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Review Article

Dietary Fatty Acids Analysis and its Relevance to Human Health

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Abstract

The problem of overweight and obesity has reached epidemic proportions worldwide, and the high prevalence is due in part to the consumption of high-fat and high-energy diet. Recent research revealed that it was necessary to consider not only the quantity, but also the quality of fat in diets. Fatty acid analysis is an important tool both for characterizing fats and oils and for determining the total fat content in foods. To date, Gas Chromatography (GC) has become an important technique in fatty acid analysis because accurate results can be obtained for complex, as well as simple, sample matrices. This review presented various analytical methods including GC developed for identification and accurate quantification of dietary fatty acids. The potential health effects of fatty acids were also discussed.

Abbreviations:

%E, Percent of Dietary Energy; AA, Arachidonic Acid; ALA, α-Linolenic Acid; AN, Acetonitrile; BF_3, Borontrifluoride; C_8, Octysilyl; C_{18}, ODS, Octadecylsilyl; CAD, Coronary Artery Disease; CE, Capillary Electrophoresis; CHD, Coronary Heart Disease; CLA, Conjugated Linoleic Acid; CVD, Cardiovascular Disease; DHA, Docosahexaenoic Acid; DPA, Docosapentaenoic Acid; DLLME, Dispersive Liquid-Liquid Microextraction; DUAE, Dynamic Ultrasound Assisted Extraction; ELSD, Evaporative Light-Scattering Detector; EPA, Eicosapentaenoic Acid; FAME, Fatty Acid Methyl Esters; FAO, Food and Agriculture Organization; FAs, Fatty Acids; FDA, Food and Drug Administration; FID, Flame Ionization Detector; FMSE, Focused Microwave Soxhlet Extraction; GC, Gas Chromatography; GLA, γ-Linolenic Acid; HDL, High Density Lipoproteins; HPLC, High Performance Liquid Chromatography; IOM, Institute of Medicine; LA, Linoleic Acid; LCFAs, Long-Chain Fatty Acids; LDL, Low Density Lipoproteins; LLE, Liquid-Liquid Extraction; LOD, Limit of Detection; MCFAs, Medium-Chain Fatty Acids; MeOH, Methanol; MS, Mass Spectrometry; MUFAs, Monounsaturated Fatty Acids; NIDDM, Non-Insulin-Dependent Diabetes Mellitus; nLC, Nanoflow Liquid Chromatography; NP, Normal-Phase; OA, Oleic Acid; PEG, Polyethylene Glycol; PFE, Pressurized Fluid Extraction; PUFAs, Polyunsaturated Fatty Acids; RP, Reversed-Phase; SBSE, Stir Bar Sorptive Extraction; SDA, Stearidonic Acid; SFAs, Saturated Fatty Acids; SFC, Supercritical Fluid Chromatography; SPE, Solid-Phase Extraction; SPME, Solid-Phase Microextraction; TG, Triglycerides; TLC, Thin-Layer Chromatography; TMS-DM, Trimethylsilyl Dizomethane; TUFA, *Trans* Unsaturated Fatty Acids; UAE, Ultrasound-Assisted Extraction; U-AMDR, The Upper Level of Acceptable Macronutrient Distribution Range; UFAs, Unsaturated Fatty Acids; UHPLC, Ultra High Performance Liquid Chromatography; WHO, World Health Organization

Introduction

Obesity has become a worldwide health problem because it is implicated in the development of many chronic diseases. Strategies recommended for weight control have generally suggested the adoption of low-fat and low-energy dietary patterns, which facilitate energy restriction and Cardio Vascular Disease (CVD) risk reduction. Recent research revealed that it is necessary to consider not only the quantity, but also the quality of fat in diets. Issues of fat quality included the potentially adverse effects of saturated (SFAs) and *Trans* Unsaturated Fatty Acids (TUFAs) on circulating cholesterol concentrations and the beneficial effects of Monounsaturated Fatty Acids (MUFAs) and Polyunsaturated Fatty Acids (PUFAs) on human health. These issues remain important aspects of public health nutrition which will be considered as part of this review. In addition, the available methods for dietary FAs analysis and problems likely to be encountered are also discussed.

It is well known that fat is the most energy dense of all the macronutrients with 1 g providing 9 kcal. Human body needs FAs for many other functions than as simply an energy source and there is an increasing awareness of penitential health benefits of specific types of FAs. FAs are

Table 1a: Common dietary saturated fatty acids, their health effects as well as regular analytical methods

	Name	Systematic name	Abbreviation	Common sources	Health effects	Analytical methods
Medium chain fatty acids (MCFAs)	Caproic acid	Hexanoic acid	C6:0	Animal fats and oils	MCFAs reduced triglyceride secretion when used in low amounts [77] High amounts of MCFAs (caprylic and capric acid) in diet increased plasma cholesterol, triglyceride, and fasting glucose levels as compared with oleic acid [78]. Besides increased fat oxidation and postprandial energy expenditure, MCFAs reduced food intake and enhanced satiety [79].	GLC-FID [80]. HPLC-FPD/RID [81]. HPLC-RID [82]
	Caprylic acid	Octanoic acid	C8:0	Milk, coconut and palm kernel oil		
	Capric acid	Decanoic acid	C10:0	Coconut oil, milk		
Long chain fatty acids (LCFAs)	Lauric acid	Dodecanoic acid	C12:0	Coconut oil, laurel oil, palm kernel oil	Lauric acid had a favorable effect on total:HDL cholesterol [16]	
	Myristic acid	Tetradecanoic acid	C14:0	Nutmeg, coconut oil, palm kernel oil, butter fat	Myristic acid increased plasma total and LDL cholesterol levels, whereas stearic acid [83]	GC-FID [86]. HPLC-FID [87]. HPLC-UV [74].
	Palmitic acid	Hexadecanoic acid	C16:0	Palm oil	Palmitic acid was more hypercholesterolemic than lauric acid [84], whereas less hypercholesterolemic than a combination of lauric and myristic acids [85].	
	Stearic acid	Octadecanoic acid	C18:0	Animal and vegetable fats and oils	Compared with other LCFAs, stearic acid lowered plasma total, LDL and HDL cholesterol concentrations [22,25].	

