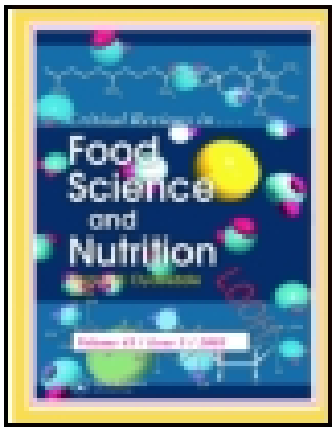

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Triacylglycerols determination by high temperature gas chromatography in the analysis of vegetable oils and foods: A review of the past ten years

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Triacylglycerols determination by high temperature gas chromatography in the analysis of vegetable oils and foods: A review of the past ten years

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Abstract

The analysis of triacylglycerols by high temperature gas chromatography, along the last ten years has been reviewed in this paper. The interest in this topic has grown along the last years due to the triacylglycerols are the main components of oils and fats and they are being used for the characterization and authentication of foods products.

The most commonly used procedures, including the official methodologies, applying high temperature gas chromatographic techniques are shown. Their importance in the characterization of different kind of samples, vegetable oils, seeds, dairy products, etc., is considered.

This review is not intended to be a comprehensive dissertation on the field of triacylglycerols analysis since that would require sufficient space to occupy a book in its own right. Rather, it will outline selected considerations and developments, where the technique has been applied.

Keywords: *Triacylglycerols, high temperature gas chromatographic (HTGC), dairy products, vegetable oils, foods.*

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INTRODUCTION

The molecular structure of a triacylglycerol (TAG) (figure 1), is important for food chemistry and technology, biochemistry and nutrition. TAGs (also commonly termed "triglycerides") consist of a glycerol skeleton, where each hydroxyl group is esterified to a fatty acid. In nature, these compounds are synthesized by enzyme systems, which determine that a centre of asymmetry is created about carbon-2 of the glycerol backbone, and they exist in different enantiomeric forms, i.e. with different fatty acids (FAs) in each position. The molecular structure of each individual TAG species can be described by a few basic attributes: (a) the total carbon number (CN), which is the sum of the alkyl chain lengths of each of the 3 FAs, (b) the degree of unsaturation in each FA, and (c) the position and configuration of the double bonds in each FA. The TAG molecule shows optical activity when the two primary hydroxyl groups are esterified with different FAs. The stereochemistry of TAGs can be described by the "stereospecific numbering" (*sn*) system as recommended by a IUPAC-IUB Commission on the Nomenclature of Glycerolipids. Due to this, it is crucial to say that the analysis of the TAG composition is a very challenging task because an enormous number of individual TAG species is possible due to the large number of possible FA combinations on the glycerol backbone.

Figure 1

Usually, the TAGs are converted into fatty acid methyl esters (FAMEs) for gas chromatography (GC) analysis. Many different methylation methods are described in literature and four of them are commonly used: acid or base-catalyzed methylation, borontrifluoride methylation,

methylation with diazomethane and silylation (ISO 5509, 2000; Seppanen Laakso et al., 2002; Mendez Antolin et al., 2008). The first method is more acceptable than other methods because it uses less aggressive reagents than other methods. This review is most concerned with the direct determination of TAGs, therefore no references will be given considering the analytical parameters of the analysis of FAMEs. The interested reader is referred to the AOAC Official Methods 996.06, 2000 and Petrovic et al., 2010.

The advantage of using TAG analysis, as compared to FA profiling, is that the genetically controlled stereo-specific distribution of the FA moieties on the glycerol backbone is preserved and, thus, the information content of intact TAGs is usually higher.

Analytical techniques for the analysis of TAGs

In the last decades, a very wide range of analytical tools has been developed for lipid analysis. Particularly powerful are the chromatographic methods, either or not in combination with spectrometric techniques such as mass spectrometry (MS) (Laakso, 2002), GC and liquid chromatography (LC) combined with MS are the key techniques in all areas of lipid analysis. The possibilities and limitations of these two chromatographic methods in TAG profiling, have been described in a number of excellent reviews. Buchgraber et al. discuss the application of GC, HPLC in normal and reversed phase mode, thin-layer chromatography and supercritical fluid chromatography for the qualitative and quantitative determination of TAGs (Buchgraber et al, 2004a). Furthermore, the chromatographic and spectrometric methods usually used for the analysis of common and less common edible vegetable oils and fats are reviewed by Andrikopoulos (Andrikopoulos, 2002a; Andrikopoulos, 2002b; Andrikopoulos et al., 2004). In general they all conclude that GC is the most restricted chromatography system for the

separation of triacylglycerols, being recommended for analyses of relatively saturated sample types, such as milk fats and oils and LC can be applied to all sample types of triacylglycerols due to separations at lower temperatures.

Techniques for the gas and liquid chromatographic separation of complex mixtures of TAGs have been reviewed in detail over the past two decades. For instance, in 1995, Myher reviewed strategies in the analysis of lipids in general, and emphasize already the importance of chromatographic methods to identify specific species (Myher and Kuksis, 1995). In the same year, Ruiz-Gutiérrez and Barron reviewed the methods for the analysis of TAGs and state the significance of the column for the separation of the TAGs (Ruiz-Gutiérrez and Barron, 1995). In particular, de la Fuente and Juárez studied the TAGs on milk samples taking into account the use of GC to separate according to the CN (de la Fuente and Juárez, 1999) and Nikolova-Damyanova reviewed the principles and applications of thin-layer chromatographic for the analysis of TAGs (Nikolova-Damyanova, 1999).

