A DISSERTATION ON

ANALYSIS OF FATTY ACID AND TRANS FATTY IN VANASPATI

SUBMITTED TO THE

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FOR THE

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IN BIOTECHNOLOGY

BY

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TO WHOM IT MAY CONCERN

This is to certify that **Mr. Himanshu Chauhan, a** student of M.Sc. Biotechnology (IV semester), Integral University has completed his four months dissertation work entitled "**Analysis of Fatty Acid and Trans Fatty in Vanaspati"** successfully. He has completed this work from 17th February to 31thMarch 2022 at FARELABS Pvt. Ltd under the guidance of **Mr. Bhushan Dole** (Head of Gas Chromatography Department).

The dissertation was a compulsory part of her M.Sc. degree. I wish her good luck and bright future.

(DR. SNOBER S. MIR)

Head,

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TRAINING CERTIFICATE

This is to certify that **Mr. Himanshu Chauhan,** S/o Mr. Hari Nath Chauhan from **Integral University Lucknow, MSc Biotechnology,** has successfully completed his dissertation on **"Fatty Acid & Trans-Fatty Aids Analysis in Vanaspati."** from 17th January 2022 to 31st May 2022 at **FARE Labs Pvt. Ltd.** and has been awarded excellent grade basis of his performance and the project report submitted.

He has accomplished the training successfully. We have found him sincere and devoted during the training.

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I, **HIMANSHU CHAUHAN**, certify that the work embodied in the training report **"Analysis of Fatty Acid and Trans Fatty Acid in Vanaspati"** to be submitted to the Master of Science in Biotechnology of Integral University, Lucknow, Uttar Pradesh, India is original and the result of analysis carried out by me under the supervision of **Mr. Bhushan Dole** (Head of Gas Chromatography Department), FARE Labs Pvt. Ltd. for the time period of January, 2022 to June, 2022. The matter embodied in Master of Science thesis has not been submitted for the award of any other degree/ diploma.

I declare that I have faithfully acknowledged and referred to the research workers wherever their works have been cited in the text. I further certify that I have not willfully lifted up some other's work, paragraph, text data, results, etc. reported in journals, books, magazines, reports, dissertations thesis, etc., or available at web sites and included them in this M.Sc. thesis and cited as my own work. I have completed all pre submission requirement as per the University rules.

Himanshu Chauhan

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Himanshu Chauhan

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ABBREVIATION'S LIST

GC- Gas chromatography
TFAs- Trans Fatty acids
SFA- Saturated Fatty acid
FID- Flame Ionization detector
ECD- Electron Capture detector
MUFA- Monounsaturated Fatty acid
PUFA- Polyunsaturated Fatty acid
FA- Fatty acid
TCD- Thermal Conductivity detector
AOAC- ASSOCIATION OF OFFICIAL ANALYTICAL COLLABORATION
MTB – Methanol, Toluene, Bromine trifluoride

ABSTRACT

The physicochemical features of different Vanaspati Samples from various commercials varieties were investigated. Fatty acid profile of different vanaspati samples were studied and the trans fatty acids of different types of vanaspati samples are discussed by many researchers. Gas chromatography is used to evaluate the fatty acid profile.

The most prevalent fatty acid was palmitic acid, which was followed by oleic, stearic, and linoleic acids. According to differential scanning calorimetry, the solid fat content of the samples was 54–67% solids at 20°C, 42–59% solids at 25°C, and 0.2–19% solids at 40°C. The melting point of the slide was between 39 and 40 degrees Celsius. The fat with the highest trans content had the highest hardness index. There was no obvious link between fatty acid composition and consistency, however.

Five different samples of vanaspati (vegetable ghee) were studied. Saturated FA, cis monounsaturated FA, and cis PUFA content in vanaspati, TFA levels in Vanaspati samples were much higher, ranging from 14.2 to 34.3 %.

Keywords: Trans Fatty acid, Vanaspati, Table Margarine, Elaidic acid, Monounsaturated fatty acid, Polyunsaturated fatty acid.