subdivided into three broad groups including SFAs, MUFAs, and PUFAs based on the number of double bonds. The sources of some common dietary FAs, their health effects, and regular analytical methods are listed in Table 1. Imbalances of FAs have been reported to lead to inflammatory and hormonal disorders, CVD, and many other conditions. Even some individuals with a dietary balance of FAs still have biochemical abnormalities that interfere with their ability to properly metabolize these FAs. On the other hand, different FAs have distinct biological properties and hence are associated with different health effects. SFAs, such as lauric, myristic, and palmitic acids, are responsible for increasing plasma total and Low Density Lipoproteins (LDL) cholesterol concentrations. Therefore, World Health Organization (WHO) and many other health associations recommended limiting the consumption of SFAs to help maintain cardiovascular health. However, replacing SFAs with MUFAs may reduce LDL cholesterol concentration and total/HDL (High Density Lipoproteins) cholesterol ratio, a powerful predictor of the risk of Coronary Heart Disease (CHD) [1]. In addition, replacing SFAs with PUFAs may similarly decrease the risk of CHD. Previous study has shown that the consumption of Omega-3 FAs, Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) reduced the risk of CHD by lowering Triglyceride (TG) levels and blood pressure [2]. In addition to the mentioned SFAs and UFAs, human diet contains TUFAs, originating from ruminant deposits and milk fats [3]. TUFAs were reported as unhealthy FAs in 2002 by Institute of Medicine (IOM) that the intake of TUFAs has a direct correlation with increased LDL cholesterol levels and increased risk of heart disease [4]. Therefore, in late 2013, the Food and Drug Administration (FDA) stated that TUFAs were not generally considered safe.

Due to the widespread health effects of FAs and TUFAs, the identification and quantification of them in biological and food materials are required. To date, the most frequently used technique for FAs analysis is Gas Chromatography (GC). On the other hand, High Performance Liquid Chromatography (HPLC) is a method for preparative scale separations of particular FAs for structural or metabolic studies. Numerous other methods, including chemical titration [5], thermometric titration [6], metal-FA complex [7], enzymatic method [8], FA binding protein [9,10], and spectroscopic method are useful for the quantitative measurement of FAs in biological and food materials [11,12].

Potential Health Effects of Fatty Acids

Saturated Fatty Acids (SFAs)

Previous metabolic studies have shown that diets high in SFAs and low in PUFAs increased blood cholesterol concentrations [13,14], which resulted in the high risk of CHD. Therefore, a high-SFA diet was considered noxious and international dietary guidelines have recommended that SFAs should not contribute more than 10% of dietary energy (%E) [15]. SFAs are hypothesized to affect many physiological pathways, including blood lipids and lipoproteins [16] and glucose-insulin responses [17].

Recent studies have shown that individual SFAs had different effects, and thus may have different effects on the risk of CHD [18]. SFAs contain largely Long-Chain Fatty Acids (LCFAs) with a chain length of 12 carbons and more. LCFAs, including lauric, myristic, and palmitic acids, tend to increase plasma total and LDL cholesterol concentrations, whereas stearic acid does not have a cholesterol-raising effect compared with oleic acid. Among these cholesterol-raising LCFAs, myristic acid is more potent than lauric acid or palmitic acid [19]. Besides LCFAs, there are some natural sources of Medium-Chain Fatty Acids (MCFAs) as shown in Table 1. Compared with LCFAs, MCFAs reduced lipoprotein secretion and attenuated postprandial TG response given in moderate amounts in diets.

Table 1b: Common dietary *cis*-monounsaturated fatty acids, their health effects as well as regular analytical methods

Name	Systematic name	Abbreviation	Common sources	Health effects	Analytical methods
Palmitoleic acid	*cis*-9-hexadecenoic acid	9*c*:16-1 or 16:1(n-7)	Animal, vegetable, and marine oils	Diet rich in palmitoleic acid increased LDL and decresased HDL cholesterol levels [88].	GC-FID [89].
Oleic acid	*cis*-9-octadecenoic acid	9*c*:18-1 or 18:1(n-9) (OA)	Animal and vegetable fats and oils, olive oil, macadamia oil	OA decreased LDL and increased HDL cholesterol level. It may hinder the progression of adrenoleukodystrophy (ALD) and possess hypotensive effects.	GC-MS [90].

Table 1c: Common dietary polyunsaturated fatty acids, their health effects as well as regular analytical methods

	Name	Systematic name	Abbreviation	Common sources	Health effects	Analytical methods
n-6 PUFA (omega-6 FAs)	Linoleic acid	*cis*-9, *cis*-12-octadecadienoic acid	18:2(n-6) (LA)	Poppyseed, safflower, sunflower, corn, and soybean oils	A higher intake of LA may reduce the incidence of CAD [91].	GC, Ag^+-HPLC, and a combination of Ag^+-TLC/GC [92].
	γ-linolenic acid	*cis*-6, *cis*-9, *cis*-12-octadecatrienoic acid	18:3(n-6) (GLA)	Evening primrose, borage and blackcurrant seed oils	The consumption of GLA may help to treat and prevent certain chronic diseases: rheumatoid arthritis, CVD, and cancer [93].	GC-FID [94].
	Arachidonic acid	*cis*-5, *cis*-8, *cis*-11, *cis*-14-eicosatetraenoic acid	20:4(n-6) (AA)	Animal fats, eggs	Increased AA intake is probably harmless in healthy adults. AA in infant formula may be helpful in growth, development and health [95].	GC-FID [63].
n-3 PUFA (omega-3 FAs)	α-linolenic acid	*all-cis* 9,12,15-octadecatrienoic acid	18:3(n-3) (ALA)	Flaxseed oil, perilla oil, canola oil, soybean oil	ALA may decrease CVD risk by inhibiting vascular inflammation and endothelial activation beyond its lipid-lowering effects [96].	GC-FID [101]. GC-MS [102]. Ag-HPLC, Ag-TLC [103].
	Stearidonic acid	*all-cis* 6,9,12,15-octadecatetraenoic acid	18:4(n-3) (SDA)	Fish oil	Supplementing SDA had little impact on modifying circulating TG and total, LDL, and HDL cholesterol [97].	
	Eicosapentaenoic acid	*all-cis* 5,8,11,14,17-eicosapentaenoic acid	20:5(n-3) (EPA)	Fish oils, marine sources	EPA can reduce inflammation and has the potential to modulate nutritional status/ body composition [98].	
	Docosapentaenoic acid	*all-cis* 7,10,13,16,19-docosapentaenoic acid	22:5(n-3) (DPA)	Fish oil	DPA reduces platelet aggregation, and improves lipid metabolism, endothelial cell migration and resolution of chronic inflammation [99].	
	Docosahexaenoic acid	*all-cis* 4,7,10,13,16,19-docosahexaenoic acid	22:6(n-3) (DHA)	Fish oils, dairy products	Dietary DHA is essential for brain functioning and it has positive effect on diseases such as hypertention, arthritis, atherosclerosis, depression, diabetes mellitus, myocardial infarction, thrombosis, heart disease and some cancers [100].	

