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# COMPREHENSIVE REVIEW ON GAS CHROMATOGRAPHIC ANALYSIS OF FATTY ACIDS IN COCONUT OIL

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**Abstract:**
*Coconut oil has several domestic uses and benefits, which can be utilized in food, pharmaceuticals and cosmetic products. Coconut oil contains mainly the constituents of several fatty acids. Fatty acids plays crucial role in biological systems. Disproportions in fatty acids cause various diseases that makes the measurement of fatty acids in coconut oil essential. Many analytical techniques have been developed to analyze fatty acids in coconut oil. This review describes the determination of fatty acids in coconut oil using gas chromatography. A lot literature is available for coconut oil preparation, which includes solvent extraction, cold pressed and hot pressed extraction. Comprehensive review on acid, base, boron trifluoride derivatization strategies and separation of fatty acids using various columns were elaborated in this article.*

***Keywords:*** *Coconut oil, fatty acids, derivatization, Gas Chromatography*

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## INTRODUCTION:

Coconut oil is an edible vegetable oil obtained from coconut tree also known as "tree of life"widely cultivated in Asian countries. Copra, the dried coconut meat scientifically called as *Cocos nucifera* L. belongs to the family Aracaceae that contains about 65-75% of oil. Coconut oil has a natural sweet taste and carries high percentage of saturated fatty acids in the form of triglycerides (90%). In addition, it is composed of medium chain fatty acid (approximately 60% of total composition) [1,2]. Eighty percent of the world production of coconut oil is used for food, whereas approximately 14% is for nonfood uses such as pharmaceuticals, cosmetics like insect repellant, soap and skin moisturizer. The fatty acid present in the coconut oil is responsible for the antiplaque, antiprotozoal, healing, anti-obesity effects. Medium chain fatty acid reduces the threat of atherosclerosis and supply energy for metabolism without increase the blood sugar level [2,3].

Medium chain fatty acids have noteworthy effect in human well being as antibiotics, particularly as antiviral and source of quick vitality without upsetting the glucose in body [3]. There is no comprehensive report available in the literature for the analysis of fatty acids by gas chromatographic technique and hence a detailed review is presented in this paper.

**Chemistry of Coconut oil:**

Coconut oil contains numerous chemical compounds including fatty acids, fatty alcohols, monoglycerides, diglycerides, triglycerides, cerebrosides, phosphatides, sterols, terpenes Vitamin E and Vitamin K [4,5].

**Types and preparation of Coconut oil:**

Based on their mode of preparation, coconut oil largely classified as refined or unrefined coconut oil. Refined coconut oil includes the solvent extract and unrefined include the virgin coconut oil obtained either by cold pressed or hot-pressed techniques. Refined coconut oil is obtained from dried coconut meat after washing, bleaching and deodorization. Cold pressed and hot-pressed coconut oil are obtained from the fresh wet coconut meat, by crushing at room temperature and next at 40°C respectively by physical or other natural ways. Physical ways include pressing, washing with water, settling, filtering and centrifugation and natural ways include fermentation by naturally occurring microorganism. Expelling, centrifugation and fermentation that is devoid of heat are the general methods for the production of commercial virgin coconut oil.

**Fatty acids in Coconut oil:**

Fatty acids (FAs) categorized primarily according to the existence or nonexistence of double bonds as saturated (without double bonds), monounsaturated (with one double bond) and polyunsaturated fatty acids (with two or up to six double bonds) (Figure 1).

Fatty acids have the elements, such as carbon, hydrogen, and oxygen that are prearranged as a long aliphatic carbon chain skeleton of uneven length with a carboxyl group at last position[7] (Figure 2). They are classified further as *cis* or *trans* based on the pattern of the double bonds and as n-3 or n-6 PUFAs depending on the location of the initial double bond from the fatty acid methyl-end [8].