INTRODUCTION

Speciality of fat with no trans fatty acids prepared by blending palm oil fraction with rice bran oil showed melting characteristics similar to those of commercial vanaspati. This fat is used in preparation of Indian traditional foods such as Mysorepak, Sohan papdi and Badusha substituting vanaspati and the quality was evaluated in comparison with those prepared using vanaspati. The appearance, texture and sensory analyses of all the products prepared with speciality fat were similar and comparable with those prepared with vanaspati. The products prepared with vanaspati and commercial samples contain trans fatty acids (0.3 - 6.3%), whereas those prepared with speciality fat did not contain any trans fatty acids. The study therefore revealed that the trans-free speciality fat prepared using palm oil fraction and rice bran oil could be used as vanaspati (trans fatty acids 17.7%) substitute to prepare a range of traditional foods and thereby improve nutritional quality.

OILS and FATS

Oils and Fats form an important part of a healthy diet. Structurally, they are esters of glycerol with three fatty acids (called either triacylglycerols or triglycerides). It is these fatty acids that give the functionality to fats. Chemically, they can be divided into four main types – saturated, *cis*- monounsaturated, *cis*-polyunsaturated and *trans*-fatty acids.

In every broad term, saturated fatty acids and *trans*-fatty acids are solid at room temperature while the *cis*-unsaturated are liquid at room temperature. Although no naturally occurring fat is either 100% saturated or 100% unsaturated (but is a mix of the two), fats are often referred to as ‘saturated’ or ‘unsaturated’ because of the predominance of one or other type of fatty acid.

From a product functionality point of view, different groups of food products will have different requirements. Bakery products (e.g., pastry and biscuits) require a fat with a moderate amount (25- 40%) of solid fat to be present during dough preparation to give a light texture without undue oil exudation in the final product. Chocolate needs to be based on cocoa butter from both a legislative and functionality point of view and any fats used to replace cocoa butter need also to conform to legislation and to melt and crystallize in the same way as cocoa butter. Although the terms 'oils' and 'fats' are often used interchangeably, they are usually used to distinguish triglycerides in the liquid state at ambient temperatures (oils) from those in the solid state (fats). They are commonly of vegetable origin (e.g., palm oil, rapeseed oil, soyabean oil, olive oil, cocoa butter, etc.) or animal origin (e.g., pork lard, beef tallow, fish oils) as well as from animal milk fats.

Fats are solid at room temperature, like Vanaspati, butter, bakery & shortening etc. Solid fats mainly come from animal foods and can also be made from vegetable oils through a process called hydrogenation.

Fats and oils are non-volatile in nature. Also, these are insoluble in water and soluble in organic solvents. Oils and fats play a vital role in our bodies' essential biochemical processes which keep us alive and well as well as in storing energy and insulating us. They are important nutrients in a normal, balanced, healthy diet and the body needs them for a variety of reasons. Fats provide a concentrated source of energy and is a carrier for fat-soluble vitamins A, D, E and K.

FATTY ACID

Fatty acid, important component of lipids (fat-soluble components of living cells) in plants, animals, and microorganisms. Generally, a fatty acid consists of a straight chain of an even number of carbon atoms, with hydrogen atoms along the length of the chain and at one end of the chain and a carboxyl group (—COOH) at the other end. It is that carboxyl group that makes it an acid (carboxylic acid). If the carbon-to-carbon bonds are all single, the acid is saturated; if any of the bonds is double or triple, the acid is unsaturated and

more reactive. A few fatty acids have branched chains; others contain ring structures (e.g., prostaglandins). Fatty acids are not found in a free state in nature; commonly they exist in combination with glycerol (an alcohol) in the form of triglyceride.