Combinations of two chromatographic dimensions have been applied for those situations where the resolving power of a one-dimensional method was insufficient. Although it is not the aim of this review, it is important to point that recently, the analytical benefits of comprehensive two-dimensional (2D) chromatography, in its various operational modes, have been exploited by the oil and fat chromatographic community to solve problems (Janssen et al., 2003; de Koning et al., 2006; Janssen et al., 2009). Thus, Tranchida et al. reports on the employment of comprehensive chromatographic methods, based on GC, LC, LC-GC and packed supercritical fluid chromatography (pSFC), in the field of lipid analysis (Tranchida et al., 2007).

For identity, control and authentication purposes of fats and oils, milk fat and special fats like extra virgin olive oil; the knowledge of the TAG composition has become an indispensable tool. On this matter, Aparicio and Aparicio-Ruiz discuss the contribution and trends in chromatography for the authentication of vegetable oils, establishing the TAG composition as a measurement of the quality and purity of vegetable oils (Aparicio and Aparicio-Ruiz, 2000). In addition, Ulberth and Buchgraber review the technical merit of different analytical platforms to establish the authenticity of oils and fats (Ulberth and Buchgraber, 2000), and the same authors review strategies to detect and analyze cocoa butter equivalents added to genuine cocoa butter or to chocolate products, for authentication purposes (Ulberth and Buchgraber, 2003).

Applications of various chromatographic techniques and electrophoretic methods employed for the analysis in macro- and micro-components in vegetable oils and dairy products are compiled and critically evaluated by Cserháti et al. as well as the employment of these methods for authenticity tests and traceability is discussed (Cserháti et al., 2005). In other reconsideration, de la Fuente and Juárez provide the advantages and disadvantages for detecting the authenticity of dairy products by new approaches such as polymerase chain reaction and isotope ratio mass spectrometry (IRMS) versus traditional procedures such as chromatographic and electrophoretic methods (de la Fuente and Juárez, 2005).

According to regulations, IUPAC-AOAC has adopted a method to resolve TAGs, based on the numbers of similar carbon atoms by HTGC, of solutions of oil and fat, under temperature-programmed conditions, where the determination is made by corrections factors and identified by reference to standard triglyceride solution (IUPAC Method 2.323, 1987; AOAC Official Method 986.19, 2000). In addition, the method for determination of TAGs by their partition

numbers (or equivalent carbon numbers, ECN) in vegetable oils by LC, was adopted by AOAC-IUPAC-AOCS (Wolffi et al., 1991; Firestone, 1994).

High temperature gas chromatography

There has always been an interest in pushing GC to the highest temperatures possible. The exact definition of "high temperature gas chromatography" (HTGC) is somewhat arbitrary and subject to some developments over the years. Normally it is considered that separations above 300ºC are HTGC. HTGC has been widely described previously in the literature, reason why it does not need to be described in detail here. For instance, the evolution of the concept is presented by Pereira et al. (Pereira et al., 2004). It offers interesting possibilities (efficiency, stability, etc.) although there are some problems associated with the injection system, deterioration of the columns, stability with temperature, etc. In the right hands and for suitable samples, HTGC is a valuable tool for determining TAGs. If it is used inappropriately, it can give meaningless results (Christie, 2005).

Any sample subjected to HTGC must be both thermally stable and resistant to thermal re-arrangement; TAGs are a group of high-boiling-range compounds that are well suited for HTGC analysis. As a result of the high molecular mass of the intact TAGs, GC analysis requires final column temperatures of 350ºC or higher. Elution is largely based on the CN, with weak selectivity towards the number of double bonds on some phases. For the highly unsaturated TAGs lower responses are generally seen. Literature is not conclusive as to whether this is due to thermal instability or the result of polymerization, however precautions should be taken to minimize losses (Christie, 2005). Thermal stress should be kept to a minimum by eluting the compounds as rapidly as possible at the lowest elution temperature feasible. In practice this

means column length should be minimized and the lowest possible film thickness should be used. The gas linear velocity should be as high as possible. To minimize the loss of column efficiency as a result of the use of high linear velocity, hydrogen is the preferred carrier gas (Kaal and Janssen, 2008). But because additional structural information about TAG composition is valuable, considerable effort has been made, along the years, to develop selective high-temperature stationary phases that resolve TAGs according to the number of unsaturated FAs composing the molecule and carbon number simultaneously.

Columns and stationary phases

The introduction of open tubular columns (capillary columns) by Golay in 1958 represented the most important breakthrough in gas chromatography. Compared to packed columns, these allow a higher resolution, i.e. higher separation capacity, and there have been further developments in column fabrication and instrument design. Originally, nonpolar stationary phases of polysiloxane type were habitually used. Those phases, which are marketed under a variety of different brand names (OV-1, OV-101, SE-30, DB-1, HP-1, Ultra-1, SPB-1, CP-Sil 5 CB, Rtx-1, BP-1, AT-1, etc. for 100% dimethyl polysiloxane and OV-3, SE-54, DB-5, HP-5, Ultra-2, SPB-5, CP-Sil 8 CB, Rtx-5, BP-5, AT-5, etc. for the slightly more polar 5% phenyl-95% dimethyl polysiloxane stationary phase) allow only separation according to the CN of TAGs, even in capillary columns (Buchgraber et al, 2004a).

For example, more than two decades ago, Geeraert et al. separated coffee oil on a 15-m glass column coated with OV-101, where the CN54 were separated into 3 fractions according to the number of unsaturated FAs in the molecule, but not by the number of double bonds within each acid (Geeraert et al., 1983). In addition, Collomb et al. also separated TAGs groups differing

with respect to the number of unsaturated FAs of different oils and fats such as palm, rapeseed, soybean, etc., on a DB-5 column (Collomb et al., 1998). Harrison et al. discuss a rapid GC-FID technique with a BP-5 column for the simultaneous quantitative analysis of FFA (free fatty acids), MAG (monoacylglycerols), DAG (diacylglycerols), TAG, sterols, and squalene in vegetable oils (Harrison et al., 2005). Molkentin controlled the purity of milk fat by gas chromatographic determination of TAGs based on separation by CN using a column filled with 3% OV-1 (Molkentin, 2007).

