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REVIEW ARTICLE

Extraction methods of fat from food samples and preparation of fatty acid methyl esters for gas chromatography: A review

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Abstract Global trends moved towards fast food consumption due to the busy lifestyle of humans. Hence, the intake of fat-related food has exceeded the daily dietary reference intake (DRI) of fat, which caused multiple diseases. Analysis of the fatty acid profile plays a vital role in nutritional labelling and helps to understand the availability of diverse fatty acids among food commodities. This article reviews, general fatty acid extraction and derivatization techniques that have been developed in the past few decades due to the structural differences of fatty acids and briefed the steps involved in the complete process of fatty acid analysis using gas chromatography-mass spectrometry (GC–MS). Hence, the review mainly focused on conventional extraction methods, followed by microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), and supercritical fluid extraction (SFE) methods and widely used acid, base, and modified derivatization techniques. Importantly, this article compares the results of the previous studies in each section which assist to decide on the most appropriate pathway for the fatty acid analysis for different selected food types. Therefore, it is hoped that this review may help researchers to develop existing experimental methods and to improve 'bad' fatty acid level mitigation techniques in future.

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Contents

1. Introduction

Fatty acids are the primary component of lipids and play a crucial role in biological systems. Fatty acids can exist as free forms and bound forms, such as cholesterol and phospholipids (Benjamins et al., 2012; De Carvalho and Caramujo, 2018). The physicochemical properties of lipids are based on their fatty acid composition. Fatty acids are carboxylic acids with saturated or unsaturated open-aliphatic carbon chains. They can be divided into short-chain fatty acids ($<$C6), medium-chain fatty acids (C6-C12), long-chain fatty acids (C13-C21), and very-long-chain fatty acids ($>$C22) based on chain length. Fats and oils found in nature randomly exist in a taxonomic pattern where saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) are located in Sn-1/Sn-3 and Sn-2 positions, respectively (Rousseau and Marangoni, 2002). For the analysis of fatty acids composition, sample preparation is a significant task. It can be done by converting the fatty acids into the methyl esters followed by gas chromatography (GC) (Chiu and Kuo, 2019; Niemi et al., 2019). This review discusses on extraction methods of fatty acids from food samples and fatty acid methyl esters (FAME) preparation methods for GC–MS analysis.

There are many biological functions associated with the fatty acids, including they act as primary constituents of cell membranes, an energy source, and regulating the activity of enzymes and inflammatory processes (Christinat et al., 2016; Corrêa-Oliveira et al., 2016; Kimura et al., 2020). Fatty acids can be presented in different sources such as fruits, vegetable oils, seeds, nuts, animal fats, and fish oils; also they can be categorized generally into four groups such as saturated, monounsaturated (MUFA), polyunsaturated (PUFA), and trans fats (TFA) in reference to the presence and number of double bonds in the carbon chain (Orsavova et al., 2015).

SFAs and TFAs ('bad' FA) are contributing to elevate the risk of coronary heart disease while MUFAs and PUFAs are acting contrary by reducing the risk of coronary heart disease (CHD) (Clarke and Lewington, 2006; Givens, 2017; Ruiz-Núñez et al., 2018). World Health Organization (WHO), has introduced the "REPLACE" action package to provide a strategic approach to eliminate industrially-produced TFA from the global food supply chain by 2023. According to WHO, there are 500,000 premature deaths from CHD per annum around the world due to TFA intake. Among these fatty acids, omega-3 and omega-6 PUFAs seem to be the most important as there are many beneficial effects, such as anti-inflammatory properties, reducing oxidative stress, presenting neuroprotection, and cardiovascular protection; however there is no definite biochemical pathway to produce these molecules on its own within the body (Fotuhi et al., 2009; Mazza et al., 2007; Sokoła-Wysoczańska et al., 2018).

Globally, for the investigation purpose of fatty acid composition, GC–MS analysis is used predominately compared to other techniques (LC-MS, GC-FID) due to the advantages in efficiency, selectivity, and cost (Krone et al.,2010; He and Aga, 2019). This method included three steps: extraction of the fatty acids from the sample matrix, derivatization of the fatty acids, and GC–MS analysis. The methodology is given in Table 1, briefly described how to extract fat from food products for GC–MS analysis (see Table 2).