Therefore, recent research attention has focused on the potential role of MCFAs for weight management [20].

Monounsaturated Fatty Acids (MUFAs)

MUFAs are distinguished from SFAs on the basis of having one double bond. The position of the hydrogen atoms around the double bond determines the geometric configuration (*cis* or *trans*) of the MUFAs. In a *cis* MUFA, the hydrogen atoms are present on the same side of the double bond, whereas in the *trans* configuration, they are on opposite sides. The most common MUFAs, including palmitoleic and oleic acid are present in considerable quantities in both animal and plant sources (as shown in Table 1). Compared with SFAs, a high-MUFA diet has been reported to reduce total and LDL cholesterol levels [21], and it decreased plasma TG concentrations and increased HDL cholesterol levels when compared with carbohydrate [22,23]. On the other hand, there is epidemiological evidence that dietary MUFAs have beneficial effects on the risk of CVD [22,24-26]. For example, previous studies have suggested that MUFAs may decrease platelet aggregation, increase bleeding time, and increase fibrinolysis, and thus protecting against thrombogenesis [27,28]. Moreover, a very-high-MUFA diet significantly decreased systolic and diastolic blood pressure in subjects with non-insulin-dependent diabetes mellitus (NIDDM) [29]. These findings, taken together, suggested that high-MUFA diets may confer benefits on CVD risk factors beyond those associated with plasma lipids and lipoproteins.

Polyunsaturated Fatty Acids (PUFAs)

Unlike SFAs and MUFAs, PUFAs have two or more double bonds. In almost all naturally occurring PUFAs, the double bonds are of *cis* configuration and arranged in methylene-interrupted (or non-conjugated) pattern. PUFAs can be divided into twelve families, ranging from double bonds located at the n-1 position to the n-12 position. In terms of extent of occurrence and human health and nutrition, n-3 and n-6 are the most important families of PUFAs. α-linolenic acid and linoleic acid are the parent fatty acids of the n-3 (omega-3) and n-6 (omega-6) families. Both of them are essential as they cannot be synthesized in mammalian tissues, and thus have to be obtained from the diet.

The various health benefits of consuming PUFAs have been widely reported and reviewed, and it is a nutritional hot topic that is attracting both public and industrial interest. PUFAs play important roles in CVD prevention [30,31], inflammation and immunity regulation [32]. Whereas a controlled-feeding study has shown that PUFAs had greater reductions in total cholesterol levels than MUFAs [33], other studies observed comparable total and LDL cholesterol-lowering effects of PUFAs and MUFAs [34,35]. The mechanisms by which PUFAs reduced CVD include the effects on blood lipid levels, blood pressure, inflammatory response, arrhythmia and endothelial function. Moreover, PUFAs affected various metabolic pathways, including those involved glycaemic control, so the types and amounts of PUFAs may have a role to play in the management of type-2 diabetes [36]. PUFAs may also reduce the risk of developing certain cancers such as colon, breast, and prostate, although the evidence is by no means conclusive [37]. In addition, a number of inflammatory conditions, such as asthma, Crohn's disease and arthritis, could be potentially be alleviated by the consumption of both omega-3 and omega-6 PUFAs [38].

According to Food and Agricultural Organization [39], the recommended range for total PUFAs (omega-6 and omega-3 FAs) is 6-11%E to decrease the risk of CHD events and to prevent risk of lipid peroxidation. However, excess supplement intake of omega-3 FAs may increase the peroxidation and reduce cytokine production.

Therefore, FAO suggested 2 g/d as the upper level of acceptable macronutrient distribution range (U-AMDR) for EPA and DHA [39].

Trans Unsaturated Fatty Acids (TUFAs)

TUFAs are mainly formed from industrial processing, being the by-product of partial hydrogenation of unsaturated vegetable oils. Major dietary sources of TUFAs are deep-fried fast foods, packaged snacks, bakery products, and margarines. Naturally occurring TUFAs are consumed in smaller amounts in meats and dairy products from ruminants; these TUFAs are produced by the action of bacteria in the ruminant stomach.

TUFAs are an independent cardiovascular risk factor [40]. Compared with the consumption of equal energy from SFAs or *cis* UFAs, the consumption of TUFAs raised levels of LDL, reduced HDL, and increased total/HDL ratio. In addition, TUFAs increased the blood levels of TG and Lp(a) lipoprotein, and reduced the particle size of LDL cholesterol, which may further raise the risk of CHD [41]. Moreover, TUFAs may promote inflammation, cause endothelial dysfunction, and influence other risk factors for CVD [42]. Therefore, the consumption of TUFAs should be minimized and the products containing TUFAs should be avoided.

FAs Analysis

It has been recognized that the FAs composition of the diet may play an important role in the development of various chronic diseases. For example, the omega-6 to omega-3 PUFAs ratio of cells or tissues has become a biomarker for monitoring the outcome of dietary interventions and for identifying the risk factors for lipid-related diseases, e.g. CVD [43]. The FA biomarkers are regarded to provide an objective measurement of some types of dietary fat intake [44], and some epidemiological studies use FAs as nutritional biomarkers to investigate the association with disease conditions, such as type 2 diabetes [45,46]. Analysis of FAs composition is usually carried out by using Gas Chromatography (GC). In recent years, several other chromatographic methods, including Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Supercritical Fluid Chromatography (SFC), and Capillary Electrophoresis (CE), have been employed for comprehensive determination of FAs composition in complex samples. When hyphenated to Mass Spectrometry (MS), these methods can provide a large amount of information on almost all of FAs in a sample.

Gas chromatography

GC has been adopted as a highly applicable method in FAs analysis for half a century. Currently, GC with high-quality capillary columns allows sensitive FAs analysis and the characterization of complex mixtures of geometric isomers when coupled with other chromatographic separations and spectroscopic identification. In general, analysis of FAs from biological or food samples involves three steps: extraction of lipids; conversion of the extracted lipids to volatile Fatty Acid Methyl Esters (FAMEs); analysis of the FAMEs using GC for the FAs profile. Choosing appropriate methods for lipid extraction and derivatization is important to prevent the occurrence of chemical changes and formation of contaminants which will then affect the accuracy of the results [47].

