AWARD LECTURES Trans- and conjugated fatty acids in food - contents and analytical aspects

Oléagineux, Corps Gras, Lipides. Volume 8, Numéro 1, 28-32, Janvier - Février 2001, Dossier : Deutsche Gesellschaft für Fettwissenschaft - Association française pour l'étude des corps gras

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Summary : The current investigations on fatty acids are focused on long chain fatty acids, trans fatty acids (TFA) and conjugated fatty acids (CFA), especially isomers of linoleic acid (CLA). This paper deals with the origins of TFA and CLA and their physiological significance. Furthermore an overview is given of analytical procedures of both TFA and CLA. In addition the contents and isomeric distribution of these groups of fatty acids in foods are presented

Keywords : TFA, CLA silver-ion HPL.

ARTICLE

Definition

The naturally occurring configuration of double bonds in fatty acids in foods is the cis-configuration. These C = C bonds take place typically in the 3rd, 6th or 9th position from the terminal methyl group *(Figure 1)*. However, some of the arising fatty acids contain one or more double bonds with trans-configuration (TFA). Conjugated fatty acids (CFA) are composed of positional and geometrical isomers of long chain fatty acids, e.g. linoleic acid or eicosadienoic acid, consisting of conjugated double bonds *(Figure 1)*.

Epidemiological studies have suggested that TFAs increase the risk of coronary heart diseases (CHD) [1]. Furthermore, clinical studies have shown that high intakes of TFA raise total cholesterol and low-density lipoprotein (LDL) cholesterol and lower high-density lipoprotein (HDL) cholesterol. In addition, TFA is discussed as affecting the lipoprotein-A-level, which may be an independent risk factor for CHD. In contrast to TFA, many beneficial effects have been reported for CLA *(Figure 2)*. In experimental animals it has been shown that a commercial isomerized CLA-mixture in the normal diet leads to reduced carcinogenesis [2-4]. The investigations showed an influence of CLA on membrane fluidity and eicosanoid synthesis. In addition, CLA reduces the LDL-level, the ratio LDL/HDL, and fatty streaks in hamsters and rabbits [5, 6]. Anabolic effects have also been described. Feeding mice and rats with CLA increases bone [7] and muscle mass [8, 9]. Further physiological properties are positive effects on the immune system and antidiabetic effects [10]. The biologically active isomer for the anabolic effect is the *trans*10, *cis*12-18:2, but for all other effects it is not known. It has been assumed to be *cis*9, *trans*11-18:2.

Article disponible sur le site http://www.ocl-journal.org ou http://dx.doi.org/10.1051/ocl.2001.0028

Formation

These two groups of unusual fatty acids (TFA and CFA) arise in the rumen of ruminants as intermediates of the hydrogenation of dietary fatty acids during bacterial fermentation. For example, linoleic acid is in the first step mainly isomerized to *cis*9, *trans*11-18:2 (rumenic acid) catalyz-ed by the anaerobic microorganism *Butyrivibrio fibrisolvens* [11], and to other minor isomers. In small amounts CLA is formed during industrial hydrogenation *(Figure 3)*.

In addition TFAs are formed in food processing systems, especially during fat hardening and refining of vegetable oils, in varying amounts.

Because CLA might change the physiological pathway of the formation of eicosanoids it results in the formation of other conjugated metabolites of linoleic acid such as conjugated linolenic acid, conjugated eicosatrienoic acid or conjugated arachidonic acid.

Analysis of CLA

Before determination of individual isomers of CLA the fat is extracted from the matrix, followed by trans-esterification *(Figure 4)*. To reduce isomerization and methoxy artefacts during converting into fatty acid methy- lester (FAME), the base-catalyzed reaction with $NaOCH_3$ show-ed best results [12].