2. Extraction methods

There are several steps involved in the preparation of a food sample for solvent extraction. It is often necessary to dry the samples prior to oil extraction using solvents because many organic solvents are immiscible with water and cannot easily penetrate foods containing much water, and therefore extraction would be inefficient (Chemat et al., 2015; Señoráns and Luna, 2012). Vacuum oven drying at low temperatures or lyophilization can be done for better lipid extraction (Nielsen, 2014). After that, dried samples are ground finely before the solvent extraction to produce a more homogeneous sample and to increase the surface area of lipid exposed to the solvent (Akoh and Min, 2008). The sample should be ground at low temperatures in order to minimize the tendency of the lipid for oxidation (Domínguez et al., 2019). Acid hydrolysis can be performed to release the bound lipids attached to protein and carbohydrates in order to enhance the extraction efficiency. According to Joslyn (1970), the extracted fat percentage of dried egg, flour, noodles, and semolina was increased by 1–6% due to pre acid digestion. For most of the food products, acid digestion is a suitable method except for dairy products and cheese, where alkaline hydrolysis and combination of two methods are implemented more effectively

Table 1 Steps of fatty acid profile analysis method for GC–MS.

No	Steps of procedures	Description	References
1	Sample drying process	Add 1 g of anhydrous Na_2SO_4 per 10 g of the sample and dry it in an oven (45 °C-55 °C). High temperatures can cause lipid oxidation and increase bound lipid concentration.	AOAC 981.11 (2000)
2	Acid hydrolysis/ digestion (Optional)	Food samples should be added to conc. HCl in methanol (10–30 mL) and heated for 15–25 min in a water bath (60–65 °C).	Nielsen (2017a)
3	Extraction process	Soxhlet, Folch, Bligh and Dyer, MAE, SFE, UAE	Wagner et al. (2008) Salimon et al. (2014) Priego-Capote et al. (2007) Ruiz-Jiménez et al. (2004) Liu et al. (2013)
4	Purification	The extraction solvent should be evaporated (rotary evaporator) and the resulted crude fat stored in freezing temperatures (−18 °C) in a capped dark bottle for further analysis.	Wagner et al. (2008) Asgary et al. (2009) Kandhro et al. (2008)
5	Derivatization	Fatty acids should be converted to fatty acid methyl esters form to obtain efficient results.	Nielsen (2017a)
6	Post procedures	Neutralization, layer separation, transferring the organic layer, water scavenging.	Nielsen (2017b)
7	Injection into GC valves	Capped, sealed and labelled valves (Completely clear with zero water bubbles)	

(AOAC 996.06), because those products may contain high composition of acid-labile conjugated linoleic acids (CLA), short-chain fatty acids and *trans,trans*-CLA (Mossoba et al., 2009). There are various well-established extraction methods, and those methods can be applied to different types of samples.

The ideal solvent for lipid extraction must be selected. The efficiency of the extraction depends on the polarity of the lipids and polarity of the solvent (Ramluckan et al., 2014). The polarity of lipids can be different; polar lipids such as glycolipids or phospholipids are more soluble in polar organic solvents such as alcohols. On the other hand, nonpolar lipids such as triacylglycerols are more soluble in nonpolar solvents such as hexane. Therefore, a combination of two or three solvents is frequently used in classical extractions, as it is impossible to select a single organic solvent (Señoráns and Luna, 2012; Servaes et al., 2015). In addition to that ideal solvent should also be inexpensive, have a relatively low boiling point, be nontoxic, and be nonflammable. Ethyl ether and petroleum ether are the most commonly used solvents, but pentane and hexane are also used in the extraction of fat in some food products (Dasari and Goud, 2013; Min and Ellefson, 2010; Thiex et al., 2003).

The section given below is focused on traditional methods which include the use of a special apparatus called Soxhlet, and some methods developed in the 1950s such as Folch, Bligh and Dyer with particular combinations of organic solvents, as well as the modern extraction techniques such as MAE, UAE and SFE methods for food matrices.

2.1. Soxhlet method

Soxhlet method is a traditional technique used for extracting lipids in foods; thereby, the sample is initially dried, ground into small particles, and placed in a porous thimble. Mainly it has three compartments; flask, extraction chamber, and condenser. The sample is placed in a thimble; once the flask is heated, the solvent is evaporated and moved up to the condenser, where it is converted into a liquid and collected into the extraction chamber containing the sample. When the solvent passes through the sample, it extracts the fats and carries them into the flask. This extraction process typically lasts several hours (6–24 h) (Nielsen, 2017b; Señoráns and Luna, 2012). After completion of the extraction, the solvent is evaporated, and the mass of lipid remaining is measured and used to analyze.