Lipid extraction and derivatization of fatty acids: Lipids extraction, aiming to remove interfering agents such as proteins, saccharides, and other small molecules from complex biological or food matrixes, is a critical step in FAs analysis. Generally, two extraction methods including Liquid-Liquid Extraction (LLE) and Solid-Phase Extraction (SPE) have been used for sample preparation. The LLE method was developed by Folch and co-workers in the 1950s using chloroform/methanol (MeOH) as the extraction solvent [48]. To date, various LLE methods have been evaluated for FAs analysis from different food products or biological materials. For example, the extraction of HCl-digested food samples, including corn beef hash, frozen turkey pie, frozen beef pie, and beef stew, with diethyl ether was more effective than chloroform/MeOH as regards the amount of total lipids, FAs distribution, and TG recovery [49]. Recently, a mixture of butanol and MeOH was used to extract total lipids from human plasma [50]. In comparison with Folch method, the extract efficiency of total lipids was comparable or better. Moreover, this method was rapid and high throughput, which can automatically extract 96 samples with 1 h. Besides LLE, SPE methods have been widely used for lipid extractions [51]. The main advantages of SPE include the reducing of solvents consumption and extraction time. However, when dealing with large volumes of samples, the recovery will be reduced due to the low peak capacity of SPE.

In recent years, many new extraction methods have been developed, such as Solid-Phase Microextraction (SPME), Stir Bar Sorptive Extraction (SBSE), Ultrasound-Assisted Extraction (UAE), Pressurized Fluid Extraction (PFE), and Dispersive Liquid-Liquid Micro Extraction (DLLME) [52-55]. They all showed high efficiency and repeatability. Ruiz-Jimenez et al. used Dynamic Ultrasound Assisted Extraction (DUAE) and Focused Microwave Soxhlet Extraction (FMSE) methods to extract lipids and found that DUAE method, when coupled with GC-MS, was two times faster and able to achieve the similar sensitivity and accuracy as Folch reference method. FMSE dramatically shortened the extraction time (3-fold) [56].

After lipids extraction, a derivatization (methylation) procedure is required to convert FAs to FAMEs prior to GC analysis. Derivatization methods that esterify both the lipid-bound and free FAs conventionally include direct esterification (HCl or H_2SO_4/MeOH), saponification-esterification (KOH, HCl/MeOH), and borontrifluoride (BF_3/MeOH) methods. The most appropriate extraction or derivatization method depends on the FAs compositions and matrixes. Salimon and co-workers compared two derivatization procedures, the base-catalysed followed by an acid-catalysed method ($KOCH_3$/HCl) and the base-catalysed followed by Trimethylsilyl Dizomethane (TMS-DM) method [57]. They found that that TMS-DM method had higher recovery values and lower variations, especially for UFAs; therefore, it is more suitable for accurate quantification of food products containing complicated mixtures of FAs. On the other hand, $KOCH_3$/HCl method required shorter time and was less expensive; thus it is more appropriate for the routine FAs analysis. When BF_3 was used as the acidic transesterification catalyst [58], some contaminants were formed from PUFAs, especially at a high concentration of BF_3. Moreover, it would lower the recoveries of EPA and DHA compared to the recovery from KOH method. It is probably due to the insufficient reaction time as the double bond close to the carboxyl group could inhibit transmethylation. Therefore, a reaction time of 1 h at 100 °C was recommended.

The conventional lipids extraction and derivatization are complicated and time-consuming; potentially cause sample loss and

contamination. In a simplified method, extraction and derivatization were combined into a single step [59]. The sample aliquot is mixed with hexane and BF_3/MeOH reagent and blanketed with nitrogen. The mixture is then heated to 100 °C and cooled to room temperature. The methyl ester in the hexane phase is then centrifuged to remove the hexane phase. A higher recovery of LCFAs from tissue samples was observed by using the simplified method, although there is no difference in relative FA composition. Moreover, this simplified method is rapid and suitable for analysis of total long chain PUFAs in various biological samples, but it is not appropriate for quantification of MCFAs. For studies requiring FAs composition of individual lipid class, prior lipids extraction and separation are still needed.

Dietary fatty acids analysis by GC: GC is one of the most powerful tools for the analysis of FAs components due to its high resolution, high sensitivity, and good reproducibility. The liquid phase used in a GC column is the principal factor to determine the nature of the separations that can be achieved. For example, flexible fused silica capillary columns coated with highly polar phases, available as CP-Sil 88, SP-2560, and BPX-70, are recommended for the analysis of fats containing complex mixtures of MUFAs isomers and a range of FAs from butyric acid to PUFAs (e.g. milk fat). The CP-Sil 88 and SP-2560 columns provide similar elution orders of FAMEs [60]. Moreover, the resolution of conjugated linoleic acid (CLA) isomers as well as PUFAs such as LA and ALA and their isomers can be improved by the CP-Sil 88 column [61]. Polyethylene Glycol (PEG) column or CarbowaxTM such as Supelcowax 10 column is suitable for analyzing PUFA as it gives a complete separation of the ALA and its isomers present in dairy fats.

For GC analysis, Flame Ionization Detector (FID) is a universal detector with linear responses for a wide range of sample sizes, gas flow and detector temperature. It is the most robust and widely used detector for FAs characterization. Nevertheless, FID does not provide any further structural information. In contrast, MS is an important biotechnological analytical method, which is capable to identify molecular species or isomeric structures. Electron impact mass spectra can be matched to compounds in extensive libraries for identification. However, GC-MS has different response factors for different compounds, such that individual FA needs its own calibration curve [47].

Table 2 summarizes the GC-based FAs analysis of food products. Fats and oils of animal origin as well as marine products contain various PUFAs, which makes analyzing the FA profiles even more complicated. In addition, as the number of double bonds increases, the number of possible isomers increases. PUFA components such as EPA and DHA can be isomerized to many possible geometrical isomers following heat treatment during processing of food or supplements. Consequently, it is more difficult to separate all of the positional and geometrical isomers of EPA and DHA. Co-elution of the isomers usually remained hidden in PUFA quantification by GC-FID [47]. To obtain good results in analysis of geometrical isomers of PUFAs, they should be converted into derivatives that are indicative of structural features such as 3-pyridylcarbinol ester and 4,4-Dimethyloxazoline (DMOX). DMOX derivatives have excellent properties for GC which can be easily resolved on all of the common polar stationary phases used in GC analysis. However, the mass spectral characteristics in the high mass range are not as good as 3-pyrifylcarbinol esters.

Prior clinical and epidemiological studies reported the association between consumption of TUFAs and the risk of CVD. As a result, the development of a fast and cost-effective detection method for TUFAs in different matrix of food products captured the widespread attention. The major encounter with GC analysis of TUFAs is in detection of positional and geometrical isomers of C18. GC is unable to separate C18 isomer and results in overlapping data. This has occurred in several cases across different variation of GC analysis. Tavella et al. compared two chromatography methodologies of different capillary column lengths and conditions to determine the amount of TUFAs found in locally available food products [62]. In their findings across 46 food items, overlapping of peaks were found in elaidic (18:1n9 *trans*) and oleic (18:1n9 *cis*) acid in the column with less resolution capacity. This study provides a stepping stone to compile a robust and comprehension food composition database. Further steps to improve the analysis include standardizing the type of instrument used, specifically the choice of column. Once reliability of the procedures is fully established, it can be used as a routine chemical analysis of food product to generate product labels. To ease the implementation of TUFAs analysis as a routine chemical analysis among industries, GC methodologies can be further innovated for the ease of usage, shorter analysis time, cost-effective and lower learning curve. Different variations of GC setting are investigated, such as inlet pressure, temperature, length of capillary columns and types of attachment.