On nonpolar columns, like those mentioned above, unsaturated TAGs eluted before saturated ones. The reason for this consists in the remarkable difference in vapor pressure between saturated and unsaturated FAs. Among unsaturated FAs differences are too small to effect further separations, thus fractionation according to the degree of instauration is not possible. The elution sequence for TAGs with a given CN is: UUU, SUU, SSU, SSS (S – saturated FA, U – unsaturated FA). A clear example of this is shown in Mayer and Lorbeer, 1997; the authors investigated the quality of different fused-silica capillary columns with a mixture of TAGs at high temperatures (figure 2).

Figure 2

Progress in separation efficiency of TAGs has been achieved by using capillary columns coated with more polar polysiloxane phases containing a higher proportion of phenyl groups (50-65%). The highly efficient separation of individual TAGs became possible due to the application of thermostable stationary phases based on polysiloxane with methyl, phenyl, and cyanopropyl

groups attached to the surface of capillary columns. The availability of cross-linked and chemical bonded phases improved the properties of columns considerably by extending the column lifetime and reducing baseline drift at elevated temperatures. The use of temperature resistant medium polarity capillary columns enhances the resolution power largely, and allows determining individual TAG species. HTGC on medium polarity stationary phases has been extensively applied for the comprehensive separation of TAGs in a wide range of fats and oils (Ruiz-Samblás et al., 2011). Capillary columns with somewhat more polar stationary phases are available commercially. These columns are known as the CB-TAP CB for TAGs from Agilent, Rtx-65-TG column (Restek, 65% diphenyl-35% dimethyl polysiloxane) from Restek (Bellefonte, PA) and DB-17ht (50%-phenyl)-methylpolysiloxane from J&W Scientific. They allow introducing additional selectivity for double bonds.

In 2002, Mayer et al. described the preparation of fused silica capillary columns coated with 75% diphenyl-25% dimethylpolysiloxane, and compare it with two capillary columns currently commercial available OV-25, PS162 (Mayer et al., 2002). The increased polarity enabled a slightly improved resolution of TAGs that differ only by one double bond, *e.g.* SOO (1-stearin-2,3-diolein) and OOO (triolein). However, this column is not available commercially, only it is possible to find this kind of coating for packed columns (Ohio Valley, Ohio, USA) which do not bear high temperatures. Thus, commercially available siphenylene-siloxane copolymers are claimed to have similar, but not identical properties as methyl, phenyl-polysiloxanes (Mayer et al., 2003a).

The properties of stationary medium-polar phases at high temperatures such as selectivity, bleeding, working range, immobilization, inertness need to be studied with other parameters

different from the usual ones. The Rohrschneider-McReynolds (Berthod et al., 1995) constants describe the selectivity of a stationary phase at low temperatures and it is restricted to analyte-stationary phase interactions at low temperatures. Additional parameters are required to describe the interactions at elevated temperatures, since temperature induced dipoles may change the selectivity. The TAG indices were used to evaluate interactions between analytes and stationary phase at elevated temperatures. The polyunsaturated TAGs OOO (triolein), LLL (trilinolein) and LnLnLn (trilinolenin) served as probes, whereas the saturated TAGs PPP (tripalmitin), SSS (tristearin) and AAA (triarachidin) were used to generate a calibration line. The TAG index, devised by Mayer et al. (Mayer et al., 2003a), was used to evaluate different medium polarity phases (Mayer et al., 2003b; Mayer et al., 2004a; Mayer et al., 2004b; Petsch et al., 2005). Table 1 shows the TAG index for different medium-polar stationary phases.

Table 1

Recently ionic liquids are being used as stationary phases in gas chromatography. Ionics liquids are class of compounds which can be defined as organic salts that are liquid below 100ºC. The properties of extremely low vapor pressure even at high temperatures, high viscosity, high thermal stability and high polarity make them suitable as phases for gas chromatography (Anderson, 2009). The first commercial ionic liquid column was launched by Supelco in 2008. A range of columns are now available with different phase types, from polyethylene glycol equivalent polarity with improved thermal stability, to phases with extremely high polarity (Whitmarsh, 2012). The use of ionic liquid for the analysis of TAGs has not been reported so

far, due to the requirement of high temperatures and the limits of these liquids, although it has been found some ionic liquid with very high stability upper 400ºC (Anderson et al., 2005). For the separation of FAMEs in a rapeseed oil sample, Supelco has developed an application with a SP-IL100 phase which is virtually equivalent in polarity /selectivity to the TCEP phase, currently one of the highest polarity/selectivity GC pahses (Sidisky and Buchanan, 2008).

Sample introduction

Other critical aspects of practical HTGC concern the method of sample introduction. In conventional GC the sample is evaporated at a temperature that is higher than the column before admission. The injection technique has been considered as being most critical for accuracy (trueness and precision), since at this stage discrimination against less volatile high molecular weight compounds can occur while the sample is being transferred from the syringe to the column (syringe discrimination) (Grob Jr. and Neukon, 1980). Many authors published detailed studies concerning the effect of the injection technique on the recovery of TAGs (Grob Jr., 1979; Poy et al., 1981; Hinshaw and Seferovic, 1986; Termonia et al., 1987). Mainly, capillary injection techniques can be divided in two groups (i) direct on column injection and (ii) injection into an externally heated vaporizer (split/splitless). Both techniques could be used in several modifications. Most of the injection techniques used in the last group have the drawback that the sample has to be transferred from the injection zone to the capillary column inlet at the oven entrance. However, with temperature programming of the external vaporizer (PTV), starting at a temperature below the solvent boiling-point to a temperature high enough to transfer the analytes to the column, can be regarded as an attempt to combine the advantages of the traditional on-column (Grob, 1979). PTV injection in both split and splitless modes were excellent sample