To quantify the whole content of CLA, capillary gas chromatography of the FAME followed by flame ionisation detection (GC-FID) was used. The commonly used stationary phase is a high polar cyanoalkyl-siloxane phase (*i.e.* CP-Sil 88 or SP2560) in columns with a length of 50 or 100 meters. Typical conditions are described elsewhere [13]. The use of only GC-FID leads to coelution of single isomers of CLA. For example the position isomers *cis/trans* 7,9 and 9,11 as well as *trans/trans* 9,11 and 10,12 are not resolved. This problem was circumvented by using Ag^+-HPLC [14]. An Ag^+-HPLC column (250 x 4.6mm) was filled with silica, bonded to silver ions via ionic bonds over phenylsulfonic acid moieties. The eluent was 0.1% acetonitrile in n-hexane and the system operated isocratically. The injection volume was 20mul with a flow of 1ml/min, and the UV-detection was performed at 234nm. Enhanced resolution was achieved by using two [15] to six [16] Ag^+-HPLC columns in series. Operating with this system it was possible to separate the hitherto not resolved *trans*8, *cis*10 from *trans*7, *cis*9 CLA-isomer *(Figure 5)* and the *cis,trans* and *trans,cis* isomers of 11,13 and 9,11.

The identification of these unusual fatty acids was performed by mass spectrometry (MS) for the position of the double bond and Fourier transform infrared spectrometry (FTIR) for determination of the configuration *(cis,cis; trans,trans; cis,trans; trans,cis*). Prior to this a preparative RP-HPLC column was used to separate and to enrich conjugated metabolites of linoleic acid from CLA [17].

To determine the position of the double bond with GC-MS the FAMEs were converted with 2-amino-2-methylpropanol (AMP) into the 4,4-dimethyl-oxazoline-derivatives (DMOX), because these products have no tendency to bond migration along the hydrocarbon chain. Furthermore DMOX derivatives have the same elution order as FAMEs and only a 10 degrees higher elution temperature than the corresponding FAME. The mass spectra of DMOX fatty acid derivatives show intensive fragments at m/z 113 and 126. For saturated fatty acids the m/z 126 was followed by a homologous series of 14 mass units (mu). In the region of a double bond the 14mu is interrupted by a 12mu.

The allylic position of the last double bond shows a higher intensive fragmentation than other positions (m/z+2; m/z+3). *Figure 6* shows the mass spectra of the 7,9 and 11,13 CLA-isomers. The parent ion of DMOX derivatives of CLA has a mass unit of 333m/z. The DMOX derivatives of conjugated eicosadienoic acid isomers contain a prominent ion with a mass unit of 361m/z.

To elucidate the configuration with GC-FTIR the DMOX derivatives or the FAMEs can be used. The *cis,trans* or *trans,cis* CLA-isomers show bands in the region of 3,020 and 3,002cm^{-1} and 988 and 950cm^{-1}. The *trans, trans* isomers have only one band in the region of 3,016cm^{-1} and the classic band at 988cm^{-1}, while the *cis,cis* isomers only have bands at 3,037 and 3,005cm^{-1}*(Figure 7)*. The distinction between *cis,trans* and *trans,cis* can be determined after partial reduction with hydrazine and separation of the resulting 18:1 FAMEs with GC-FID.

Contents of TFA and CLA in foods

The major sources of TFA in the diet are partially hydrogenated vegetable oils, dairy products and meat from ruminants. Therefore processed food products with high contents of these partially hydrogenated oils or dairy fat like cakes, pastries, French fries and crisps contain the highest amounts of TFA *(Figure 8)*. Current values of TFA in margarines are significantly reduced in comparison to contents of TFA from earlier investigations [18]. A look at the isomeric distribution of TFA in milk fat shows that it is totally different to that of the partially hydrogenated vegetable oils. While in milk fat *trans*-vaccenic acid makes up more than 60% of total *trans* octadecenoic acids, in hydrogenated oils a Gaussian distribution occurs, which is centered around C18:1*t*9 to C18:1*t*12 [19].