This method is one of the most commonly used techniques because of its unattended and straightforward use, but there are some disadvantages, such as; hazardous and flammable organic solvents, potential emissions of toxic compounds during extraction, usage of more costly and higher purity solvents, laborious procedures, time-consuming, and method requires three different weights; the weight of the sample, weight of the empty flask, and weight of the flask with final extraction (Fakirov, 2006; López-Bascón and Castro., 2020).

2.2. Folch method

Folch method is the most well-known fatty acid extraction method proposed by Jordi Folch and the most reliable method for the quantitative extraction of lipids (Liu et al., 2018). A mixture of chloroform and methanol at a ratio of 2:1 (v/v) was used as the extraction solvent, and the final volume must be 20 times of the 1 g sample. After that, water or a salt solution was added to cause the phase separation. The lower phase was used in analyzing fatty acids (Fernández et al., 2011). This protocol can be used to extract lipids from common lipid-rich foods such as avocados, eggs, and mayonnaise (Cheng et al., 2019; Kato et al., 2017; Rodríguez-Carpena et al., 2011; Shinn and Proctor, 2013).

Table 2 Commonly used Fat extraction and derivatization methods in different food categories.

Food matrix	Food sample	Extraction method	Derivatization method	Reference
Meat	Cooked turkey breast Fresh pork loin Cooked ham Dry-cured ham Mortadella Beef burger Fresh sausage Dry-cured sausage and salami Lamb meat	Soxhlet method (PE/EE) Bligh and Dyer method Folch method Hara–Radin Method Rapid microwave solvent extraction SFE	Base catalyst sodium methoxide method Acid catalyst BF_3 method (BF_3 -MeOH method)	AOAC, Official Method 960.39, 2012 AOAC, Official Method 991.36, 2012 AOAC, Official Method 985.15, 2012 AOAC, Official Method 969.33, 2012, Pérez-Palacios et al. (2008) Fiori et al. (2013) Hara and Radin, (1978) O'fallon, et al. (2007) King et al. (1996.)
Nuts and seeds	Almond Apricot Cashew Hazelnut Peanut Pecan Pistachio Walnut Chia seeds oleaginous seeds	Soxhlet method (EE/Hex) UAE	BF_3-MeOH method TMSH method	Al Juhaimi et al. (2018) Aremu and Akinwumi (2014) AOAC, Official Method 948.22 (2012) Luque and De Castro (2004) Mello et al. (2017)
Butter	Animal butters Plant butter	Soxhlet method (Hex) Solvent extraction (EE/PE) Rose-Gottlieb method Bligh and Dyer method Folch method	Acid catalyst conc. H_2SO_4 (95%) method BF_3 -MeOH method	Abdul-Mumeen et al. (2019) Rutkowska and Adamska (2011) Ozcan et al. (2016) AOAC, Official Method 938.06, 2012 Nazari et al. (2012)
Animal feeds	Forages Cereal grains	Solvent extraction (EE/Hex) Soxhlet (diethyl ether)	BF_3-MeOH method Base catalyst sodium methoxide method	Kan (2015) Leiva and Granados (2020) AOAC, Official Method 2003.05 AOAC, Official Method 2003.06
Fast food	Burger French fries Pizzas Potato chips Snacks	Non-meat foods – Solvent extraction (PE/Hex) Meat foods– Folch method MAE SFE	Base catalyst sodium methoxide method BF_3 -MeOH method	Musaiger et al. (2008) Fernández and Juan (2000) Nazari et al. (2012) Virot et al. (2008) Devineni et al. (1997)
Dairy products	Milk powder ice cream high-fat milks low-fat milks high-fat yoghurts low-fat yogurts high-fat cheeses low-fat cheeses	Modified Folch method - Chloroform:Methanol (2:1, v/v) Modified Bligh and Dyer- Chloroform:Methanol:Water (1:4:0.8, v/v).	BF_3 -MeOH method Base catalyst sodium methoxide/ KOH method TMAH method	Nazari et al. (2012) Castanha et al. (2013) Martin-Hernández et al. (1988) Amores and Virto (2019)
Flour products and Chocolate	Cake cream biscuits simple biscuits cream chocolates Simple chocolates	Solvent extraction (PE/Hex) Folch method Soxhlet extraction (Hex) MAE Bligh and Dyer	Base catalyst sodium methoxide method BF_3 -MeOH method TMSH method	Fernández and Juan (2000) Nazari et al. (2012) Virot et al. (2008) Patrignani et al. (2015) Bahrami et al. (2014)