introduction systems for high temperature GC in the bibliography. A main advantage over on column injection is that the column can be maintained at a relatively high temperature during injection (Buchgraber et al, 2004a). Results of two intercomparison studies for TAG analysis by GC, where the performance of different sample introduction techniques, was compared (Buchgraber et al, 2004d), suggest that all injection techniques considered were equivalent in terms of repeatability and accuracy, as judged by the magnitude of the obtained FID response factors. These response factors depend to a certain extent on the ratio of the fuel gases and the used carrier gas. The major part of the papers found in bibliography for the analysis of TAGs use He as carrier gas and the most common detector used in bibliography is the flame ionisation detector (see tables 2, 3 and 4 for more information).

Finally, we have just given brief information on the history and the state of the art of the HTGC technique and some aspect related to it. Thus, a variety of samples, along the last years, have been successfully analyzed by HTGC. The purpose of this paper is to present and discuss the ten last year's contribution as well as recent trends in HTGC to the analysis of TAGs in different samples such as oils, seeds, dairy products and plants.

1. ANALYTICAL APPLICATIONS

HTGC is an important analytical technique for qualitative and quantitative analysis in a wide range of application areas. Though, as it has been mentioned before, there are some obstacles for obtaining reliable quantitative results in HTGC analysis of TAGs. For instance, the sample introduction is critical and discrimination against boiling point can occur if the injection mode is not properly selected or optimized. Quantification of the TAGs, either as pure compounds or as

carbon number clusters, requires accurate response factor if no chemometrics tools are applied. In addition, the possible thermal degradation of TAGs, at elevated temperatures, should be considered. However, and despite of these obstacles several applications, to diverse kind of samples, have been developed successfully. Recent research has resulted in better chromatographic columns and methods for sample preparation that enable a significant expansion of the molecular application range of HTGC (Kaal and Janssen, 2008). This work intends to provide an updated and extensive overview since 2001 on the principal applications. For organizational reasons, this paper has been structured based on broad sample categories analyzed by this technique.

1.1 DAIRY PRODUCTS

Determination of intact TAGs is regarded as advantageous in dairy product analysis because the genetically controlled specific distribution of the FA moieties on the glycerol backbone is preserved; the information content is, thus, higher. More than 400 different FAs have been identified as components of milk fat (Buchgraber et al, 2000).

Despite the advantages of using capillary columns, the official European Union method for the determination of the purity of milk fat is based on determination of TAG composition by packed column (Commission Regulation EEC No 454/95). Its revised versions (Commission Regulation EC No 2771/99: Commission Regulation EC No 213/2001; Commission Regulation EC No 273/2008) foresee capillary columns as an alternative technique, where the same results are obtained. In addition, other alternatives using capillary GC, previously to the ten years revision, have also been described (Molkentin and Precht, 1995; Ulberth et al., 1998).

The EU official method, based on the original idea of Timms (Timms, 1980) later developed by Precht (Precht, 1991) is based on an evaluation algorithm ("TG formulae") using TAG data obtained by chromatographic determination. Precht and Molketin later studied the equivalence of packed and capillary GC columns by the use of this method (Molkentin and Precht, 2000).

The current international standard or the analysis of TAGs in milk and milk products created by International Organization for Standardization (ISO) and International Dairy Federation (IDF) jointly (ISO 17678/IDF 202:2010) specifies HTGC coupled with FID for TAG determination. In this international standard, two kinds of chromatographic columns are specified, either 500 mm x 2 mm internal diameter glass column packed with 3% OV-1 stationary phase or 5 m non-polar phase capillary column. The preparation, silanization, packing and conditioning of the packed column are described.

Examples of the use of HTGC to separate milk fat according to CN and TAG determination in dairy products over the past ten years are presented in table 2. Information regarding sample type, chromatographic column, carrier gas and injection mode are listed for each study (when information available) and some of these studies are discussed more fully below.

Table 2

In 2003, Buchgraber et al. reported an intercomparison study in which 13 laboratories applied HTGC on columns coated with medium-polarity stationary phases to determine the TAG profile of cocoa butter (Buchgraber et al., 2003). The results showed that this technique was robust and reliable for this purpose. Previously, the same authors had studied this sample by comparing

HPLC and GC techniques (Buchgraber et al., 2000). The same matrix was studied also by Guyon et al. and compared HTGC with MS, being both comparable but, showing this last technique more advantageous related to the detection of each compound (Guyon et al., 2003).

Milk fat TAG composition is affected by seasonal and regional variations in feeding. Due to this fact, determination by HTGC of classes of milk fat into groups of identical numbers of acyl-C atoms (C24-C54) has been reported to be a more effective criterion than FA composition for determining their origin (Fontecha, 2006a, Fontecha, 2006b). The authenticity assessment of dairy products has become a very important issue. Most current analytical approaches to authenticity issues in milk fat constituents are essentially based on the GC separation of its components. Nowadays, the best way of revealing the presence of foreign fats in milk fat includes the study of the TAG profile. Many authors have researched this subject, for instance, de la Fuente and Juárez provide an extensive review from 1991 on the principal applications for detecting the authenticity of dairy products (de la Fuente and Juárez, 2005). Subsequently, a method to detect adulteration of milk fat with partially hydrogenated vegetable oils was developed by Destaillats et al. (Destaillats et al., 2006). Later, Povolo et al. studied the authenticity of pure milk fat, together with mixtures of milk fat containing two levels of four foreign fats (coconut fat, sunflower oil, lard and beef tallow), using a method based on the application of formulae deriving from multiple linear regressions (Povolo et al., 2008).