Because of the "carry-over-effect" CLAs are predominantly found in milk, milk products, meat and meat products. Foods produced with the above mentioned products therefore contain higher amounts of CLA, while edible oils, margarines and frying fats contain only negligible amounts of CLA *(Figure 9)*.

The distribution of CLA isomers in cheese shows one major isomer C18:2 *c*9*t*11 with nearly 84% of total CLA. Further occurring isomers of CLA with portions of 2-5% are *t*11*t*13, *t*7*c*9 and cis/trans and trans/cis isomers of 11,13. In contrast to the naturally occurring distribution, in a commercially available CLA-mixture four isomers of the *c/t* group with nearly the same content comprise the highest amount at about 90% with minor amounts of all other isomers [15].

From these data Fritsche and Steinhart estimated the daily intake of CLA based on a German nutrition study to 0.35g/d for women and 0.43g/d for men. Whereas the estimated daily intake of TFA in Germany in 1992 was 3.4g/d for women and 4.1g/d for men, the up-dated TFA intake shows lower values with 1.9g/d for women and 2.3g/d for men because of the decreased TFA contents in margarines and the lower meat consumption [13, 20, 21].

CONCLUSION

Operating with two or three Ag^+-HPLC columns in series is a powerful technique to determine the CLA isomeric distribution in foods. GC-MS and GC-FTIR are complementary techniques to confirm the double bond position and configuration in individual CLA isomers or other conjugated fatty acid isomers.

Predominant sources of TFA and CLA are foods from ruminants (*i.e.* milk, milk products, meat) and partially hydrogenated vegetable oils.

Because of their good physiological properties CLAs are predestined for use in functional food. This will be achieved by enrichment of fat containing products directly with CLA enriched oils or indirectly with raw material with higher amounts of CLA. One aspect of this is the genetic engineering of plants to produce oils with higher contents of CLA. Another possibility is the formation of starter cultures, which produce higher amounts of CLA in dairy products. For all of the above it is necessary to perform physiological studies with individual CLA-isomers to reassess the beneficial properties and the toxicological studies of individual CLA-isomers. Further, it will be possible to understand the mechanism of the effects of the benefits and to identify the biologically active compound. Further investigations should include as well the determination of single TFA isomers and their significance as the determination of single CLA isomers in other biological matrices such as human fluids and tissues.