Abbreviations: EE – ethyl ether; PE – petroleum ether; Hex- hexane; TMAH- Tetramethylammonium hydroxide; TMSH- Trimethyl sulfonium hydroxide; MeOH-methanol.

2.3. Bligh and Dyer method

Bligh and Dyer's method applies chloroform:methanol: water mixture at 2:2:1.8 ratio for total extraction of lipid from fish muscle (Bligh and Dyer, 1959; Jensen, 2008). Although the procedure was developed using cod muscle, it states that it can be applied to any tissue containing (or modified to contain) 80% water (Breil et al., 2017). Despite this solvent reduction, the Bligh and Dyer method are still thought to yield recovery of 95% of total lipids. Low solvent consumption, while providing high recovery, is the major advantage of this method (Kumari et al., 2011). In this method also, chloroform was used as an extraction solvent, which is having a concern due to its high toxicity (Hussain et al., 2014).

These two extraction methods have found general acceptance as standard procedures for the recovery of total lipids (Axelsson and Gentili, 2014). In these two methods, a monophasic solvent system of chloroform and methanol was formed to extract and dissolve the fats. Thereafter, water was added to produce a biphasic system for separation of polar and nonpolar compounds into an upper and lower phase,

respectively (Breil et al., 2017). The Folch method is regarded as the most reliable method for complete recovery of total lipids and the Bligh and Dyer method is more widely known for the extraction of lipids from tissues of vascular plants (Iverson et al., 2001).

Alternative solvent systems have been developed to overcome the toxicity of chloroform, but these are generally less efficient and sensitive to the water content of the sample (Byrne et al., 2016). One study proposed the use of methyl-tert-butyl ether (MTBE), and it proved that MTBE provided faster and clean lipid extraction (Matyash et al., 2008). The overall recovery percentage of the MTBE method was 90–98%, which is almost similar to the Folch method. Besides, Hara and Radin (1978), have proposed an extraction method using the low toxicity solvents hexane and isopropanol. This method is more effective than other methods because of higher extraction amounts of total fatty acid and less sample preparation time (Serafim et al., 2019).

Butanol: Methanol (BUME) method is another lipid extraction method. Initially, one-phase extraction with 300 mL butanol: methanol (3:1) mixture followed by a two-phase extraction with 300 mL heptane: ethyl acetate (3:1) and 300 mL 1% acetic acid (Quehenberger et al., 2010). Since chloroform is not used for the extraction, this method is more environment-friendly and less toxic.

Pérez-Palacios et al. (2008), determined the fat content in nine meat products using six different fat extraction methods; standard Soxhlet method with and without previous acid hydrolysis, continuous Soxhlet method (with and without previous acid hydrolysis), and Folch method and Bligh and Dyer method. According to their results, the Folch method and the Soxhlet with previous acid hydrolysis method were the most effective. The fat content extracted from the Bligh and Dyer method reported the lowest lipid content for all meat.

Shin et al. (2013), studied fat classes and content in bakery products by adapting the Folch method, the automated Soxhlet method, and the AOAC 996.06, 2002. According to their results, the automated Soxhlet and Folch methods (gravimetric methods) were able to a high amount of total fat than AOAC 996.06 method. Besides the Folch and automated Soxhlet method showed almost no difference in fat (crude) content, and the automated Soxhlet method produced a higher amount of SFAs, MUFAs, and TFAs than that of the Folch method for three samples. The automated Soxhlet method did not result in a higher amount of PUFA than that of the Folch method in any sample.

Ewald et al. (1998), studied both Bligh and Dyer extraction using chloroform/ methanol and Soxhlet extraction using hexane/acetone for extracting total lipid and polychlorinated biphenyls (PCBs) from muscle tissue in four species of fish, caught in the southern part of the Baltic Sea. According to their results, a higher amount of total lipid was extracted from the Bligh and Dyer method than Soxhlet method with hexane/acetone.