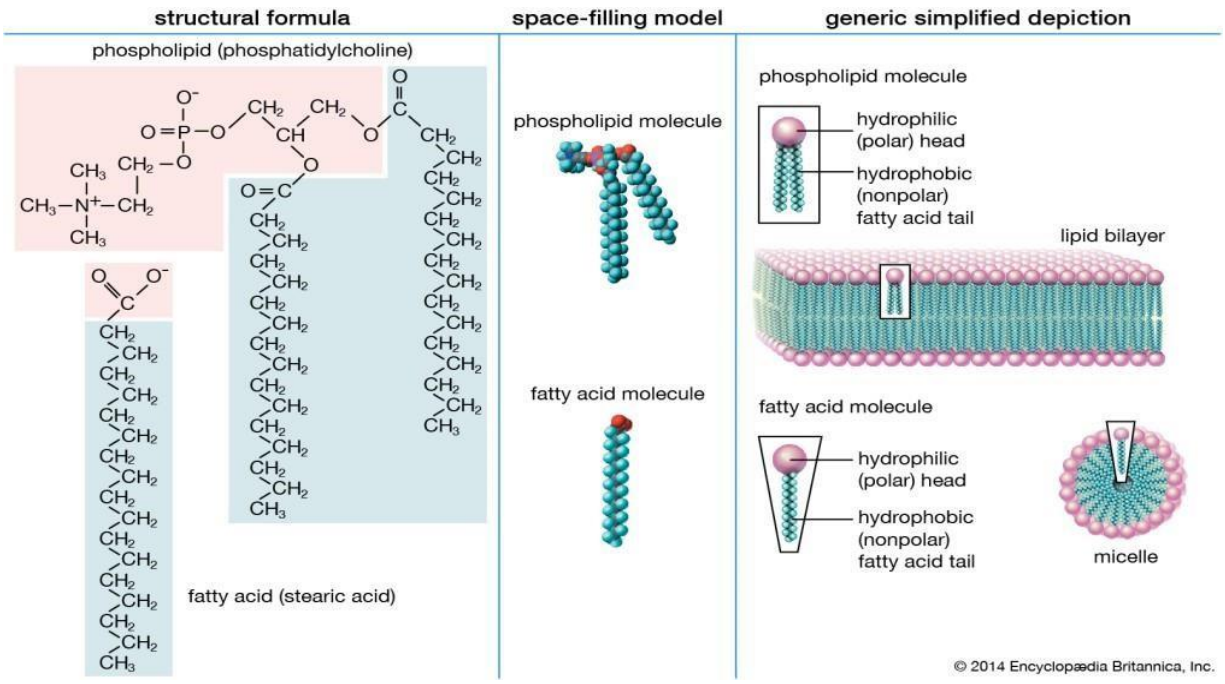


Figure 1- structure of fatty acid

Fatty acids can be divided into four general categories: saturated, monounsaturated, polyunsaturated, and trans fats. Saturated fatty acids and trans fats are associated with an increased risk of coronary heart disease. Monounsaturated fatty acids and polyunsaturated fatty acids are associated with a decreased risk of coronary heart disease, although these associations are not uniformly supported in the literature. Omega-3 fatty acids, which are a type of polyunsaturated fatty acid, have been studied as potential therapy for a variety of medical conditions because of their suspected anti-inflammatory properties. Omega-3 fatty acids have also been shown to provide some benefit to patients with cystic fibrosis, and may have a protective effect against dementia. Physicians should counsel patients about the importance of avoiding hydrogenated oils and foods containing trans fats because of their association with coronary heart disease in observational studies.

Different forms of Fatty acids

One is Saturated fatty acids, and these forms are those that have all single bonds (having the greatest number of hydrogen atoms).
Saturated fatty acids have a linear structure and are solidified at room temperature. They are derived from animal fats. Example fats. Monounsaturated (one double bond) or polyunsaturated (more than one double bond) fatty acids have one and more than one double bonds.

Unsaturated fatty acids have a bent structure and are fluid at room temperature. They are derived from plants. Example oils.