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Illustrations

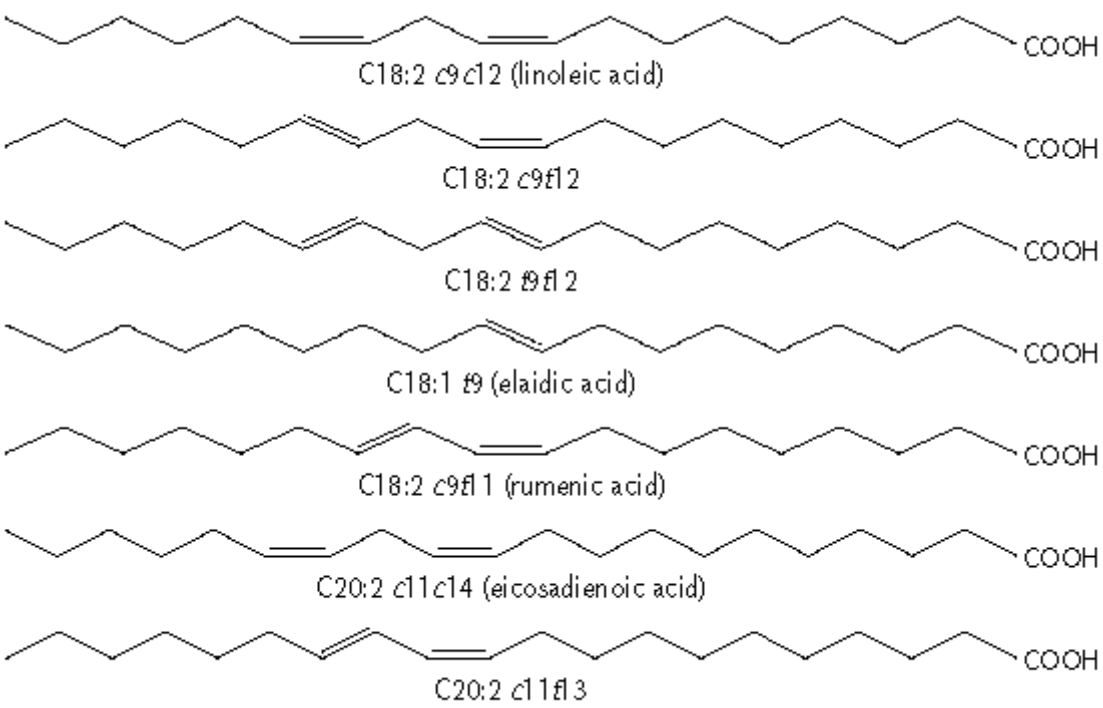

Figure 1. *Structures of linoleic acid isomers, elaidic acid, rumenic acid (CLA-isomer) and eicosadienoic acid isomers.*