Cruz-Hernandez et al. used a highly polar capillary column to achieve an accurate separation of *cis* and *trans* isomers [63]. In this study, they demonstrated the importance of gathering absolute data of human milk, which contains complex FAs. This is crucial as normalized data sets are lack of inter-individual variability thereby loss of precious data. A GC method that is capable of analysing large sample size with small sample amount was developed and validated. In a different work, Destaillats et al. employed a short and highly polar capillary column to analyze milk fat in order to shorten the analysis time. Although the methodology was capable to separate FAs in milk fat and rat red blood cell and plasma, the separation condition caused an overlapping of positional and geometrical isomers of oleic acids (18:1n9 *cis*) [64]. To develop an accurate quantification of TUFAs in milk fat, various analytical methods were compared and it was found that vaccenic acid (18:1n11 *trans*) and *trans*-18:1 isomers could be accurately determined by direct GLC under optimal conditions. However, the method was unable to determine the isomeric distribution of *trans*-18:1. This disadvantage can be resolved with an additional HPLC fractionation step [65].

In recent years, the comprehensive multidimensional GC system becomes more and more widely used to analyze complex samples. The main advantage of multidimensional GC is improvement of the chromatographic resolution due to the increase of peak capacity [66]. Mondello et al. explored several GC conditions, including the usage of different parameters (e.g. inlet pressure and temperature) and different GC arrangements (e.g. GC-MS and two GCs). To test the effectiveness of various conditions, different lipidic matrices ranging from simple butter to complex menhaden oils are used as test samples. Fast GC (Supelcowax-10, 60-70 min) showed promising results; symmetric, narrow and high peak shape giving no signs of column overloading. The developed method was shown to be applicable in routine applications on complex natural products [67]. On the other hand, ionic liquid

Table 2: Gas chromatography based fatty acids analysis of food products.

Name	Matrix	Sample Preparation	Column	Carrier gas	Method	LOD/LOQ	Reference
10:0; 12:0; 14:0; 14:1n-7; 16:0; 16:1n-7; 18:0; 18:1n-9t; 18:1n-9c; 18:1n-7; 18:2n-6; 18:3n-3	White bread, margarine, butter, cookies, crackers, potato crisps, cheetos, cheese flavored sticks	Lipids extraction: Folch technique Cholesterol extraction: Saponification with KOH/MeOH	(1) CPSil-88, capillary column coated with 100% cyanpropylpolysiloxne (50 m x 0.25 mm) (2) Omegawax 250, capillary column (30 m x 0.25 mm)	He with 19 psi Split ratio: 1:70	Detector: FID Detector T: 250 °C Injector T: 250 °C Oven T: 185 °C for 3 min, Increase at 3 °C/min to 230 °C	<0.3 % content	[62]
14:0; 16:0; 16:1t; 18:0; 18:1t; 18:2t,t; 18:2c,t; 18:2t,c; 18:2; 20:0; 18:3; 22:0	Bakery Products	Lipids extraction: Folch Technique FAME preparation: Sodium methylate in MeOH Microwave-assisted Soxhlet extraction	SP-2380, fused silica capillary column (60 m x 0.25 mm) coated with stabilized poly (90% biscyanopropyl/10% cyanopropylphenyl siloxane)	He at 1 mL/min Spilt ratio: Non	Detector: MS Detector Condition: electron impact ionization positive mode, transfer line 170 °C and ion trap at 170 °C Injector T: 250 °C Oven T: 50 °C held for 2 min, increased at 5 °C/min to 250 °C and held 15 min	0.98-3.93 µg/g (14:0 = 2.28/7.51; 16:0 = 0.98/3.23; t16:1 = 2.97/9.79; 18:0 = 1.18/3.89; t18:1 = 1.01/3.33; 18:1 = 1.2/3.97; tt18:2 = 2.08/6.86; tc18:2 = 1.64/5.41; ct18:2 = 0.98/3.23; 18:2 = 2.29/7.56; 20:0 1.10/3.62; 20:0 = 1.1/3.62; 18:3 = 1.19/ 3.93; 22:0 = 3.93/12.98)	[104]
10:0; 12:0; 14:0; 16:0; 16:1 n-7; 18:0; trans-18:1; 18:1 n-9; 18:1 n-7; 18:2 n-6; 18:3 n-3; 20:0; 20:1 n-9; 20:2 n-6; 20:3 n-6; 22:1 n-9; 20:4 n-6; 20:5 n-3; 24:0; 24:1 n-9; 22:6 n-3	Human milk	FAME preparation: HCl/ MeOH	Fused-silica CP-Sil 88 capillary column (100% cyanopropylpolysiloxane; 100 m x 0.25 mm)	H_2 at 1.5 mL/min Spilt ratio: 1:25 Temperature: 250 °C	Detector: FID Detector T: 300 °C Oven ramp: at 60 °C isothermal for 5 min, increased to 165 °C at 15 °C/min and held isothermal for 1 min, increased to 195 °C at 2 °C/min and held isothermal for 14 min, increased to 215 °C at 5°C/min and held isothermal for 8 min		[63]
14:0-24:0	Butter, lard, tallow, peanut, corn, sunflower, soya, olive menhaden oils	FAME Preparation	Supelcowax-10 (10 m x 0.10 mm)	He at 30.1 cm/s Split ratio: 1:100 Inlet pressure: 100 kPa Sampling rate: 12.5 Hz	Detector: FID Detector T: 300 °C Injection temperature: 250 °C Injection volume: 1.0 µl Oven T: 50-280 °C at 3 °C/min Column flow: 1.26 mL/min		[67]
4:0, 6:0, 8:0, 10:0-18:0, 15:1 n-5, 16:1 n-7, 17:1 n-7, trans-18:1 n-9, 18:1 n-9, trans-18:2 n-6, 18:2 n-6, 20:0, 18:3 n-6, 20:1 n-9, 18:3 n-3, 21:0, 20:2 n-6, 22:0, 20:3 n-6, 22:1 n-9, 20:3 n-3, 20:4 n-6, 22:2 n-6, 24:0, 20:5 n-3, 22:6 n-3, 24:1 n-9	Milk fat, Cocoa butter, Tuna oil	FAME	Fused-silica BPX-70 Capillary column (10 m x 0.1 mm)	H_2 at 1 mL/min split ratio: 500:1 Sampling rate: 100 Hz	Detector: FID Oven T: 50 °C isothermal for 0.2 min, increased to 180 °C at 120 °C/min and isothermal for 1 min, increased to 220 °C at 20 °C/min and increased to 250 °C at 50 °C /min		[64]
4:0-22:0, 14:1 n-5, 15:1 n-5, 16:1 n-7, 17;1 n-7, trans-9 18:1, 18:1 n-9, all trans-18:2 n-6, 18:2 n-6, 18:3 n-3, 20:2 n-6, 20:3 n-6,22:1 n-9, 20:3 n-3, 20:4, n-6, 22:2 n-6, 24:0, 20:5 n-3, 24:1 n-9, 22:6 n-3, trans-18:1	Dairy milk	FAME	Fused silica CP-Sil 88 capillary column (100 m x 0.25 mm)	H_2 Inlet pressure: 200 kPa	Detector: FID Oven T: 60 °C isothermal for 5 min, increased to 165 C at 15 °C/min and isothermal for 1 min, increased to 225 °C at 2 °C/min and isothermal for 225 °C at 17 min		[64,65]
15:0, 16:0, 18:1, 18:1t9, 18:2c9, 18:2c12, 18:2t9, 18:2t12	Biscuit, cracker, bread with filling, cake	Lipids were extracted using Soxhlet method. FAME were prepared using base followed by trimethylsilyl diazomethane ($NaOCH_3$/TMS-DM)	BPX-70 fused silica capillary column (30 m x 0.25 mm)	He at 1 mL/min Split ratio: 30:1	Detector: FID Injection T: 260ºC Detector T: 280ºC Column T: 100ºC at 10ºC/ min to 160ºC, ramped at 3ºC/min to 220ºC, held for 5min, increased at 10ºC/ min to 260ºC, held for 5min	LOD: 0.03-0.09 µg/mL LOQ: 0.1-0.2 µg/mL	[105]
18:1, 18:2, 18:3	Potato crisps fried in olive oil	Single step extraction and transesterification using toluene and methanolic HCl	Fisons-8000 fused silica capillary column (30 m x 0.32 mm)	He at 1.2 mL/min Split ratio: 30:1	Detector: FID Injection T: 240ºC Detector T: 260ºC Column T: 160ºC held for 1 min, ramped at 3.5ºC/min to 230ºC, held for 14 min	LOD (µg/ml) 18:1 (5.00), 18:2 (3.00), 18:3 (1.00)	[70]