Recently, Haddad et al. have demonstrated improved separation of camel milk fat using HTGC with a Rtx-65TG column compared to the HPLC method (Haddad et al., 2011). Using the same specification column Romano et al. showed similar TAG and FA patterns between commercial

samples of Mozzarella di Bufala Campana (a protected designation of origin cheese) and the parent milk and bovine counterparts (Romano et al., 2011).

1.2 VEGETABLE OILS AND SEEDS

As triacylglycerols are the main component of all the fats and oils of commercial importance, a great deal of effort has been applied to their analysis. TAGs and other constituents are the main components of vegetable oils. Each oil has a characteristic pattern of TAG and the physical and chemical properties of a particular oil are determined mainly by the abundance of different TAG molecular species. Typical chain lengths of FAs, found in vegetable oils, range from 14 to 20. Here the carbon number is defined as the sum of the alkyl chain lengths of the three FAs. This results in TAGs with total carbon numbers typically between 46 and 56. A considerable number of oils have been characterized by GC measuring the composition of FA.

Whilst TAGs species are associated with the chemical and physical properties of oils, the regio-types of TAGs species are associated with the intrinsic properties such as biochemical and nutritional aspects. Regarding this, Martínez-Force et al. proposed the asymmetric α coefficient, which is calculated from the FA, sn-2 FA, and TAG composition of the oil, to calculate the stereochemical asymmetry of FA in TAG molecular species (Martínez-Force et al., 2004). Previously, Fernandez Moya et al. identified the TAG molecular species of oils obtained from different sunflower mutant lines with increased amounts of palmitic or stearic acids in linoleic or high-oleic backgrounds (Fernandez-Moya et al., 2000). TAGs were identified and data corrected for the relative response of the FID according to the method described in 1993 (Carelli and Cert, 1993).

As previously mentioned, TAGs have been historically used as a tool of detection of adulterations in oils. Andrikopolous et al. studied individual TAG species of olive oil as well as several seed oils (corn, cottonseed, palm, peanut, soybean, and sunflower), using a WCOT TAP CB fused-silica capillary column, to detect adulterations in olive oil (Andrikopolous et al., 2001). They achieved verification of adulteration of olive oil with a low content (< 5%) of these seed oils (except peanut oil). This was achieved by detection of the increasing levels of OOO or PPP in olive oil in which these TAG species are normally absent or present at very low levels (< 0.5%). An adulteration with over 20% peanut oil could be detected by the increasing levels of PLL (1-palmitin-2,3dilinolein). Lately, Woo Park et al. have studied mixtures of sesame oil with soybean oil and how the relative percentage of linolenic acid increased rapidly compared to those of oleic and linoleic acids as the ratio of soybean oil increased in the blended sesame oil (Park et al., 2010). They propose the HTGC–FID as an appropriate tool for the TAG analysis from dairy foods with high contents of short chain length fatty acids. Ruiz-Samblás et al. were able to distinguish between extra virgin olive oil from different varieties of olive oil fruit with the aid of HTGC and multivariate statistical tools (Ruiz-Samblás et al., 2011).

Table 3

Related to the analysis of seeds, Yoshida et al. isolated TAG by TLC, and analyzed them by GC with the method of Matsui, Watanabe and Ikekawa, from 1973 (Yoshida et al., 2000). They evaluated the changes in the molecular species composition and FA distribution of TAG from the seed coat of roasted soybean and compared the results with obtained from unroasted soybean

seed coats. Subsequently, the same authors, applied the same method to TAGs in sunflower seeds (Yoshida et al., 2002), peanuts oils, kernels of pumpkin seed, peas, adzuki beans etc. (see table 3). Destaillats et al. developed a simple, fast, and accurate method for the regiospecific analysis of petroselinic, oleic, and cis-vaccenic acids in TAGs and validated it with natural oils of basil and coriander seeds (Destaillats et al., 2002). This method constituted an improvement over existing methods for the analysis of these acids in fats and oils since it provided a better separation of the isomers by GC and also provided information on TAG structure.

Recently, Moldoveanu and Chang describe (for the first time according to the authors), the FA and TAG profile for selected Nicotiana species (tobacco seed extracts) (Moldoveanu et al., 2011). Ten years previously Waheed et al. investigated the same matrix and report the FAs and TAGs profiles of this species (Waheed et al., 2001).

For the analysis of essential oils Marriot et al. reviewed the GC technologies applied for the analysis of essential oils (Marriot et al., 2001). More recently, Pinto and Lancas, have described a method for the analysis of TAGs (total carbon only) in Azadirachta Indica essential oil (Pinto and Lancas, 2010).

1.3 OTHERS

Some papers that either use HTGC for TAG analysis of samples different to those previously described or have special features that warrant mention are included in this final section. A summary is found in table 4.

Table 4

In 2004, van Vliet and van Kempen developed a mathematical framework to estimate the TAG distribution based on any subset of GC and LC data since they claimed that, although notable separations could be obtained on some stationary phases, separation of all TAGs present in a sample using a single LC or GC column was impossible (van Vliet and van Kempen, 2004). Within this computational estimation, three different approaches are described: a least squares, a genetic algorithm and expectation maximisation. They applied the developed algorithms by estimating the TAG distribution of palm oil, palm stearin and evening primrose. Gutiérrez et al. illustrate a paper where chromatographic conditions are described that provide well-developed peaks of TAG of low molecular weight (C28 to C32) up to high molecular weight (C34 to C54) with a low polar column, so they do not distinguish between number of insaturations (Gutiérrez et al., 2004). The profiles were obtained from the oils from: peanut, canola, sunflower, maize, olive and fish, and the fats of; raw milk, pork and beef. Recently, van Ruth et al, describe the analysis of TAG composition (and other compounds) of different animal and vegetable fats as well as oils in order to predict their identities (van Ruth et al, 2010).