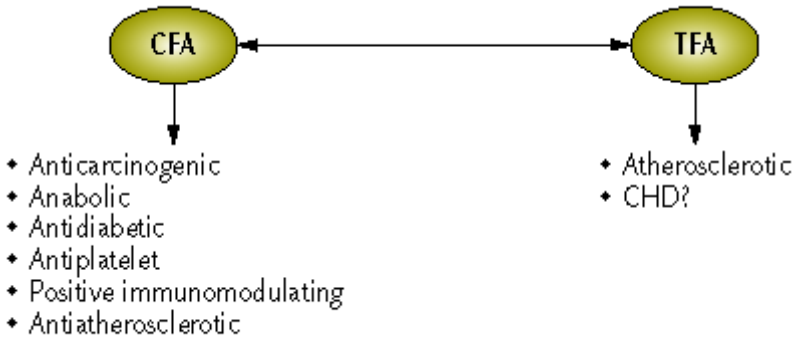

Figure 2. *Physiological significance of TFA and CLA.*

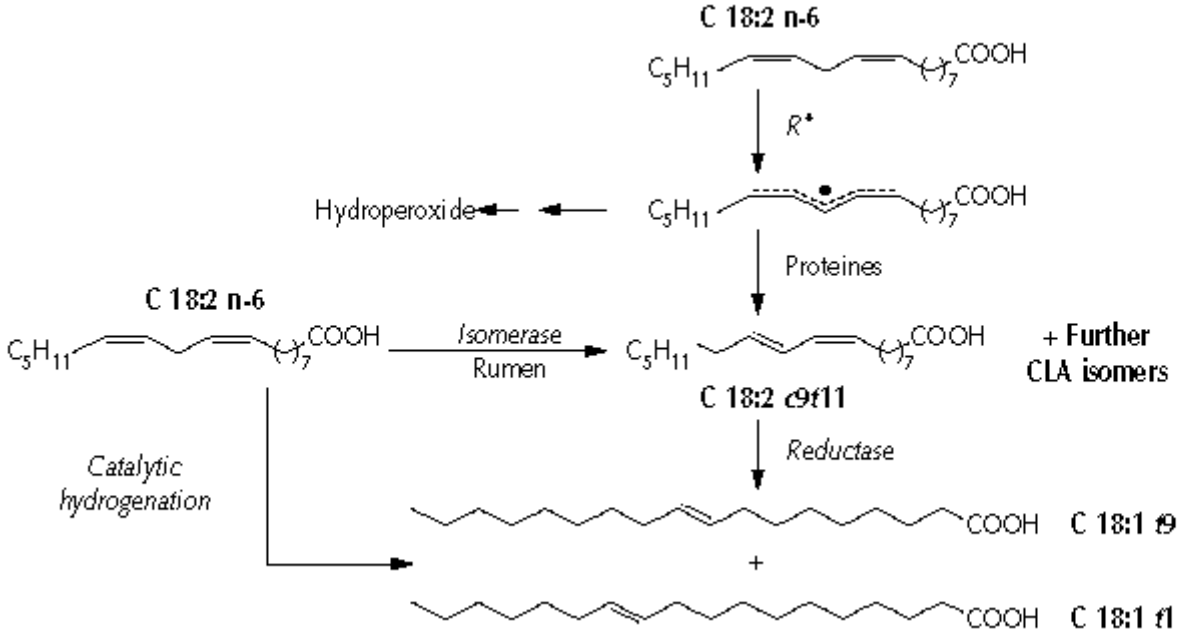

Figure 3. *Formation of TFA and CLA (from Fritsche and Steinhart [13]).*

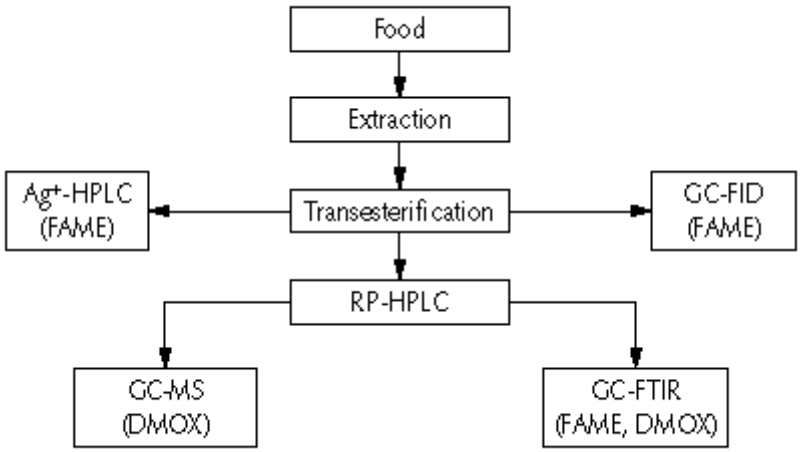

Figure 4. *Idendification and determination of TFA and CFA.*

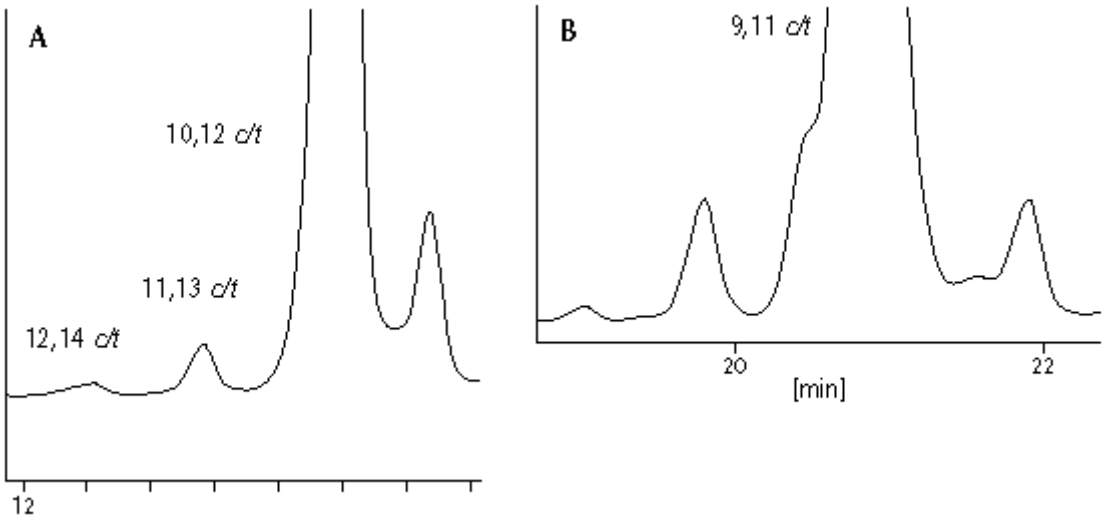

Figure 5. *Improvement of the separation of CLA isomers by tandem-column Ag^+-HPLC (cheese fat) (from Ricket* et al. *[15]).*