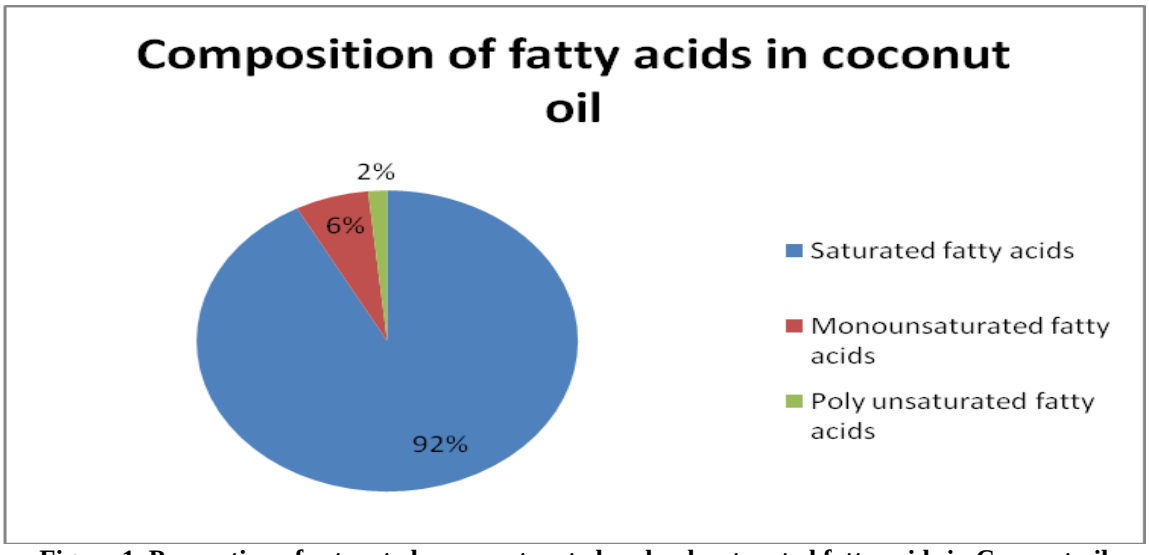


**Figure 1. Proportion of saturated, monosaturated and polysaturated fatty acids in Coconut oil**

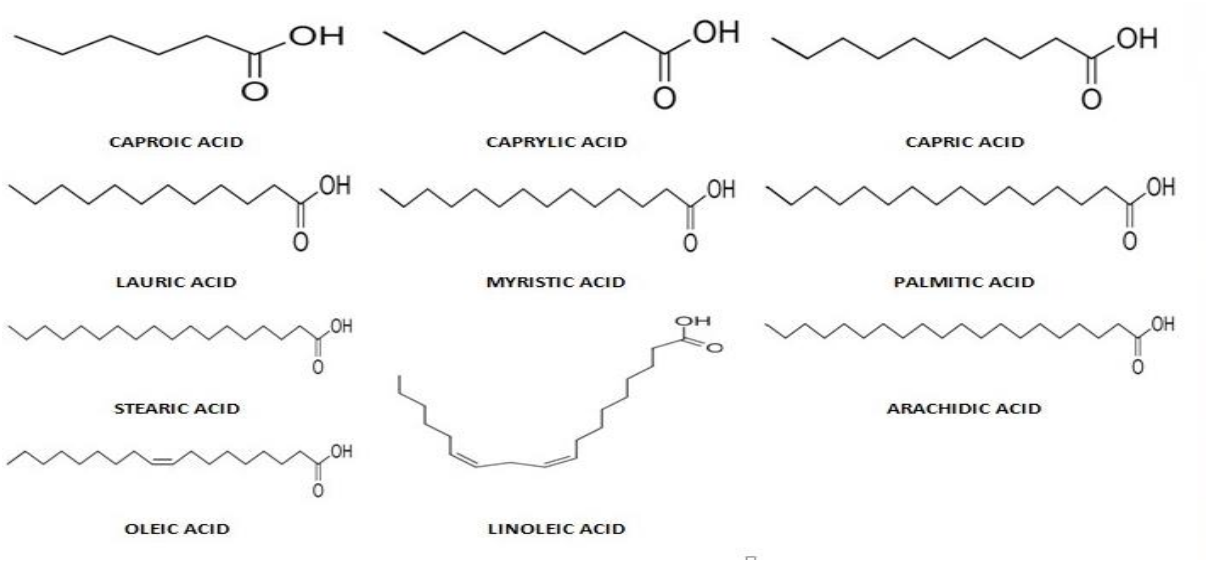


**Figure 2. Chemical structures of fatty acids present in coconut oil**

Saturated fatty acids include lauric acid (45-52%), myristic acid (16-21%), caprylic acid (5-10%), capric acid (4-8%), caproic acid (0.5-1%), palmitic acid (7-10%) and stearic acid (2-4%). Unsaturated fatty acids include oleic acid (5-8%), linoleic acid (1-3%) and linolenic acid (0.2%) [6,9] (Table 1).