CONCLUSIONS

The use of HTGC in the analysis of triacylglycerols over the past decade has been reviewed and discussed.The progress in chromatographic techniques, as well as stationary phases in columns, have enormously facilitated the analysis of TAGs in different kinds of sample and have favored the use of GC instead of LC as preferred official methods. Therefore, according to the results

found in the literature, HTGC seems to be a simple, reliable and satisfactory methodology for the TAGs analysis.

Finally, it also has been observed that for authentication purposes, TAGs have shown to be good markers for prediction of identities, origin, varieties, etc. of fats, oils and dairy products. The challenge for researchers now is to give a step ahead and to develop new methods to get a better insight into the TAGs representing oils, fats, seeds and dairy products.

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Figure Captions

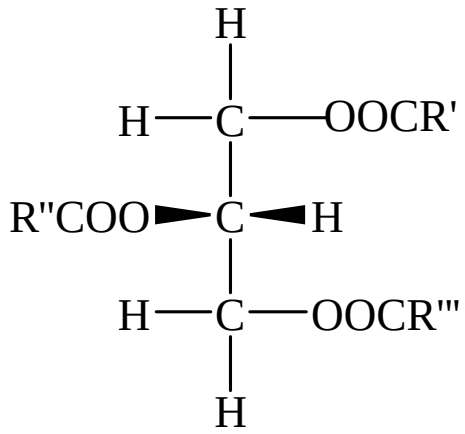
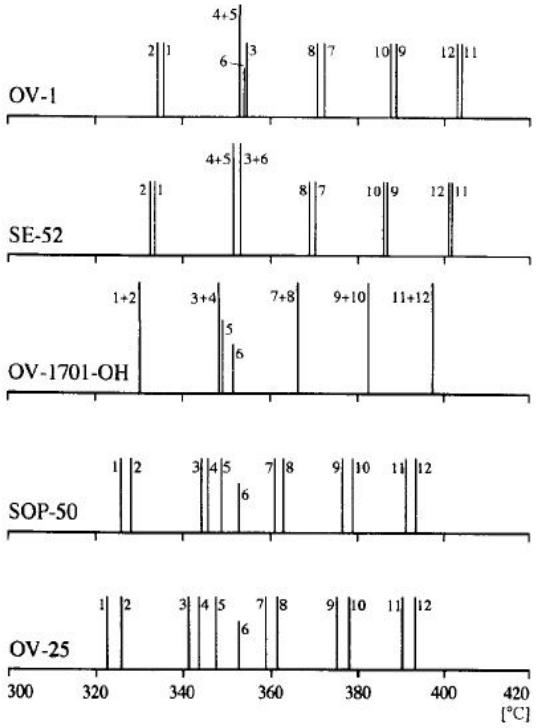


Figure 1. Fischer projection of a triacyl-sn-glycerol



Triacylglycerol	Abbreviation	CN:Bonds
1 Tripalmitin	PPP	48:0
2 Tripalmitolein	PoPoPo	48:3
3 Tristearin	SSS	54:0
4 Triolein	OOO	54:3
5 Trilinolein	LLL	54:6
6 Trilinolenin	LnLnLn	54:9
7 Triarachidin	AAA	60:0
8 Trigadolin	GaGaGa	60:3
9 Tribehenin	BeBeBe	66:0
10 Trierucin	ErErEr	66:3
11 Trilignocerin	LgLgLg	72:0
12 Trinervonin	NNN	72:3

Figure 2. Elution pattern of the TAG mixture of five stationary phases: OV-I, SE-52, OV-1701-OH, SOP-50 and OV-25. (taken from Mayer and Lorbeer, 1997; with permission).

Table 1. TAG index for different stationary phases

Stationary phase	*TAG index*			*Reference*
	OOO	**LLL**	**LnLnLn**	
OV-1	53.16	53.16	53.72	(Mayer et al., 2003a)
SE-52	53.40	53.63	54.30	
OV-17	54.26	55.25	56.54	
SOP-50	54.73	55.87	57.42	
007-65HT	54.88	56.17	57.86	
SOP-75	55.29	56.98	59.01	
SM-30	53.61	53.80	54.29	
SM-50	53.85	54.18	54.60	
SP-50	54.51	55.39	56.58	
SP-43	54.63	55.68	56.93	
SP-25	54.99	56.39	58.14	
SP-20	55.06	56.49	58.25	
SMP-5	55.22	56.80	58.67	
SphTFP-25	53.84	54.19	54.51	(Mayer et al., 2003b)
SDPE-33	53.83	54.25	54.95	(Mayer et al., 2004a)
BGB-Silaren	54.01	54.56	55.41	
OV-25	54.87	56.17	57.99	(Mayer et al., 2004b)
SP-10	55.34	57.06	59.11	
Dexsil 300	53.54	53.67	54.27	(Petsch et al., 2005)
Dexsil 400	53.69	54.00	54.51	