Type of Fatty Acid	Double Bonds	Diagram
Saturated	None	
Monounsaturated	One	
Polyunsaturated	Multiple (>1)	

Figure 2- types of fatty acid

SATURATED FATTY ACIDS (SFA)

A saturated fat is a type of fat in which the fatty acid chains have all or predominantly single bonds. Saturated fatty acids are extremely stable i.e., they do not easily become rancid, meaning they have good keeping properties (shelf life). Saturated fat is solid at room temperature, which is why it is also known as "solid fat."
Most animal fats such as meat, butter, cheese and cream contain relatively high levels of saturated fat. Saturated fat is also in tropical oils, such as coconut oil, palm oil, and cocoa butter. Foods made with butter, margarine, or shortening and many baked goods such as cakes, biscuits, cookies, pastries and other desserts can also be high in saturated fat. Some of these are:
Butyric (C4:0), Lauric (C12:0), Myristic (C14:0), Palmitic (C16:0), Stearic (C18:0), Arachidic (C20:0), Behenic (C22:0).
Molecular Formula: $C_{12}H_{24}O_2$

Chemical Structure of Lauric Acid is:

Figure 3. Chemical structure of lauric acid

UNSATURATED FATTY ACIDS

Unsaturated fatty acids have at least one or more double bonds in their chemical structure. There are three types of unsaturated fatty acids:

- Mono Unsaturated (MUFA)
- Poly Unsaturated (PUFA)
- Trans Fatty Acid (TFA)

MONOUNSATURATED FATTY ACIDS (MUFA)

Fatty acids in this category have one double bond in their chemical structure. They are relatively stable to oxidation and the development of rancidity and are now considered, in nutritional terms, as being the best type of fat to eat.

Monounsaturated fats are found in high concentrations in Olive, peanut, and canola oils. Erucic (C22:1) and oleic (C18:1) acids are the most commonly found monounsaturated fatty acids inedible oils.

Molecular Formula: $C_{18}H_{34}O_2$

Chemical structure of oleic acid is:

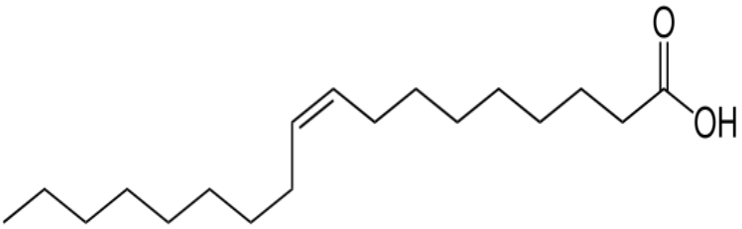


Figure 4. Chemical structure of oleic acid

POLYUNSATURATED FATTY ACIDS (PUFA)

Polyunsaturated fatty acids contain two or more double bonds in their chemical structure. They are least stable fatty acids to oxidation and as such are best used in cold applications. Polyunsaturated fats are found in high concentrations in Sunflower, Corn, Soybean, and Flaxseed (Linseed) oils. Most common polyunsaturated fatty acids found in edible oils are linoleic (C18:2) and linolenic (C18:3).

Molecular Formula: $C_{18}H_{32}O_2$

Chemical structure of linoleic acid is:

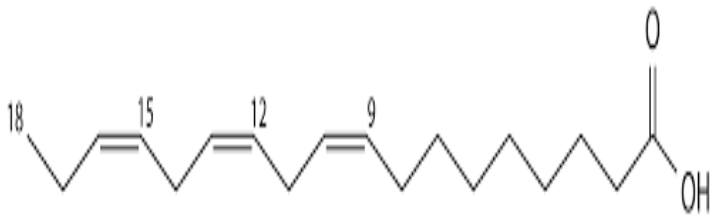


Figure 5. Chemical structure of linoleic acid

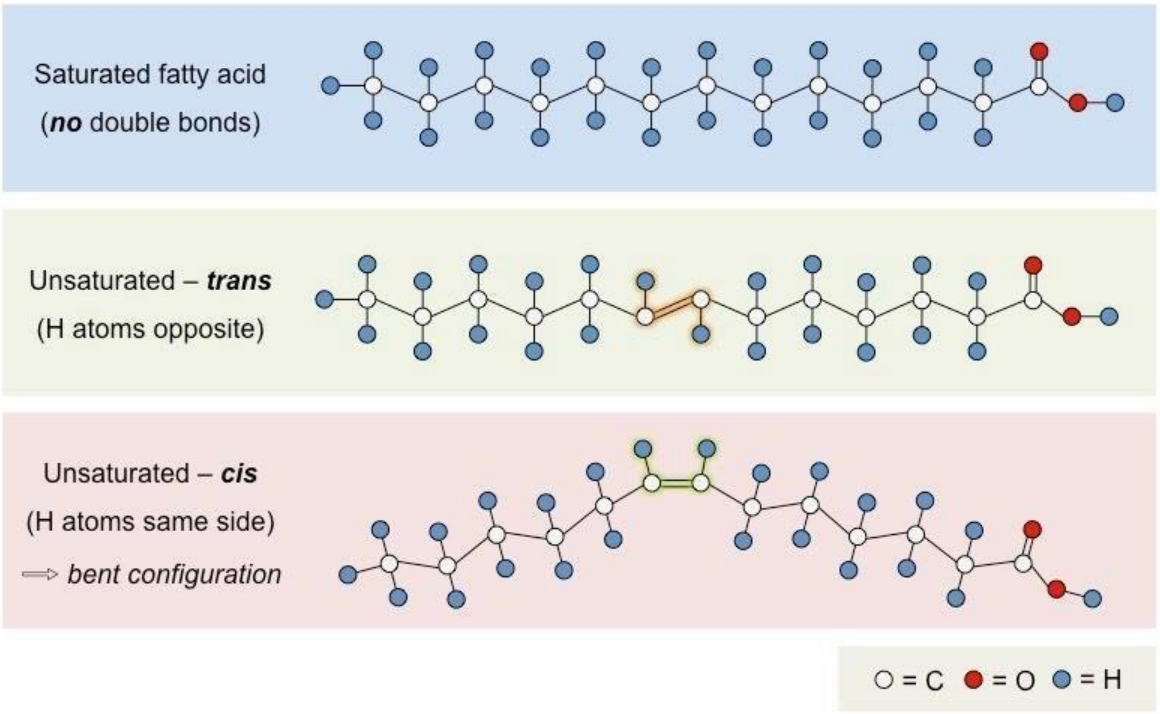


Figure 6 - Types of Fatty Acid Configurations

TRANS FATTY ACIDS (TFA)

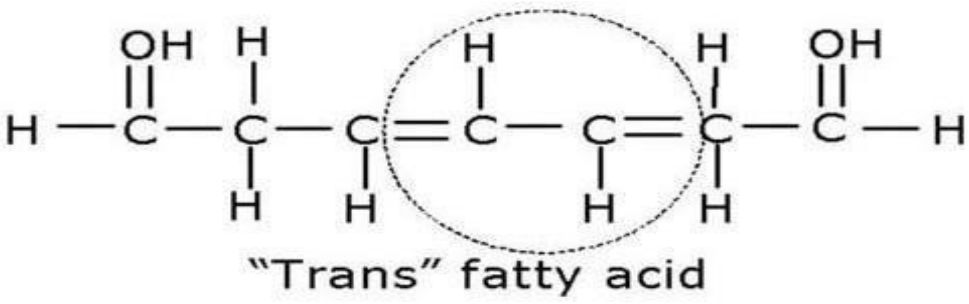


Figure 7- Trans fatty acid

Trans fatty acids, more commonly called trans fats, are made by heating liquid vegetable oils in the presence of hydrogen gas and a catalyst by the process called hydrogenation. This process increases the shelf life of fat and makes the fat harder at room temperature.

Trans fats are rare in nature. Trans fats are also created during the manufacture of some baked products such as pies, pastries, cakes, biscuits and buns. It is the trans fats that are produced during food manufacturing that you should be most concerned about, not the small

amounts of trans fats naturally found in healthy foods like low-fat dairy products. High concentrations of trans are found in Vanaspati, shortening and margarine.