**Table 1. Fatty acids present in coconut oil [**10]

| S.No | Fatty acids | Common name | Abbreviation |
|---|---|---|---|
| 1 | Hexanoic acid | Caproic acid | C6:0 |
| 2 | Octanoic acid | Caprylic acid | C8:0 |
| 3 | Decanoic acid | Capric acid | C10:0 |
| 4 | Dodecanoic acid | Lauric acid | C12:0 |
| 5 | Tetadecanoic acid | Myristic acid | C14:0 |
| 6 | Hexadecanoic acid | Palmitic acid | C16:0 |
| 7 | Octadecanoic acid | Stearic acid | C18:0 |
| 8 | *Cis*-9-octadecenoic acid | Oleic acid | C18:1 |
| 9 | *Cis*-9,12-octadecadienoic acid | Linoleic acid | C18:2 |
| 10 | Eicosanoic acid | Arachidic acid | C20:0 |

**Gas chromatographic analysis of coconut oil:**

Analysis of coconut oil provides a valuable data about their qualities. Gas chromatography, a very common investigative procedure in various research laboratories, reveals a quick and reliable technique for analysis of coconut oils. The fatty acid composition is determined as methyl esters of fatty acids using gas-liquid chromatography [11]. Gas chromatography is an isolated and flexible technique [12] in which separations are attained between a moving gas phase and a liquid stationary phase coated in the column after a mixture of compounds injected in very small amount. Components split in the column and detector detects the number of components that leaves the column [13,14,15,16]. The gas chromatography can able to optimize by initialize from a lower temperature, then gradually raising it to higher temperature during the analysis[6].

Gas chromatographic separation can be possible only for volatile samples and solutes having free polar groups will be difficult to be analyzed by gas chromatographic technique [17]. Free, underivatized type of fatty acids may be hard to analyze by gas chromatography because the highly polar compounds likely to form hydrogen bonds, leading to adsorption issues. Minimizing the polarity will make the solute to analyze easily and effectively [18]. Coconut oil being non volatile, it will be difficult to perform GC analysis directly and hence derivatization makes the coconut oil more suitable for the analysis by changing the oil into volatile and thermally stable compound.

**Derivatization:**

Gas chromatography, a technique for separation of volatile compounds which are thermally stable, is unfortunately not always easy to apply for compounds of biomedical and environmental interest, particularly for those of high molecular weight or containing polar functional groups. These groups are difficult to analyze by GC either because they are not sufficiently volatile, tail badly, are too strongly attracted to the stationary phase, thermally unstable or even decomposed.

Derivatization is the process of chemically modifying a sample to produce new molecule that is suitable for analysis using GC. Derivatization is

generally required for fatty acids with carbon numbers more than 10 by gas chromatography[18].
Chemical derivatization prior to analysis is generally done to

1. increase the volatility and decrease the polarity of compounds; -
2. reduce thermal degradation of samples by increasing their thermal stability;
3. increase detector response by incorporating functional groups which lead to higher detector signals.
4. improve separation and reduce tailing
5. enlarge substrate spectrum
6. improve chromatographic behavior or delectability

Derivatization reaction should be rapid, quantitative and produce minimal bye-product.
The reagents used for derivatization should comply the following requirements

1. The reagent should produce more than 95 % complete derivatives.
2. It should not cause any rearrangements or structural alterations of compounds during formation of the derivative.
3. It should not contribute to loss of the sample during the reaction.
4. It should produce a derivative that will not interact with the GC column.
5. It should produce a derivative that is stable with respect to time.