18:3, 18:2, 18:1 *cis*, 18:1 *trans*, 16:0, 18:0	Cotton, canola, sunflower, corn and soybean oils	Transesterification using KOH and MeOH	SP™ 2380 fused silica capillary column (30 m x 0.25 mm)	N_2 at 20 cm/min Split ratio: 100:1	Detector: FID Injection T: 260°C Detector T: 260°C Column T: 140-240°C at 5°C/min in 30 min	LOQ (mg/L) 18:3 (2.9), 18:2 (12.7), 18:1 *cis* (92.1), 18:1 *trans* (90.6), 16:0 (216.5), 18:0 (198.1)	[106]
14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:1n-9, 18:4n-3, 20:4n-6, 20:5n-3, 22:5n-3, 22:6n-3	Molluscs, crustaceans, fish	Direct saponification with KOH/ MeOH and double petroleum ether clean followed by derivatization with TMS-DM	HP-88 J&W fused silica capillary column (100 m x 0.25 mm)	H_2 at 2.7 mL/min Split ratio: 17.7:1	Detector: FID Injection T: 250°C Detector T: 270°C Column T: 140°C increased at 3°C/min to 190°C, ramped at 1.5°C/min to 220°C, increased at 10°C/min to 240°C, held for 7min.	LOD/LOQ (mg/mL) 14:0, 20:4n-6, 22:6n:3 (0.006/0.016); 16:0 (0.009/0.018); 16:1n-7 (0.005/0.015); 18:0, 18:2n-6 (0.005/0.014); 18:1n-9 (0.004/0.013); 18:3n-3 (0.005/0.015); 20:1n-9, 18:4n-3 (0.004:0.013); 20:5n-3 (0.006/0.015); 22:5n-3 (0.005/0.016)	[107]
Wide range of different FAs (short-chain FA, branched-chain FAs, CLA and isomers, saturated, MUFA and PUFA)	Steak	Direct saponification with KOH/ MeOH followed by derivatization with TMS-DM	CP-SIL 88 fused silica (100 m x 0.25 mm)	He at 2 mL/min	Detector: FID Injection T: 250°C Detector T: 300°C Column T: 100°C at 2°C/min to 170°C,held for 15min, ramped at 0.5°C/min to 180°C, 10°C/min to 200°C, held for 10min, 2°C/min to 230°C, held for 10min	LOD/LOQ (mg) isoC15:0, C18:0, cis9trans11CLA, C20:5n-3 (0.015/0.022); C18:3n-6 (0.115/0.167)	[94]
16:0, 16:1, 17:0, 17:1, 18:0, 18:1, 18:3, 18:2, 20:0, 20:1, 22:0, 24:0,	Virgin olive oil	Transesterification with methanolic KOH solution	DB-23-capillary column (60 m x 0.25 mm)	He at 83.7 mL/min Split mode	Detector: FID Injector T: 280°C Detector T: 250°C T program: 165°C held for 35min, ramped up at 10°C/5 min to 220°C for 62 min		[108]
8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 18:1, 18:2, 18:3	Corn oil, palm oil, coconut oil, olive oil	Transesterification with NaOH/MeOH and BF_3/ MeOH	INNOWax capillary column (30 m x 0.25mm)	H_2 at 40 mL/min, with air 400 mL/min and make up gas of He at 30ml/min Split ratio 50:1	Detector: FID Injector T: 230°C Detector T: 250°C Column T: Initial T of 100°C 3°/min to 250°C	LOD/LOQ (mg/L) 8:0 (5.8/ 19.3), 10:0 (6.1/ 20.3), 12:0 (28.0/93.4), 14:0 (9.6/32.1), 16:0 (6.4/21.5), 18:0 (6.4/21.2), 18:1 (11.9/39.8), 18:2 (4.4/14.7), 18:3 (6.8/22.7)	[58]
14:0, 16:0, t16:1, 18:0, t18:1, 18:1, tt18:2, tc18:2, ct18:2, 18:2, 20:0, 18:3, 22:0	Bakery products	Two extraction methods were compared: i) Folch extraction method using chloroform-MeOH mixture ii) Dynamic ultrasound-assisted extraction using hexane as leaching carrier FAME were prepared by transesterification using sodium methylate-MeOH	SP-2380 fused-silica capillary column (60 m x 0.25 mm) coated with stabilisedpoly (90% biscyanopropyl/10% cyanopropylphenyl siloxane)	He at 1 mL/min Splitless injection mode	Detector: Saturn 2200 ion trap MS Electron-impact ionization (EI) positive mode Transfer line T: 170°C Ion trap T: 170°C Injector T: 250°C Column T: 50°C held for 2min, increased at 5°C/min to 250°C and held for 15min	LOD/LOQ (µg/g) 14:0 (2.28/7.51), 16:0, ct18:2 (0.98/3.23), t16:1 (2.97/9.79), 18:0 (1.18/3.89), t18:1 (1.01/3.33), 18:1 (1.20/3.97), tt18:2 (2.08/6.86), tc18:2 (1.64/5.41), 18:2 (2.29/7.56), 20:0 (1.10/3.62), 18:3 (1.19/3.93), 22:0 (3.93/12.98)	[56]
14:0, t14:1, t15:1, 16:0, t16:1, 16:1, 17:0, t17:1, 18:0, t18:1, 18:1, t18:2, 18:2, t19:1, 18:3, t20:1, t20:2, t22:1	Shortening	FAME was extracted using sodium methoxide and concentrated sodium chloride solution	AT-Silar-90 capillary column (30 m x 0.25 mm)	He at 0.6 mL/min Split ratio 50:1	Detector: Shimazu GCMS-QP2010 MS Electron ionization 70eV Column T: Isothermal at 180°C OR 150°C held for 10min, ramp at 5°C/min, 200°C held for 2min OR 150°C held for 10min, ramp at 2.7°C/min, 210°C held for 3min.		[109]
6:0, 8:0, 10:0, 12:0, 13:0, 14:0, 14:1c, 15:0, 16:0, 16:1c, 16:1t, 17:0, 18:0, 18:1c, 18:1t, 18:2c, 18:2t, 18:3c, 18:3t, 19:0, 20:0, 20:1c, 22:0	Biscuits and cakes, fats and oils, chocolate, snack bars, pies and pastry, partially cooked frozen wedges	Lipid extraction was performed using Soxhlet method. FAME was prepared using transesterification	SP2560 capillary column (100 m x 0.25 mm)	He at 0.5 mL/min Split ratio of 50:1	Detector: MS Injector T: 225°C MS: SCAN mode: 35-500m/z Column T: 150-200°C at 1°C/min, 200°C held for 40min	LOD: 0.1 g/100 g	[110]