2.4. Microwave-assisted extraction

MAE approach is more effective than conventional methods, and the MAE has the advantages of being fast and robust and consuming a small amount of solvent for extracting lipids and does not require samples devoid of water (Batista et al., 2001). The improvement of microwave extraction was obtained by irradiating the mixture of a sample and an appropriate solvent several times for a short time, for instance, 30 s every time (Destandau et al., 2013). Costa and Bragagnolo (2017) compared the MAE method and the Folch method for lipid extraction from fish samples. Their results indicated that there were no significant differences in fatty acids content in the Folch method and MAE method. Furthermore, there are some drawbacks of this method include lipid oxidation and fatty acid compositional modification (Hu et al., 2008).

Ramalhosa et al. (2012), compared MAE method against Soxhlet, Bligh and Dyer, Modified Bligh and Dyer, Folch, Modified Folch, Hara and Radin, Roese-Gottlieb for quantification of the total lipid content of three fish species. Their results illustrated that the MAE, Bligh and Dyer, Folch, Modified Folch, and Hara and Radin were provided the highest fat extraction efficiency, and although they were not significantly different. Furthermore, MAE was shown the highest repeatability; however, Roese-Gottlieb, Soxhlet, and Modified Bligh and Dyer's methods were very low in terms of efficiency and repeatability.

2.5. Supercritical fluid extraction

SFE is an essential alternative for lipid extraction at the analytical scale and industrial scale (Akanda et al., 2012). Based on the principle of the instrument, the food sample to be analyzed heat slightly in a pressurized chamber and then mix supercritical CO_2 (SC-CO_2) fluid with it (Sairam et al., 2012). The CO_2 extracts the lipid fraction and forms a solvent layer, which is separated from the polar components. Then under reduced pressure and temperature of the solvent, which causes the CO_2 to turn back into a gas, leaving the lipid fraction with no organic solvent. In this method, used only SC-CO_2 which usually yields good recoveries of nonpolar lipids, but polar lipids may remain partially because of their lower solubility in SC-CO_2, and therefore samples containing a certain quantity of these types of lipids which may impart a limitation of this method (Sahena et al., 2009). To improve the extraction of polar lipids, the polarity of SC-CO_2 to be altered by using co-solvents such as methanol, ethanol, or even water, in small proportions (Patil et al., 2018). The presence of water, dissolved in the supercritical fluid in a very small percentage, also increases the solubility of polar compounds, and it has been used successfully to analyze several dairy products (Lebovka et al., 2016). The particle size of the food products affects the lipid recovery since it influences the surface area of the sample exposed to SC-CO_2. The moisture content of samples also affects the extraction efficiency by conditioning their surface structure. High moisture content reduces SC-CO_2-sample contact due to the low solubility of water in SC-CO_2 and moisture acts as a barrier to the diffusion of SC-CO_2 in the sample as well as to the diffusion of lipids outside the sample. Therefore, samples with high moisture content are usually freeze-dried before SC-CO_2 extraction to improve efficiency (Señoráns and Luna, 2012). SFE has several distinct properties, and it is regarded as a promising alternative technique to conventional solvent extraction methods. Some of its significant advantages are as follows; No organic solvent use, the possibility of reusing carbon dioxide as a solvent, selective extractions, mild extraction conditions, especially low temperature for thermolabile compounds.

Berg et al. (1997), reported that SFE could be used in the analytical laboratory for extraction of total fat as well as giving the relation between different lipid classes in meat and meat products with accuracy equal to conventional solvent extraction methods. Under optimal conditions with ethanol carbon dioxide being modified as the supercritical fluid, with the hydro matrix as water adsorbent and with a small amount of cyclohexane added to the sample, the extraction time is reduced to 30 min. This time is considerably shorter than the Bligh and Dyer extraction.

2.6. Ultrasonic-assisted extraction

Some modified approaches have been proposed to improve extraction speed or reduce solvent consumption. Briefly, 1.0 g of milled sample was placed in a 100 mL Erlenmeyer flask and mixed with 40 mL of extraction solvent (isopropanol/ n-hexane). After that, the flask was placed in the sonication bath for extraction. Under this method, the extraction efficiency was enhanced by ultrasound pressure waves and resulting in cavitation phenomena. Thereafter, the extract was removed from the vessel and filtered using Grade 1 Whatman filter paper under vacuum conditions. The filtrate was dehydrated using anhydrous sodium sulfate and diluted to 50 mL using the same organic solvent used in the extraction process (Teng et al., 2016). Liu et al. (2013), used the UAE to extract fat from tissue samples. Their results indicated that this method was comparable to the conventional liquid-liquid extraction method but with the advantage of being environmentally friendly due to the lower solvent consumption.