The various derivatization methods employed in gas chromatographic analysis are

1. Esterification
2. Alkylation
3. Acylation
4. Silylation

**Esterification:**
It is the method used for derivatization of alcohols and carboxylic acids.

**$RCOOH + R'OH \longrightarrow RCOOR' + H_2O$**

Preparation of methyl ester derivatives of fatty acids must be by far the commonest chemical reaction performed by lipid analysts as there is no need to hydrolyze them to obtain the free acids before preparing esters, as most lipids can be transesterified directly. Esters prepared by the methods mentioned in Table 2 can be purified by adsorption chromatography.

Acids can be esterified by treating them with an appropriate alcohol using an inorganic acid to catalyze the reaction. A portion of the ancient rarity arrangement during acid derivatization could be decreased by abstaining from utilizing high response temperatures or measures of derivatization reagent, or including some dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) during the response [19-20]. Notwithstanding being derivatized after lipid extraction, the HCl, $H_2SO_4$ and $BF_3$ derivatization strategies could likewise be utilized for one-step extraction-derivatization approach.

**Acid catalyzed esterification method:**
Free fatty acids are esterified and *O*-acyl lipids transesterified by heating them with a large excess of anhydrous methanol in the presence of an acidic catalyst. Hydrochloric acid (HCl), sulfuric acid ($H_2SO_4$) and Boron trifluoride ($BF_3$) are the reagents by and large used for the acid derivatization.

$$RCOOR'+CH_3OH \underset{}{\overset{H^+}{\rightleftharpoons}} RCOOCH_3+R'OH$$

$$RCOOH+CH_3OH \underset{}{\overset{H}{\rightleftharpoons}} RCOOCH_3+H_2O$$

In HCl derivatization, methanolic HCl is added to the dried lipid extract and the arrangement is warmed for a specific period. The $H_2SO_4$ derivatization strategy has additionally been broadly utilized for the examination of fatty acids in organic examples.

**Base catalyzed esterification method:**
Basic derivatization strategies provide the advantages of quick derivatization, no double bond isomerization issue, and smooth operation and use less competitive reagents, however, they are not appropriate for derivatizing free fatty acids [21-23]. The sodium meth oxide ($NaOCH_3$) derivatization method has been used in numerous studies. Typically, 0.5 M $NaOCH_3$ in anhydrous methanol is added to the lipid extract, and the resultant solution is reacted at 45° C for 5 min and neutralized with sodium hydrogen sulphate ($NaHSO_4$, 15%). Finally, the fatty acid methyl esters (FAMEs) are extracted with a natural solvent and analyzed by way of GC. The protocol is simple and the response time is very short.

**Boron trifluoride catalyzed esterification method:**
The $BF_3$ derivatization technique has been utilized for fatty acid investigation quite a few years, and it is presently utilized for derivatizing different oils. This protocol utilizes the Boron trifluoride-methanol reagent at 80-100°C for 45-60 min, has the benefit of a short response time. In spite of the fact that the BF3 strategy gives proficient derivatization, its unsteadiness and the development of antiques have been subjects of worry in a few investigations [24,25,26].

Boron trifluoride-methanol is the method of choice for the preparation of methyl esters of fatty acids when plenty of samples are available. Sample sizes of 100-1500 µl are easily derivatized, and the isolation of the methyl esters is simple and

quantitative when dealing with acids having chain lengths from C8 to C24. Special techniques are needed for the successful isolation of the more volatile fatty acid methyl esters below C8 [28,29,30].

**Table 2. Fatty acid derivatization method, reagent used and their reaction conditions**

| Derivatization method | Reagent | Reaction condition | Reference |
|---|---|---|---|
| Acidic derivatization | Methanolic Boron-trifluoride | 100°C for 5 min | 32 |
| Acidic derivatization | Sulphuric acid | Room temperature for 5 min | 1 |
| Basic derivatization | Methanolic potassium hydroxide | Room temperature for 5 min | 25 |
| Acidic derivatization | Hydrochloric acid | Room temperature | 33 |
| Base catalysed transferication | Methanolic sodium hydroxide | 50°C for 10 min | 34 |
| Base catalysed transferication | Methanolic sodium hydroxide | 55°C for 10 min | 5 |
| Acidic derivatization | Methanolic Sulphuric acid | 50°C for 2 h | 3 |
| Base catalysed transferication | Methanolic sodium hydroxide | 50°C for 20 secs | 3 |
| Boron trifluoride catalyzed derivatization | Boron trifluoride | 80°C for 25 min | 3 |
| Boron trifluoride catalyzed derivatization | Methanol-toluene-Boron trifluoride | 70°C for 1 h | 35 |
| Boron trifluoride catalyzed derivatization | Boron trifluoride | 100°C for 5 min | 36 |
| Acidic derivatization | Sulphuric acid | 100°C for 30 min | 37 |
| Basic derivatization | Sodium methoxide | Room temperature | 38 |
| Acidic derivatization | Hydrochloric acid | Room temperature | 39 |
| Basic derivatization | Sodium methoxide | Room temperature | 40 |