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Table 2. Dairy products

Sample	*Technique, Detector, Carrier gas*	*Column, Stationary phase, Manufacturer, Injection mode*	*Reference*
Milk fat	GC	—	(Ito et al., 2000)
Foreign fat in butterfat	HTGC-FID, N_2	50 cm 3% OV-1 100/120 mesh Gas ChromQ 5 m HT-SimDist CB Ultimetal (Chrompack), on-column	(Molkentin and Precht, 2000)
Goat's milk	HTGC-MS(FID), He	30 m Rtx-65TG (Restek), split-splitless	(Fontecha et al., 2000)
Cocoa butter	HTGC-FID, H_2	30 m DB-17-HT (J&W Scientific) 25 m CP-TAP (Chrompack), on-column	(Buchgraber et al., 2000)
Salatrim	HTGC-FID, He	15 m DB5-HT (J&W Scientific Inc.), on-column	(Jasper, 2000)
Chocolate formulations	HTGC	—	(Simoneau et al., 2000)
Bovine milk fat	HTGC-(EI)MS	30 m Rtx-65TG (Restek), on-column	(Mottram and Evershed, 2001)
Cocoa butter	fast GC	5 m nonpolar capillary column	(Barcarolo and Anklam, 2001)
Milk and milk products	HTGC-FID, N_2	50 cm 3% OV-1 100/120 mesh Gas ChromQ or capillary column, hot injection	(EC, 2001)
Butter flavouring	HTGC-FID	50 cm 3% OV-1 100/120 mesh Gas ChromQ or non polar capillary column 5 m	(Bononi et al., 2001)
Butterfat	HTGC-(EI)MS, He	30 m HP-5MS (Hewlett Packard), split	(Zou, 2002a)
Caprylic/capric triglycerides	HTGC-(EI)MS, He	30 m HP-5MS (Hewlett Packard), split	(Zou, 2002c)

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Cocoa butter	HTGC, N_2	10 m DB1 (J&W Scientific), on-column	(Guyon et al., 2003)
Cocoa butter (review)	—	—	(Ulberth and Buchgraber, 2003)
Cocoa butter	HTGC-FID, He ,N_2, H_2	30 m DB-17-HT (J&W Scientific),25 m CP-TAP (Chrompack)	(Buchgraber et al., 2003)
Butter purity	HTGC	30 m Rtx-65TG (Restek), split, PTV, OCI Rtx-65TG (Restek)	(Naviglio and Raia, 2003a)
Butter oil	HTGC-FID, N_2	50 cm 3% OV-1 100/120 mesh or capillary column	(Zeleny and Schimmel, 2003)
Butter	HTGC	30 m Rtx-65TG (Restek)	(Naviglio and Raia, 2003b)
Ewe's milk fat	HTGC	2.5 m Rtx-65TG (Restek), split	(Goudjil et al., 2003)
Milk fat	HTGC-FID	Petrocol EX 2887	(Tateo and Bononi, 2003)
Cocoa butter validation method	HTGC	—	(Buchgraber et al., 2004b)
Cocoa Butter added to chocolate bars	HTGC, N_2	10 m DB-1 (J&W Scientific), on-column	(Guyon et al., 2004)
Mozzarella di Bufala Campana	GC	—	(Romano et al., 2004)
Butter	GC	Petrocol EX 2887	(Barattero and Lucero, 2004)
Cocoa butter equivalent samples	HTGC-FID, H_2	25 m CB-TAP (Varian), on-column	(Buchgraber et al., 2004c)
Cocoa butter interlaboratory	HTGC-FID, He,N_2, H_2	30 m DB-17-HT (J&W Scientific), 25 m CB-TAP (Varian-Chrompack, 30 m Rtx-65TG (Restek), split, PTV, OCI	(Buchgraber et al., 2004d)
Ovine milk fat	HTGC-MS, He	30 m Rtx-65TG (Restek), split-splitless	(Fontecha et al., 2005)
Dairy Products (review)	—	—	(de la Fuente and Juárez, 2005)
Dairy Products (review)	—	—	(Cserháti et al., 2005)
Butterfat	HTGC-FID; GC-MS, He	25 m phenyl(65%)methylsilicone (Quadrex), on-column	(Kemppinen and Kalo, 2006)
Milk fat	HT(UFM)GC-FID	4 m DB5-HT (J&W Scientific Inc.), split	(Destaillats et al., 2006)

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Origin cheeses (milk fat authenticity)	HT(UFM)GC-FID	2.5 m Rtx-65TG (Restek), split	(Fontecha et al., 2006a)
PDO cheeses	HT(UFM)GC-FID	2.5 m Rtx-65TG (Restek), split	(Fontecha et al., 2006b)
Tofu	HTGC-FID	2.5 m Rtx-65TG (Restek)	(Prestamo and Fontecha, 2007)
Foreign fat in milk fat	HTGC-FID, N_2	50 cm 3% OV-1 100/120 mesh , splitless	(Molkentin, 2007)
Milk fat	UFM-GC	----------	(Povolo et al., 2007)
Cow milk fat	HTGC-FID, H_2	4 m HP-1 (Agilent), on-column	(Povolo et al., 2008)
	HT(UFM)GC-FID, H_2	2.5 m 100%dimethylpolysiloxane column, split-PTV	
Mozzarella di Bufala Campana	HRGC	—	(Romano et al., 2008)
Milk and milk products	HTGC-FID, He,N_2, H_2	50 cm 3% OV-1 100/120 mesh Gas ChromQ, hot needle or non polar capillary column 5 m, on-column	(EC, 2008)
Cocoa butter	HTGC	—	(Wang et al., 2009)
Milk fat	Ultrafast GC	2.5 m 100% dimethylpolysiloxane	(Povolo and Contarini, 2009)
Non-milk fat in milk fat	HTGC-FID, He	2 m HP-5 (Hewlett Packard), split-splitless	(Gutiérrez et al., 2009)
Milk and milk products	HTGC-FID, He,N_2, H_2	50 cm 3% OV-1 100/120 mesh, hot needle or non polar capillary column 5 m, on-column	ISO 17678 – IDF 202 (2010)
Camel milk	HTGC-FID, He	30 m Rtx-65TG (Restek), split	(Haddad et al., 2011)
Mozzarella di Bufala	HTGC-FID, He	30 m Rtx-65TG (Restek), split-PTV	(Romano et al., 2011)