Da Porto et al. (2013), compared UAE and Soxhlet method for the extraction of oil from grape seeds. According to their results UAE carried out (at 20 kHz, 150 W) for 30 min produced similar oil recovery as Soxhlet method using n-hexane for 6 h (max. 70 °C). The fatty acid compositions of the oil were not affected significantly by the application of ultrasound. Compared to the conventional extraction methods, the high yield of fat obtained with a lower solvent consumption and a shorter extraction time are the major advantages of UAE (Chemat et al., 2017; Pico, 2013; Tiwari, 2015).

3. Derivatization methods

The fatty acid composition is determined as the methyl esters of fatty acids by GC–MS. The conversion process of fatty acids into FAMEs is called derivatization (Asperger et al., 2001). It is challenging to analyze fatty acids in their free form as these highly polar compounds tend to form hydrogen bonds, leading to adsorption issues. Reducing their polarity may make them more amenable for analysis (Iwasaki et al., 2012). Thermally stable compounds such as aroma compounds, PCBs, common vegetable and animal fats/oils and fatty acids, and volatile contaminants can easily be extracted from the food samples and subjected to GC analysis, but samples which, contain more considerable amounts of hydroperoxy, epoxy, aldehyde, ketone, cyclopropenyl, cyclopropyl, and conjugated polyunsaturated groups should be avoided due to the obliteration of these compounds (AOAC 969.33; Nielsen, 2014). To distinguish very slight differences in UFA, the polar carboxyl functional groups must first be neutralized. This process then allows column chemistry to perform separations by boiling point elution, and by the degree of unsaturation, position of unsaturation, and even the cis vs trans configuration of unsaturation (Santha and Napolitano, 1992). Derivatization is usually necessary for fatty acid analysis by GC–MS, especially for fatty acids with carbon numbers larger than 10 (Chiu and Kuo, 2019). In this section, the most frequently used methods for fatty acid derivatization are going to introduce. AOAC 969.33 and AOAC 996.06 methods are used for the fat analysis in a laboratory for the qualitative analysis experiments and nutritional labelling purposes with special emphasis in trans fatty acids (includes complex calculations and more extended analysis period for sample) respectively (Nielsen, 2017b).

3.1. Acid derivatization methods

Generally, acid derivatization methods can be applied to total fatty acids (free and bound). The commonly used acid derivatization reagents are hydrochloric acid (HCl), acetyl chloride (CH_3COCl), sulfuric acid (H_2SO_4), and boron trifluoride (BF_3) (Ichihara and Fukubayashi, 2010).

HCl derivatization is one of the most widely used fatty acid analysis methods because HCl is a relatively mild reagent and gives almost quantitative yields and its operational simplicity (Mohamed and Bakdash, 2017). Anhydrous methanolic 5% (w/v) HCl was prepared by mixing acetyl chloride with methanol, the produced HCl act as the catalyst of the esterification reaction. Then, 2.0 mL of prepared solution was added to a lipid sample in a screw-capped glass test tube, and the mixture was heated at 80 °C with occasional shaking. Afterwards, the sample was evaporated to dryness at 80 °C under gentle airflow followed by addition of toluene (1 mL), and the tube was again tightly closed and heated at 80 °C for 3 min, and finally, the FAMEs were prepared for GC analysis (Antolin et al., 2008; Orata, 2012).

The acetyl chloride derivatization method was introduced in 1986. When using this method, lipid extract was dissolved in 600 mL of acetyl chloride in methanol (1:9) and 400 mL hexane, and the sample was heated to 90–95 °C for one hour in a tightly closed vial. After derivatization, the samples are neutralized, and the FAMEs are extracted with an organic solvent for further GC analysis (Chiu and Kuo, 2019). Some potential problems and safety issues may need to be considered when using the acetyl chloride derivatization method. For example, acetyl chloride derivatization is an exothermic reaction, causing the sample to spill out of the vial, which could be dangerous. In addition, some PUFAs are relatively unstable at high temperatures (Yanamadala and Praveen, 2014; Xu et al., 2010).