**Alkylation:**

Alkylation reduces the molecular polarity by replacing active hydrogen by alkyl groups usually by an aliphatic or aliphatic-aromatic.This technique is used to modify those compounds containing acidic hydrogen's such as carboxylic acids and phenols. The principal chromatographic use of this reaction is the conversion of organic acids into esters that produce better chromatograms than the free acids. The principal reaction employed for preparation of these derivatives is nucleophilic displacement. Alkylation reactions can also be used to prepare ethers, thioethers and thioesters, N-alkyl amines, amides and sulphonamides. As the acidity of the active hydrogen decreases, the strength of the alkylating reagent must be increased. As the reagents and conditions become harsher, the selectivity and applicability of the method become more limited [31].

In general, the products of alkylation are less polar than the starting materials because active hydrogen has been replaced by an alkyl group. Probably the largest application of alkylation for analytical derivatization is the conversion of organic acids

into esters, especially methyl esters [32]. Base catalyzed alkylation, diazo alkylation, flash alkylation and extractive alkylation are the methods used in gas chromatographic derivatization process. Base catalyzed reaction is reported for the preparation of methyl derivatives of barbiturates, phenytoins and sulphonyl ureas using dimethyl sulphate reagent [27]. Drugs containing heterocyclic NH groups like sulphonamides, theophylline and thiabendazole undergo derivatization by alkylation reaction. Extractive alkylation is advantages over all the alkylation techniques, as base catalyzed and diazo reaction uses toxic reagents like dimethyl sulphate and diazomethane. The flash alkylation is not as mild as extractive alkylation and hence the possibility of producing more than one product.

**Acylation:**
It is the simplest and most preferred method for derivatization reaction as it results in the quantitative production of derivatives with superior chromatographic performance than that of the original compounds. Acylation, an alternative to silylation, allows the conversion of compounds with active hydrogen such as -OH, -SH, and - NH into derivatives that can be easily analyzed by Gas chromatography. A very popular example of this method is the insertion of perfluoracyl groups to enable electron capture detection (ECD) since fluorinated, reagents provides derivatives that are highly volatile than the corresponding non-halogenated analogues. Another benefit of acylation is the formation of fragmentation-directing derivatives for GC/MS analysis. Acylation reduces the polarity of amino, hydroxyl and thiol groups and hence phenols, primary alcohols, secondary alcohols, primary amine and secondary amines can be easily derivatized to highly volatile and thermally stable compounds. Acyl derivatives can be formed with acyl anhydrides, acyl halides and activated acyl amide reagents.

The acyl halides and acyl derivatives are highly reactive and are suitable for use where steric hindrance may be a factor. The anhydrides and acyl halides form acid by-products which must be removed before GC analysis. Activated amide reagents, such as N-Methyl-bis(trifluoroacetamide **(**MBTFA), have the advantage of not yielding acid by-products. Fluorinated acyl groups, containing trifluoracetyl and heptafluorobutyryl have the advantage of high volatility, high reactivity, ease of removal (in case of excess quantity) and high electronegativity. Acylation converts these compounds with active hydrogens into esters, thioesters, and amides. Acylations are normally carried out in pyridine, tetrahydrofuran or any other solvent capable of accepting the acid by-product.