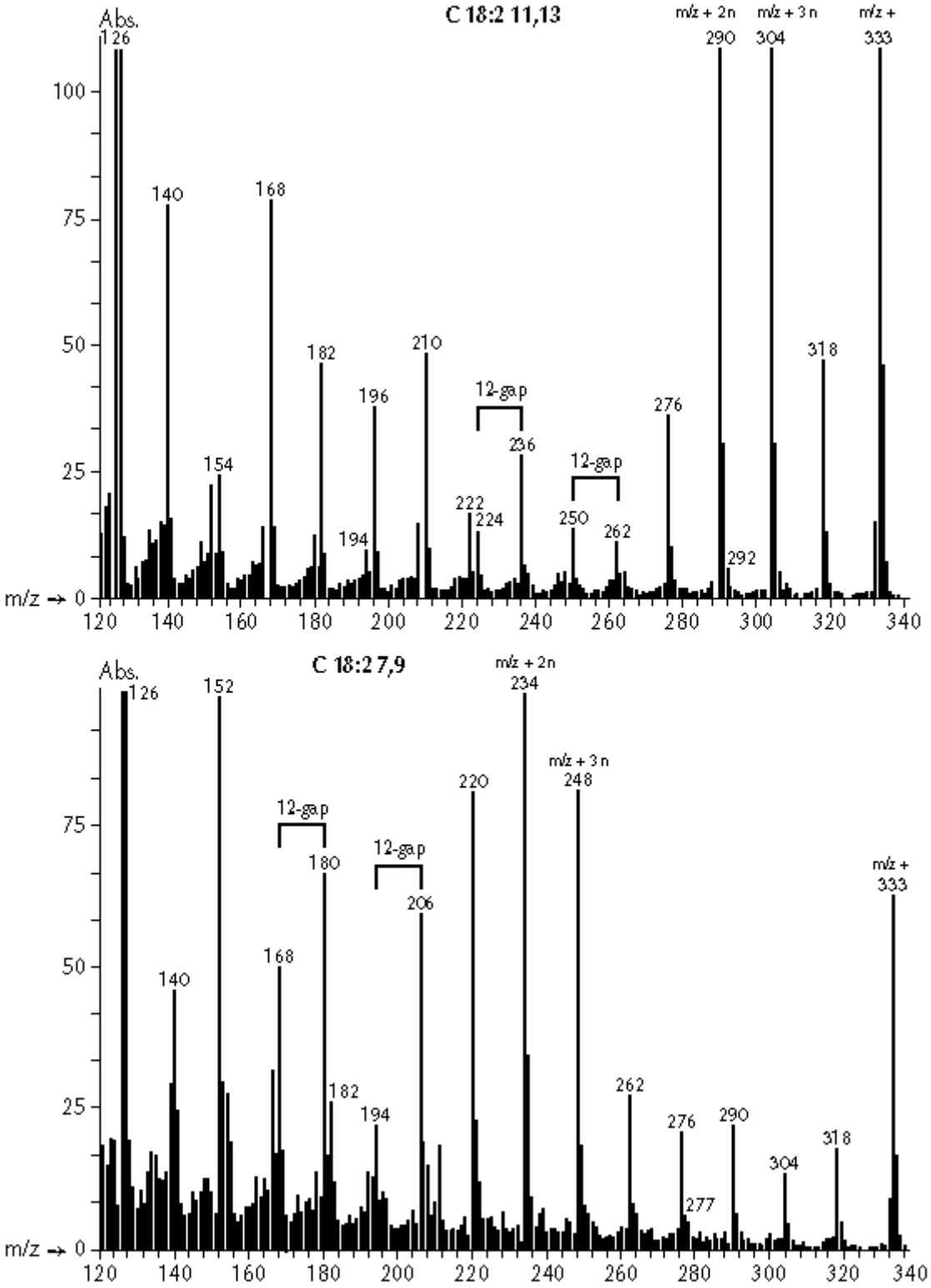

Figure 6. *Mass spectra of the two CLA isomers of 11,13 and 7,9 (DMOX-derivatives).*