The H_2SO_4 derivatization method has also been widely used for the analysis of fatty acids in biological samples. H_2SO_4 -methanol 2% (v/v) was added into a vial containing previously weighed 10 mg of fat. The vial was heated at 80 °C with occasional shaking. Afterwards, 0.25 mL of the neutralized aqueous solution (sodium hydroxide 1 M) was added, and it was smoothly shaken. Before the analysis of the n-hexane phase, the sample was allowed to rest for 5 min (Masood et al., 2005). The reaction procedure is similar to that of other derivatization methods. Because H_2SO_4 is a strong oxidizing agent, this method is not recommended for PUFA analysis (Kuksis, 1994; Michalski and Łyko, 2010).

The BF_3 derivatization method has been used for fatty acid analysis for the last several decades, and it is now widely being used for the derivatization of food samples with the advantage of short reaction time (Salimon et al., 2014). Generally, the derivatization is performed by adding BF_3-Methanol reagent (14%, w/v) into the lipid sample and heating at 80–100 °C for 45–60 min. Finally, the FAMEs are extracted with an organic solvent and analyzed by GC (Flores and Doskey, 2015; Zhang et al., 2015). Although the BF_3 method provides efficient derivatization, its instability and the formation of artefacts have been subjects of concern in several studies (Knittelfelder and Kohlwein, 2017; Kramer et al., 1997).

Furthermore, these artefact formations during acid derivatization could be reduced by eluding elevated reaction temperatures or amounts of derivatization reagent and adding some dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) during the reaction (Little, 1999). In addition, the HCl, acetyl chloride, H_2SO_4, and BF_3 derivatization methods could also be used for a one-step extraction-derivatization approach (Meier et al., 2006).

3.2. Basic derivatization methods

Basic derivatization methods have distinguished advantages; short derivatization time, less double bond isomerization issue, ease of operation, and use of less oxidative reagents (Kramer et al., 1997). However, they are not suitable for the derivatization of free fatty acids (FFA) (Koohikamali et al., 2012).

The sodium methoxide ($NaOCH_3$) derivatization method has been used in several studies. Typically, 0.5 M $NaOCH_3$ in anhydrous methanol is added to the lipid extract, and the solution is reacted at 45 °C for 5 min. Sodium bisulfate ($NaHSO_4$) (15%) is then added to neutralize the mixture. Finally, the FAMEs are extracted with an organic solvent and analyzed by GC (Aldai et al., 2005).

Potassium hydroxide (KOH) is also used for the basic derivatization. Implementation of the procedure undergoes a quite simple pathway with shorter reaction time. Methanolic KOH (2 mol/L) is added to the lipid extract, and the mixture is incubated at room temperature or heated to 50 °C for a few minutes for fatty acid derivatization followed by the addition of $NaHSO_4$. Finally, the supernatant is collected and analyzed by GC (Ostermann et al. 2014).

3.3. Other derivatization methods

Apart from acid and base derivatization methods, some modified techniques were applied for biological samples which can be implemented more with food samples. Due to the novelty of these methods lack of experiments have been studied with respect to food samples to date. Hence research studies should be continued using these methods to develop and validate more about the outcome for food samples in the future.

Trimethylsulfonium hydroxide (TMSH) is a good example that comprises a single step rapid derivatization process with lesser artefact formation comparatively (Müller et al., 1998). Due to the elementary procedure, this method was useful for large batch analysis. But this modified method exists few limitations which include lower derivatization efficiency for PUFA (Pflaster et al., 2014). Ačanski et al. (2015), used TMSH in a GC–MS system for the derivatization of liposoluble samples extracted from different types of cereals and pseudocereals for qualitative analysis.

Quehenberger et al. (2011), suggested the pentafluorobenzyl bromide (PFB-Br) method specifically for the analysis of FFAs (Kawahara, 1968; Kuiper et al., 2018). By this method, FAs were modified into easily detected halogenated derivative and analyzed using negative chemical ionization (NCI) GC–MS (Pawlosky et al., 1992). Briefly, a mixture of PFB-Br and N, N-Diisopropylethylamine (DIPEA) at a ratio of 1:1 was mixed with dried lipid extract at room temperature for 15–30 min, and pentafluorobenzyl esters of fatty acids (PFB-FAs) were formed (Kulkarni et al., 2012). Though this reagent primly experimented with biological samples, it was used to investigate the presence of malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), which are less concerned but highly toxic reactive carbonyl compounds formed due to the lipid oxidation in food matrix because of the strong electronegative nature of the reagent (Zhou et al., 2020).