**Silylation:**
It is the most widely used method, which produces silyl derivatives which are more volatile and more thermally stable. It replaces the active hydrogen with dimethyl or trimethyl silyl groups. They are usually formed by the replacement of the active hydrogens from acids, alcohols, thiols, amines, amides and enolizable ketones and aldehydes with the trimethylsilyl group. Trimethyl silylation is an important derivatisation technique widely applicable to pharmaceutical compounds, as they are formed easily, thermally stable and provide excellent chromatographic performance. A wide variety of reagents are available for the introduction of the trimethylsilyl group. Hexamethyldisilzane, Trimethylchlorosilane, Trimethylsilylimdiazole, N-Trimethylsilyldiethylamine, NO-Bis(trimethylsilyl) acetamide and NO-Bis(trimethylsily1) trifluoroacetamide are used as silylating agents. These reagents differ in their reactivity, selectivity, side reactions and the character of the reaction byproducts from the silylation reagent. Both silylation reagents and trimethylsilyl derivatives are hydrolytically unstable (they react faster with alcohol and water) and must be protected from moisture. It often is possible to prepare derivatives in the presence of small amounts of moisture or to isolate and purify derivatives by extraction in an organic solvent followed by washing with aqueous solutions. Reagents that introduce a t-butyldimethylsilyl group in place of the trimethylsilyl group were developed to impart greater hydrolytic stability to the derivatives.

**Fatty acids analysis of coconut oil by Gas Chromatography:**
Gas chromatographic analyses of fatty acids in oils are performed on the methyl esters of fatty acids with coatings of liquid phases. Gas chromatography is generally employed method for the determination of the common saturated and unsaturated fatty acids[29]. Accurate estimation of all fatty acids becomes extremely important when attempting to rebuild the overall composition of a lipid mixture (Table 3).

**Table 3. Some representative examples of the GC instrument and columns for the analysis of fatty acids in coconut oil**

| GC instrument | Column | Carrier gas | Injector temp | Detector temp | Detector | Reference |
|---|---|---|---|---|---|---|
| PerkinElmer GC-MS 680 | Elite-5MS(30 m × 0.25mm×0.50 µm) | Helium | 250°C | 210°C | MS | 32 |
| Trace Ultra 3300 Thermo Scientific | CP-7420(100 m × 0.25 mm×0.25µm) | Hydrogen | 230°C | 250°C | FID | 1 |
| Shimadzu, QP2010 Ultra | Silica Column (100 m x 0.25 mm x 0.2 µm) | Nitrogen | 250°C | 260°C | FID,MS | 25 |
| Agilent technology | DB-FFAP (30 m x 0.25 mm x 0.25 µm) | Helium | 250°C | 250°C | FID | 33 |
| Shimadzu 2010 plus | CP-SIL 88 (100 m ×0.25 mm×0.2µm) | Helium | 250°C | 260°C | FID | 34 |
| Agilent 7890GC | HP-5 ms (30m x 0.25mm x0.25µm) | Helium | 280°C | 280°C | MS | 5 |
| Shimadzu GC-2014 | Rtx-Wax (30 m x 0.25 mm x 0.25 µm) | Helium | 240°C | 260°C | FID | 3 |
| Thermo Quest-Trace GC | SP-2560 (100 m × 0.25 mm×0.2 µm) | Nitrogen | 225°C | 260°C | FID | 35 |
| Shimadzu GC-MS QP 2010 | Zebron ZB-FFAP (60 m X 0.25 mm X 0.25 µm) | Helium | 250°C | 210°C(ion source) | MS | 36 |
| HP model 5890 series II (plus) | SPB™-1 (30 m × 0.32 mm X 1 µm) | Nitrogen | 250°C | 280°C | FID | 37 |
| Perkin Elmer Gas chromatograph | SP™ 2380 (30 m X 0.25 mm X 0.25 µm) | Nitrogen | 250°C | 250°C | FID | 39 |
| Shimadzu GC-2010 | RTX-5 (30 m X 0.25 mm X 0.2 µm) | Helium | 200°C | 200°C | FID | 40 |

In 2019, Haron et al., evaluated fatty acid composition and antimicrobial activity of methyl esterified virgin coconut oil and activated virgin coconut oil on *Streptococcus* mutans using GC-MS with helium as carrier gas helium at 1.99 ml/min flow rate. It was found that medium chain fatty acids present in virgin coconut oil are caproic acid, caprylic acid, capric acid, and lauric acid. The long chain fatty acids are myristic acid, palmitic acid, arachidic acid and oleic acid. Medium fatty acids in activated virgin coconut oil are found to be caproic acid, caprylic acid, capric acid, lauric acid and long

chain fatty acids are myristic acid, palmitic acid, oleic acid and stearic acid [32].