For instance, the trans fatty acid of oleic(C18:1) is Elaidic, trans(C18:1) and that of linoleic(C18:2) is Linolelaidic, trans(C18:2).

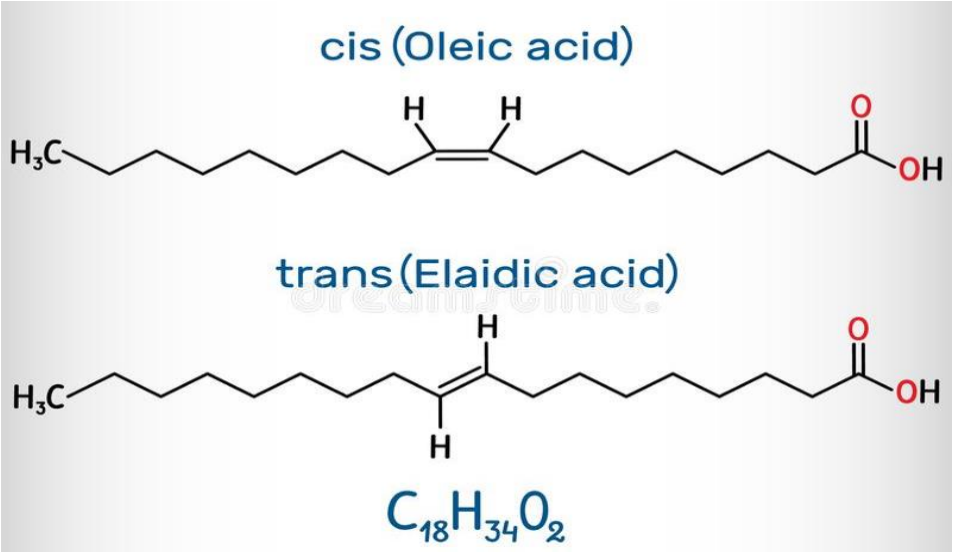


Figure 8. Chemical structure of trans of oleic acid

FSSAI limits for Trans Fatty acids

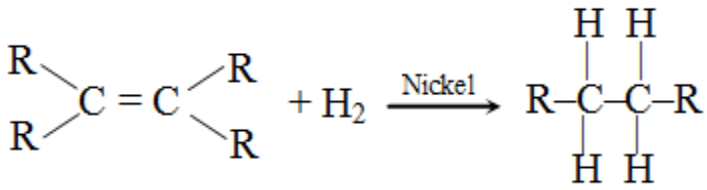
FSSAI has set a limit for industrial TFAs in edible oils and fats of not more than 2% effective.
January 1, 2021, and the limit would be further decreased in all fats and oils to not more than 2% by January 1, 2022, as per its 2018 plan.

Beginning January 1, 2022, food products holding more than 2% industrial Trans Fatty acids will be banned. With India's Food Safety and Standards Authority (FSSAI) establishing a deadline of January 1, 2022 for restricting trans-fat levels in food items and edible oils and fats to 2%, India will join a group of roughly 40 countries around the world adopting policies to eliminate trans-fats from their food supply chains

HYDROGENATION

The process was originally introduced to convert some of the unsaturated fatty acids in vegetable oils and animal fats to make them more stable to oxidation. In this process, the unsaturated double bonds in the fatty acids of the oil molecules react with hydrogen atoms in

the presence of a catalyst (as shown in the below reaction). Nickel catalyst is used in commercial hydrogenation of edible oils.



Other catalysts, such as platinum, palladium, copper, etc., have also been applied in hydrogenation applications. These are not used in commercial hydrogenation of edible oils.
Hydrogenation has been used for a long time to improve oxidative stability of vegetable oils for improved shelf life and to modify the melting characteristics of the oil to formulate products like shortening, margarine and Vanaspati with the desired physical properties.