Detection of the volatile thiols available in some food and beverages (especially in wine) is a challenge due to the reactivity and complexity of the samples. Hence, for the analytical evaluation novel methods have been developed to overcome the issues by wine researchers and PFB-Br was used as an effective reagent for derivatization process (Chen et al., 2019).

Ostermann et al., (2014), compared different fatty acid derivatization methods, including HCl, BF_3, KOH, TMSH derivatization, combined NaOH, and BF3 with biological samples. Their results have shown that each derivatization method has its limitations·BF_3 was failed to the derivate cholesterol as well as for triacylglycerols (TAGs) and, KOH derivatization method led to insufficient derivatization of free fatty acids. TMSH derivatization has low efficacies in the derivatization of PUFA, ($< 50\%$). Moreover, HCl derivatization and combination of BF_3 with NaOH (NaOH-BF3) were comparable with the derivatization of FFA, polar lipids, and TAGs.

Kramer et al. (1997), compared several acids and base derivatization methods for fat analysis of rumen milk and found that the best derivatization can be obtained with $NaOCH_3$, followed by HCl or BF_3, or diazomethane. Certainly, such multiple-step methods that combine both acid and alkaline catalyzed methylation have been widely adopted in milk TFA analysis (Liu et al., 2018).

Since tetramethylammonium hydroxide (TMAH) able to instantaneously convert methyl esters of glycerides; it was commonly used for adapting FFA into FAME and form salts of FFA in separate phases. Due to this, both components of the lipid extract can be analyzed without prior separation (Mannion et al., 2016). Though, the use of TMAH has limitations because the glyceride component of extracted lipids was revealed to prevent FFA determination (Amores and Virto, 2019).

Antolin et al. (2008), studied about five derivatization methods for determining very-long-chain fatty acids by gas chromatography (GC), using diazomethane, H_2SO_4, HCl, BF_3 and TMSH. Compared to other methods, the HCl method needed more time (90 min) to complete the derivatization. Considering the cost, speed, safety, and GC response, the method H_2SO_4 was found the most appropriate for determining the very-long-chain fatty acids.

Salimon et al. (2014), analyzed FA and TFA availability in bakery and fast food products using GC. In this study, they have compared two modified derivatization methods; Combi-

nation of base/acid catalyzed method ($KOCH_3$/HCl) and base catalyzed followed by trimethylsilyl-diazomethane (TMS-DM). They have found that the first method was less expensive, convenient and rapid compared to the other method, and it was suitable for the comprehensive analysis of cis/trans UFA rich food samples.

In another study, robustness, precision and accuracy of the base catalyzed followed by TMS-DM method was analyzed. The authors have reported that the method provided satisfactory results for the fatty acid composition, including TFA for the analyzed margarine samples. The introduction of TMS-DM reagent or HCl after the base catalysis was useful for the complete methylation of FFA (Salimon et al., 2014; Salimon et al., 2017).

4. Conclusion

GC–MS is one of the most reliable methods for the analysis of total fatty acids, PUFA, MUFA, SFA and TFA available in a variety of food products. Liquid-liquid extraction, MAE, and UAE methods are widely used for the fatty acid extraction followed by catalytic derivatization. Commonly used catalysts are within the acidic type or basic type with their own capabilities and limitations. But there is no any specific system of fat extraction and derivatization for food varieties. Hence, at the initiation stage of the experiment for the investigation of the FA profile, the researcher should select the proper analyzing pathways to proceed, which depends upon the selected food commodities. Both qualitative and quantitative fatty acid analysis required to follow the critical derivatization step. For the complete FAME preparation, a combination of acid and base catalyzed methods are now being used along with novel extraction processes. It is important to follow precaution guidelines, especially at the FAME preparation and need to select the cost-effective, precise and efficient methods for the experiment.

CRediT authorship contribution statement

Geeth G. Hewavitharana: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Dilini N. Perera:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **S.B. Navaratne:** Conceptualization, Writing - review & editing, Resources, Supervision. **I. Wickaramasinghe:** Conceptualization, Writing - review & editing, Resources, Supervision.

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