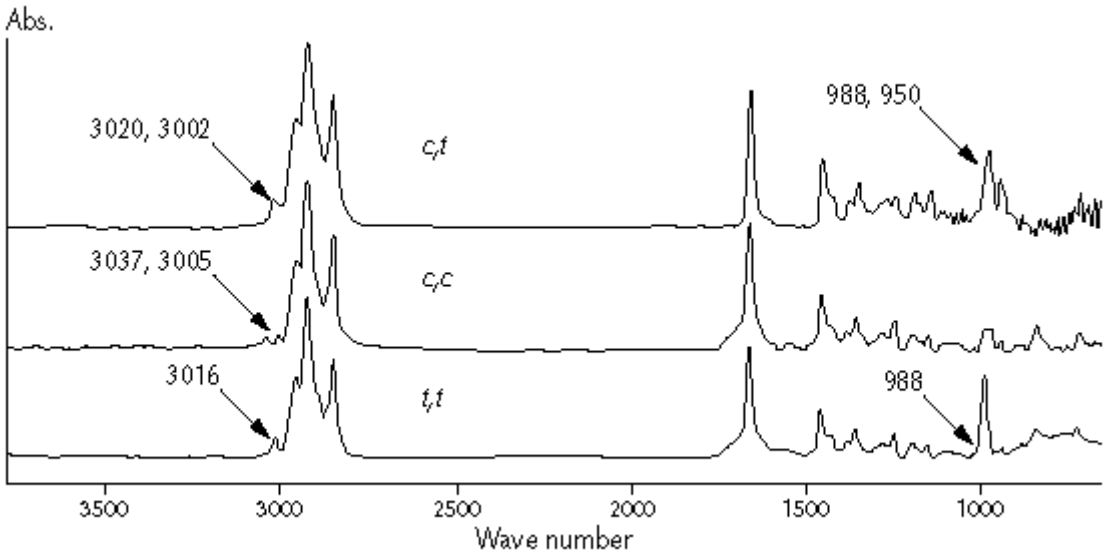

Figure 7. *GC-DD-FTIR spectra of CLA isomers (DMOX-derivatives) (from Sehat* et al. *[14]).*