ESSENTIAL FATTY ACID

Essential fatty acids, or EFAs, are unsaturated fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them in the body and therefore must be obtained from the diet. Studies have shown that increasing the intake of
Certain essential fatty acids, either alone or in combination with other fats and compounds, can increase health, help in treating certain diseases, or even improve body composition, mental and physical performance. They are:

- **OMEGA-3**
  Fatty acids have the first double bond at the third carbon
  Eicosapentaenoic (EPA) (C20:5n3)
  Docosahexaneoic (DHA) (C22:6n3)
  Alpha-Linolenic (ALA) (C18:3n3)
- **OMEGA-6**
  Fatty acid have the first double bond at the sixth carbon atom
  Linoleic Acid (C18:2n6)
- **OMEGA-9**
  Fatty acids have the first double bond at the ninth carbon atom.
  Oleic Acid (C18:1n9)
  Erucic (C22:1n9)

GAS CHROMATOGRAPHY

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture.

In some situations, GC may help in identifying a compound. Only volatile compounds are tested in GC. GC is the most sensitive and accurate method for fat analysis.
There are three components of GC, these are:

- Injector
- Oven
- Detector
-

Also, there are two phases in GC:

- Stationary phase - Column (different types of columns according to the compounds).
- Mobile phase - Gas

The gases used in GC are Nitrogen, Hydrogen and Zero Air. Nitrogen goes in column and is used as carrier gas. Hydrogen is the fuel gas while zero air is used for ignition and contains 20% O2.
In gas chromatography, the mobile phase is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. Helium remains the most commonly used carrier gas in about 90% of instruments although nitrogen is preferred for improved separations. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The instrument used to perform gas chromatography is called a gas chromatograph. The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.
The process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled. Finally, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas. Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point differences.

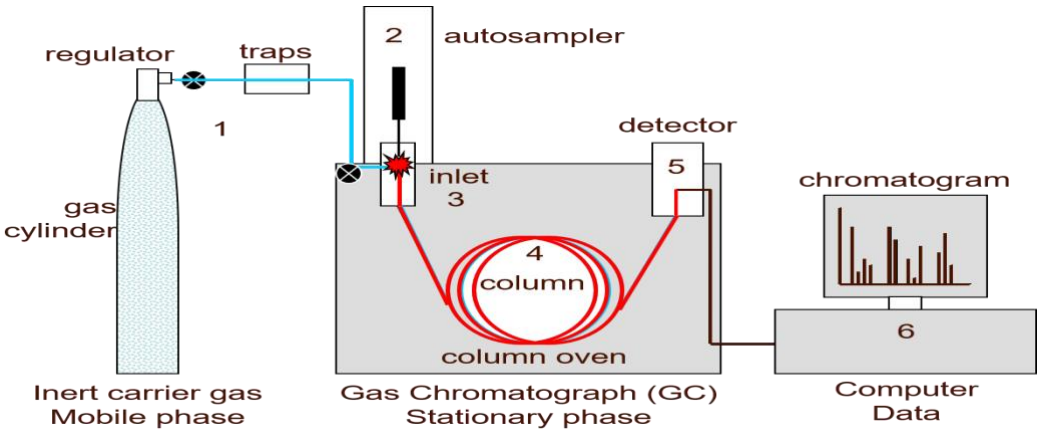


Figure 9. Diagram of Gas chromatography instrument

REVIEW OF LITERATURE

Edible Oils are in 3 forms on the basis of processing viz. Crude Oil, Refined Oil and Vanaspati. The uninitiated, based on the terms used, will perceive

- Crude oil is natural and not yet fit for consumption
- Refined oil is improved and made perfect for consumption
- Vanaspati, in Sanskrit means 'lord of forest', should be very healthy

This is where consumers are misguided by the names and their literal meanings. The fact though is

- Crude Oil that is just filtered after extracting oil from the oil seed is the healthiest. Unrefined oils still have healthy bioactive components, Vitamin E, flavour and aroma.
- Refined Oil is highly processed with the use of chemicals and heat to just retain the oil. Even oils extracted from lower quality oil seed can be refined. So, refined oil though pure is not as healthy as corresponding crude/unrefined oil.
- Vanaspati is formed by partial hydrogenation of refined vegetable oil is extremely harmful. They have Trans fats which are formed as a by-product of hydrogenation.