8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 23:0, 16:1n9, 18:1n8, 18:1n9t, 18:2n10,13, 18:2n9,12t-t, 18:2n9,12, 18:3n9,12,15, 20:1n11, 20:3n1,4,8t-t-t, 22:1n13	Margarine	FAME was prepared using transesterification	HP-5MS (5% phenylmethylsiloxane) (30 m x 0.25 mm)	He at 0.8 mL/min Split ratio of 50:1	Detector: MS Electron impact mode at 70eV Injector T: 240°C Detector T: 260°C Column T: 150°C held for 2min, increased to 230°C at 4°C/min, 230°C held for 5 min		[111]

has been used as a novel stationary of GC column to separate lipids, especially the isomers. Delmonte et al. used the ionic liquid GC capillary column to separate the geometric and positional isomers of MUFAs. More than 10 individual peaks were obtained as different isomers of 18:1 FAs [68]. The ionic liquid GC capillary column could be recommended as the most suitable column for the analysis of total FAs and it may offer more molecular species information for FAs analysis.

High performance liquid chromatography

HPLC is a commonly used technique to compare with GC for the analysis of FAs. Although GC-FID is the most commonly used, it has several limitations. The short-chain FAMEs may be lost during esterification due to their volatility. Besides, PUFAs can be thermally degraded during their conversion to FAME. FID also destroys the FAs impeding their recovery for further analysis. On the other hand, HPLC systems are relatively isolated from the environment, a characteristic that can effectively reduce the contact between sample and air, thus avoiding the self-oxidation and degradation of FAs. Moreover, HPLC uses lower temperature which reduces the risk of isomerization of UFAs. Almost all of the FAs species can be separated by HPLC. Besides, the separation components can be collected for further analysis [69]. Sanches-Silva et al. reported the performance of HPLC systems including sensitivity and precision for the routine determination of 18:1, 18:2, and 18:3 in potato crisps fried in olive oil [70]. However, when a more complete study of FA profile is required, GC is more suitable as HPLC may present co-elution of peaks. The FAs analysis of food products by using HPLC is summarized in Table 3.

Most of the separations of FAs by HPLC are Reversed-Phase (RP) systems, i.e. hydrophobic stationary phase and polar mobile phase. With the development of stationary phases, Octadecylsilyl column (C_{18}, ODS) is most commonly used due to its commercial availability and high retention and better resolution. Moreover, octysilyl (C_8) and aminopropyl (NH_2) columns can also be used for FAs separation, depending on the substance polarity [69]. Recently, Ultra High Performance LC (UHPLC) columns and core-shell columns have been used to obtain faster separation and much higher resolution compared with traditional HPLC [71]. Besides the revolution of stationary phases, the reduction of the inner diameter of the column, accompanied with the reduction of the flow rate of the mobile phase, was reported to increase the detection sensitivity of trace amounts of samples. For example, nanoflow LC (nLC) has shown good performance for lipidomics analysis with the limit of detection (LOD) close to femtomoles [72].

Since the mobile phase of HPLC is liquid, the sample is extracted to dissolve in the liquid. Several sample preparation methods are available including SPE, precipitation, evaporation, filtration and LLE. The most common method is LLE. Extraction solvent containing chloroform may artificially raise the measured concentration of certain FAs while the use of isopropanol and heptane avoids the contaminants during extraction [73]. The mobile phases are usually based on a polar solvent, e.g. water, to which a less polar solvent such as Acetonitrile (AN) or MeOH is added. Several HPLC detectors are commercially available, i.e. UV-vis detection, electrochemical detection, fluorescent detection, chemiluminescence detection, evaporative light-scattering detection and MS detection [69]. Derivatization with chromophores is often used in analysis by HPLC to facilitate the sensitivity and selectivity of the detection by UV-Vis absorption or direct fluorescence. The derivatives include benzyl, *p*-nitrobenzyl, phenacyl, *p*-bromophenacyl, *p*-methylthiobenzyl and *1*-napthylamine esters. The absence of chromophores groups often makes the detection difficult in UV-vis region. Guarrasi et al. validated a method using AN, MeOH and *n*-hexane as mobile phase. This simple chromatographic technique using UV detector allows the detection without a preliminary derivatization with chromophores [74]. Guo et al. compared HPLC with Evaporative Light-Scattering Detector (ELSD) with GC-FID. HPLC showed better separation and precision without the need to derivatize the sample components. Besides, HPLC-ELSD detector was able to quantitate the UFAs more accurately with the comparatively low LOD [75].

