R2 m/z (intensity %)
8,10	407	308	264
	(100)	(570)	(1,351)
9,11	407	322	250
	(100)	(638)	(1,333)
10,12	407	336	236
	(100)	(520)	(1,144)
11,13	407	350	222
	(100)	(295)	(754)

Table 3. *Composition (% of total CLA) of the different isomers of CLA.*

	Sunflower oil			Peanut oil	Rapeseed oil
	#1	#2	#3		
8 *trans*, 10 *cis*	3.9	3.2	4.9	6.8	7.8
9 *cis*, 11 *trans*	12.4	11.8	7.7	9.7	14.3
9 *trans*, 11 *cis*	6.5	6.1	7.9	6.0	5.4
10 *cis*, 12 *trans*	5.7	5.0	6.3	5.6	5.5
10 *trans*, 12 *cis*	9.1	11.0	6.8	6.9	8.4
11 *cis*, 13 *trans*	4.0	3.9	5.0	3.9	3.1
8 *cis*, 10 *cis*	tr	tr	tr	0.9	1.0
9 *cis*, 11 *cis*	1.9	2.1	1.9	2.4	2.4
10 *cis*, 12 *cis*	1.5	1.6	1.7	1.5	1.1
11 *cis*, 13 *cis*	0.2	0.1	0.2	0.2	0.3
8 *trans*,10 *trans*	2.1	2.6	1.5	6.4	9.2
9 *trans*,11 *trans*	26.3	26.3	27.8	23.3	18.0
10 *trans*,12 *trans*	25.8	25.9	27.6	20.9	14.5
11 *trans*,13 *trans*	0.4	0.3	0.4	0.5	1.3
Other CLA[a]	0.5	0.1	0.4	4.8	7.8

[a]Tentatively identified as 7-9 and 12-14 in each geometrical position.