Table 3. Oils and seeds

Sample	*Technique, Detector, Carrier gas*	*Column, Stationary phase, Manufacturer, Injection mode*	*Reference*
Soybeans	HTGC-FID	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2000)
Sunflower	HTGC-FID, H_2	15 m 400-65HT 65% phenyl silicone (Quadrex), split	(Fernández-Moya et al., 2000)
Authenticity fats and oils (review)	—	—	(Ulberth and Buchgraber, 2000)
Authentication vegetable oils (review)	—	—	(Aparicio and Aparicio-Ruíz, 2000)
Nicotiana tabacum L. seed oil	GC	—	(Waheed et al., 2001)
Essential oils	GC	—	(Marriott et al., 2001)
Olive and corn oil	Fast GC	—	(Frega and Bocci, 2001)
Olive oil (corn, cottonseed, palm, peanut, soybean, and sunflower)	GC-FID	WCOT TAP-CB (Chrompack), split, on-column	(Andrikopoulos et al., 2001)
Oils and fats (review)	—	—	(Andrikopoulos, 2002a)
Common edible vegetable oils (review)	—	—	(Andrikopoulos, 2002b)
Sunflower seeds	HTGC-FID	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2002)
Coconut oil	HTGC-(EI)MS	30 m HP-5MS (Agilent)	(Zou, 2002b)
Olive oils	HTGC-FID, H_2	25 m HT-5 (SGE), split	(Park and Lee, 2003)
Virgin olive oil (review)	—	—	(Ollivier, 2003)
Edible oils and fats	Comprehensive	7 or 25 m DB-1-HT (J&W Scientific),	(Janssen et al., 2003)

	LC×GC, H_2	on-column	
Peanut oils	HTGC-FID	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2003)
Plant tissues	HTGC-FID, H_2	15 m DB-17-HT (J&W Scientific), split	(Ruiz-Lopez et al., 2003)
Less common edible vegetable oils (review)	—	—	(Andrikopoulos et al., 2004)
Oils of rice, soybean, walnut hazelnut,olive and sunflower	HTGC-FID, H_2	15 m DB-17-HT (J&W Scientific), split	(Martinez Force. et al., 2004)
Kernels of pumpkin seed	HTGC-FID,	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2004a)
Pumpkin seeds	HTGC-FID	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2004b)
Vegetable oils (review)	—	—	(Cserháti et al., 2005)
Kidney beans	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2005)
Palm Oil	HTGC-FID, He	15 m BPX 5 (SGE), on-column	(Lik et al., 2005)
Soybean	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2006)
Edible oils	HTGC-FID; gLC×GC, He	5 m Ultimetal SIMDIS (Chrompack)	(de Koning et al., 2006)
Sesame seeds	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2007a)
Peas	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2007b)
Adzuki beans	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2008a)
Broad beans	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2008b)
Edible oils and fats (review)	Comprehensive chromatography	—	(Janssen et al., 2009)

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Adzuki beans	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2009a)
Adzuki beans	HTGC-FID¸ He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2009b)
Apiaceae seeds oils	HTGC-FID, H_2	30 m Rtx-65TG (Restek), split	(Ngo-Duy et al., 2009)
Broad beans	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2010b)
Animal fats and vegetable oils	HTGC-FID, He	50 cm 3% OV-1 100/120 mesh Gas ChromQ, hot needle or non polar capillary column 5 m, on-column	(van Ruth et al., 2010)
Sesame and soybean oils	HTGC-FID, He	15 m 400-65 HT (Quadrex), split	(Park et al., 2010)
Olive oil	HTGC-(EI)MS, He	30 m Rtx-65TG (Restek), split	(Ruiz-Samblás et al., 2010)
Azadirachta indica essential oil	HTGC-FID, H_2	9 m Croma-5 HT 5%, split	(Pinto and Lancas, 2010)
Nicotiana species seed extracts	HTGC-FID ; ($CI^{(+)}$)-MS, H_2	30 m Rtx-65TG (Restek); 25m CP-Tap (Varian), on-column/PTV	(Moldoveanu and Chang, 2011)
Olive oil	HTGC-(EI)MS, He	30 m Rtx-65TG (Restek), split	(Ruiz-Samblás et al., 2011)

Table 4. Others

Sample	*Technique, Detector, Carrier gas*	*Column, Stationary phase, Manufacturer, Injection mode*	*Reference*
Monitoring of interesterification	HTGC-FID, H_2	25 m 65 HT (Quadrex), on-column	(Mu et al., 2000)
Derivatization of monoacylglycerols	HTGC-FID, H_2	30 m Rtx-65TG (Restek), split	(Destaillats et al., 2002)
Vegetable fats	Computational estimation	—	(van Vliet and van Kempen, 2004)
Animal and vegetable fats	HTGC-FID, He	2 m HP-5 (Agilent), split	(Gutiérrez et al., 2004)
Sardine meat	GC-MS, He	25 m Supercap (Quadrex)	(Jittrepotch et al., 2006)
Fattening diet if Iberian pig	HTGC-FID, H_2	30 m DB-17-HT (J&W Scientific), split	(Viera-Alcaide et al., 2007)
Subcutaneous adipose of Iberian pig	HTGC-FID, H_2	30 m DB-17-HT (J&W Scientific), split	(Viera-Alcaide et al., 2008)
Black and red rices (review)	HTGC-FID, He	50 cm 2% OV-17 80/100 on silanized Shimalite mesh	(Yoshida et al., 2010a)
Animal/vegetable fats and oils	HTGC-FID, He	50 cm 3% OV-1 100/120 mesh Gas ChromQ, hot needle or non polar capillary column 5 m, on-column	(van Ruth et al., 2010)