The progress of LC methods for FAs analysis is generally going in two directions, targeted and comprehensive analysis. For targeted analysis, LC-MS methods have been developed for highly sensitive detection of FAs [76]. For comprehensive analysis, two-dimensional LC is the best way with normal-phase (NP) LC to discriminate different classes of lipids and RPLC to differentiate FAs chain in each class.

Applications of Dietary Fatty Acids Analysis in Human Nutrition

Although the GC and HPLC methods described here are satisfactory for analyzing a wide range of FAs of food and biological samples, we believe further modifications to the methods may provide increased resolution and efficiency. However, the pursuit of ideal analytical methods is not the ultimate goal of dietary FAs analysis. FAs analysis is one of the most important tools for investigation of human nutrition and health.

It is clear that FAs, especially UFAs, are critical nutrients and may help in preventing the progression of some illnesses. FAs are present in wide range of food matrices. In order to gain a better understanding on the FAs, their role in health can be incorporated to nutritional guidelines. In addition, with the increasing incidence of chronic diseases and increasing understanding the health impact of FAs, food fortification and supplementation with PUFA are becoming more popular. Besides that, consumers nowadays are more health conscious and tend to go for healthier products. As a result, the provision of clear fatty acid information on product label would help consumers to make their food choices to maximize the

Table 3: High performance liquid chromatography based fatty acids analysis of food products.

Fatty acids	Matrix	Sample preparation	Column and detector	Mobile phase	LOD/ LOQ	Reference
18:1, 18:2, 18:3	Potato crisps fried in olive oil	Saponification with NaOH and extracted with HCl and MeOH	HP1100 system, UV detector set at 195 nm. Tracer Extrasil ODS2 column (25 cm, 0.4 cm, 5 µm) at 60°C	AN-water and AN. Flow rate: 1.2 mL/min until 6 min then increased to 2.0 mL/ min	LOD (µg/mL) 18:1 (0.01); 18:2 (0.23); 18:3 (0.74)	[70]
18:3, 18:2, 18:1 *cis*, 18:1 *trans*, 16:0, 18:0	Cotton, canola, sunflower, corn and soybean oils	Transesterification using MeOH and KOH	CTO-20A HPLC equipment with a UV detector set at 205 nm Single Shim-Pack VP-ODS C_{18} RP column (250 mm, 4.6 mm, 5 µm) at 40°C	A: Gradient elution of MeOH and 2-propanol-hexane at 1 mL/min (20min) B: isocratic elution of AN for 34 min at 1 mL/min	LOQ (mg/L) 18:3 (2.9); 18:2 (12.7); 18:1 *cis* (92.1); 18:1 *trans* (90.6); 16:0 (216.5); 18:0 (198.1)	[106]
Palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid	Olive oils, soy bean oils, sunflower oils, peanut oils and palm oil	KOH alcohol solution H_2SO_4	A: HP1050 with a RI-71 detector at 30°C LiChrospher 100 RP-18 (250 mm, 4 mm, 5 µm) at 40°C B: HPLC S1100, RI detector Erma 7512 at 30°C LiChrospher 100 RP-18 (250 mm, 4 mm, 5 µm) at 35°C C: HP1050 with a diode array detector (206 nm) LiChrospher 100 RP-18 (250 mm, 4 mm, 5 µm) at 30°C	A: Water and MeOH at 1.05 mL/min B: water and AN at 1.2 mL/ min C: AN and water, AN at 1.0 mL/min		[112]
Myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid	Vegetable oils	Saponification using 2M sodium methanoate and diluted using MeOH.	Waters-Alliance (model 2695) with C^4D detector (peak-to-peak amplitude of 100 V and frequency of 100 kHz) Fused silica capillary with 250 µm i.d. and 360µm o.d. ODS C_{18} analytical column (250 mm, 4.6 mm, 5 µm) at 45°C	MeoH, sodium scetate at flow rate of 0.6mL/min	LOD (µg/mL) Myristic acid (0.6); palmitic acid, stearic acid, oleic acid and linoleic acid (0.1)	[113]
18:0, 18:1, 18:2, 18:3	Aniseed oil	Conversion of FAs to p-methoxyphenacyl derivatives	1100 series HPLC system with multiple-wavelength detector. Nucleosil 100-5SA column (250, 4.6 mm) was converted to silver ion form	1% ammonium acetate solution at 0.5 mL/min for 1 h and then with distilled water at 1 mL/min for 1h. While water was pumped, silver nitrate in water was injected.		[114]
Myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, stearidonic, eicosapentaenoic, docosahexaenoic	Pumpkin seed oil, rice bran oil, soybean oil, algal oil	Base-catalysed transesterification with MeOH solution of KOH	Alltech ELSD 500MKIII Evaporative Light-scattering Detector. Two Inertsil C_{18} RP columns (250 mm, 4.6 mm, 5 µm) ELSD drift tube temperature at 75°C. N_2 flow of nebulizer at 2.75 L/min and 1.38 bar.	MeOH-water at 1 mL/min	LOD (mg/mL) Lauric (0.0010) Myristic (0.0050), palmitic (0.0031), palmitoleic(0.0014), stearic (0.0016), oleic (0.0015), linoleic (0.0024), linolenic (0.0002), stearidonic (0.0009), EPA (0.0028), DHA (0.0001)	[115]
14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 22:1	Extra virgin olive oil, seed oil	Saponification with NaOH and petroleum ether and HCl	Shimadzu LC-2010 AT Prominence equipped with a UV-Vis photodiode array detector (SPD-M20A); RP HS-C_{18} column (250 mm, 4.6 mm, 5 µm)	AN, MeOH and *n*-hexane at flow rate of 1 mL/min	LOQ (mg/mL) 14:0 (0.232), 16:0 (0.093), 16:1 (0.039), 18:0 (0.056), 18:1 (0.068), 18:2 (0.004), 18:3 (0.0005), 22:1 (0.067)	[74]

benefits of dietary fatty acid intake. This review has discussed several chromatographic and extraction methods of analyzing various dietary FAs. Future studies can use the methods discussed to analyse different fatty acids in existing food products as well as the novel sources of FAs. Excellent fatty acids analysis would also be significant in improving food qualities and nutritional values of a product.

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