In 2019, Jessica et al., determined the coconut oil adulteration by direct infusion electrospray ionization mass spectrometry by esterification. Chromatographic analysis was performed in a cyanopropyl column, with split mode set at 40:1 ratio and hydrogen gas as the mobile phase. Saturated fatty acids were found predominantly in coconut oil where, lauric acid was the most abundant saturated fatty acid, ranging from 48-53%. The monounsaturated oleic acid was found in the range of 3-5%, and the polyunsaturated linoleic acid was found in the range of 0.65-1.5% [1].

In 2019, Cansel et al., reported the analysis of fatty acid in coconut oil using GC-FID and GC-MS using silica column (100 m X 0.25 mm X 0.2 µm) and nitrogen as mobile phase at a flow rate of 30 ml/min. It was found that coconut oil contain large amount of lauric acid myristic acid [25].

In 2018, Van Nguyen et al., isolated the fatty acids from virgin coconut oil using lipase and fatty acid by GC analysis. The main component of fatty acid was medium chain fatty acid, lauric acid [33].

In 2018, Martini et al., compared the lipid profile of coconut oil by GC-FID and Raman spectroscopy after base catalyzed transferication. Major fatty acids lauric, myristic, palmitic, caprylic, capric and oleic acid found to present in coconut oil [34].

In 2018, Idu MacDonald et al., analyzed the methyl ester of cold and hot pressed coconut oil by the method published by AOAC. Cold pressed oil reported to produce high yield of lauric acid compared to hot pressed oil [2].

In 2017, Alica et al., developed a GC-MS method for the determination of fatty acids in oils after esterification using methanolic sodium hydroxide. Methyl esters of lauric acid and myristic acid are found as major constituent. Capric acid, palmitic acid, oleic acid, linoleic acid and stearic acid found in minor quantities [5]

In 2016, Julius Pontoh et al., examined the medium chain fatty acids in coconut oil by acid catalyzed, base catalyzed and Boron trifluoride catalyzed derivatization method. For analysis capillary fused silica column (Rtx-Wax: 30 m X 0.25 mm X 0.25 µm) was used with helium set at 75 kPa. Injection mode was set as 1:10 split mode. Base catalyzed derivatization was found to be efficient with highest conversion efficiency and but boron trifluoride catalyzed derivatization found to be the best [3].

In 2016, Dawrul Islam et al., identified composition of fatty acids in coconut oil using AOAC GC-FID method. Coconut oil reported to contain high amount of saturated fatty acids and less amount of mono and poly unsaturated fatty acids [35].

In 2013, Diana Moigraden et al., quantitatively identified the fatty acids from coconut oil using GC-MS method after esterification with $BF_3$-MeOH method. The study results showed coconut oil contains 87% saturated fatty acid and 8% unsaturated fatty acid. Unsaturated fatty acid composed mainly by oleic acid [36].

In 2013, Vesna Kostik et al., studied the fatty acids of coconut oil by modified ISO method. Lauric acid was the major fatty acids found in Coconut oil, besides the other saturated fatty acids caprylic acid, capric acid, myristic acid, palmitic acid and stearic acid [37].

In 2012, Mansor et al., virgin coconut oil and reported high content of medium chain fatty acids, majority with lauric acid. Lauric acid present in different coconut oil ranged between of 46-48% and highest in coconut oil extracted by fermentation process [38].

In 2009, Henna et al., compared the storage stability in virgin coconut oil and extra virgin oil upon thermal treatment. Virgin coconut oil showed higher stability with no significant changes in fatty acid proportion throughout the storage [39].

In 2009, Rohman et al., reported coconut oil contains high percentage of saturated fatty acids, particularly lauric acid, compared with palm oil [40].

## CONCLUSION:

Gas chromatography is a rapid, sensitive and versatile technique widely used for the separation of volatile oils and fatty acids in nonvolatile oils after derivatization. The information provided in this review will be useful for the researchers who wish to pursue the studies in fatty acid analysis of coconut oil using gas chromatographic technique.

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