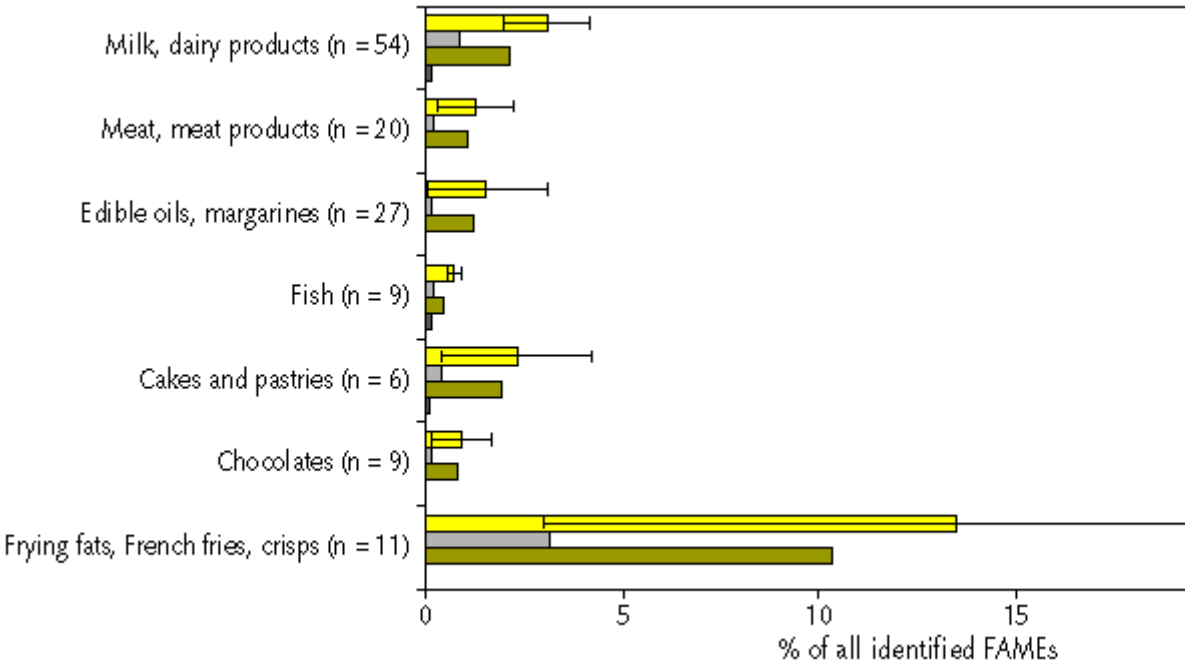

Figure 8. *Contents of TFA in various foods (from Pfalzgraf and Steinhart [21]).*

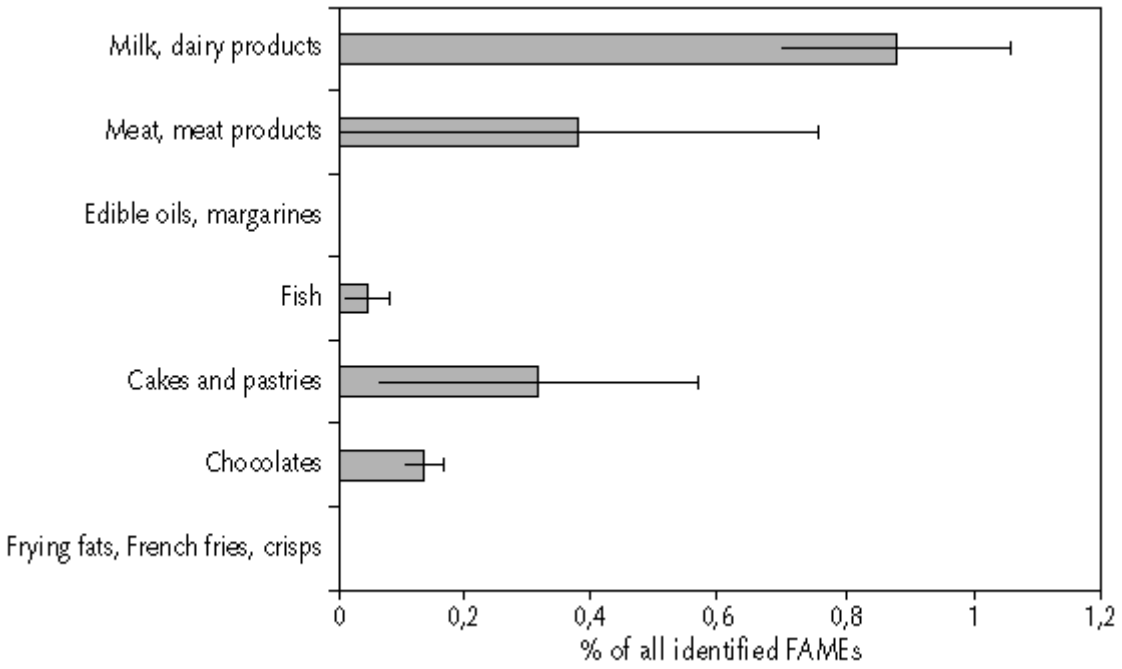

Figure 9. *CLA amounts in various foods (from Fritsche and Steinhart [13]).*