In India, of the total 13 million tons of edible oil consumed, 10% is in the form of Vanaspati and 45% each in refined and unrefined form. Doctors and Health Departments warn consumers against intake of saturated fats, but the real culprit for all the heart problems and obesity are trans fats. Chemical analysis of trans fats gives a logical reason why trans-fats are harmful.

Chemistry of fats

Fatty acids are characterized as either saturated or unsaturated based on the presence of double bonds in its structure. If the molecule contains no double bonds, it is said to be saturated; otherwise, it is unsaturated to some degree. A saturated fat has no double bonds, has the maximum number of hydrogens bonded to the carbons, and therefore is "saturated" with hydrogen atoms. The more double bonds in the fatty acid the more vulnerable it is to rancidity, as free radicals attack double bonds.

In most naturally occurring unsaturated fatty acids (and all saturated fatty acids), the hydrogen atoms are on the same side of the double bonds of the carbon chain (cis configuration — from the Latin, meaning "on the same side"). However, partial hydrogenation reconfigures most of the double bonds that do not become chemically saturated, twisting them so that the hydrogen atoms end up on different sides of the chain. This type of configuration is called trans, from the Latin, meaning "across." The trans conformation is the

lower energy form, and is favoured when catalytically equilibrated as a side reaction in hydrogenation.

The trans configuration is straighter, while the cis configuration is noticeably kinked. Trans fats have a much higher melting point (45 °C), due to the ability of the trans molecules to pack more tightly, forming a solid that is more difficult to break apart. This notably means that it is a solid at human body temperatures (37 °C).

In essence, hydrogenated oil/trans fats are stable making the food products last longer and are solids at body temperature making it harder to digest.

Hydrogenated oils are more stable than corresponding natural oils with unsaturated fats. Saturated fats are mostly found in animal sources (Butter, Ghee etc) which are scarce and hence expensive. Oils from vegetable sources are mostly unsaturated, abundant and less expensive. So, partial hydrogenation of less expensive unsaturated fats from vegetable sources is an attractive commercial proposition. They are not only stable and make the fried food product last longer but also adds to the taste. That is why we find widespread use in commercial cooking in Breads, Cookies, Cream Biscuits, Sweets, fried snacks, chocolates and ice creams.

Health Risk

The primary health risk identified for trans-fat consumption is an elevated risk of coronary heart disease. The reason is trans-fat increases the level of LDL or bad cholesterol and decreases HDL or good cholesterol. Other ill-effects are Alzheimer's disease, Cancer, Diabetes, Obesity, Liver dysfunction and infertility in women.

Prevention of Food Adulteration Act specifies that the melting point of Vanaspati be strictly less than 41 °C. That means manufactures must ensure that hydrogenation is stopped before melting point reaches that point. Also, the labels in packed food products must show the amount of trans fats in the food sample. ITC, Britannia and the likes who are branded manufacturers of such food products clearly mention that Trans fats are bad for health just like Cigarette packs mention 'Smoking is injurious to health'.

It is highly advised that consumers avoid all commercially prepared foods (Bakeries, Hotels and Packed foods) to the extent possible. One should also carefully read the labels for its ingredients and nutrition values. Food items containing Edible Vegetable Fat or having Trans fats value more than 2% should be avoided.

Trans Fatty Acid

Trans fatty acid (TFA) is isomers of monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, respectively) and are not synthesized by the human body, nor are they

essential to the human diet. Moreover, dietary intake of most TFAs is well recognized as an independent risk factor for the development of cardiovascular diseases (CVDs) (Mozaffaria, 2016) and is also associated with mortality from all causes (Kiage et al., 2013). While some TFAs are naturally produced by the bacteria in the rumen and as such found in the meat, milk and dairy products of ruminants (MacGibbon and Taylor, 2006), their contribution to overall TFA consumption is minimal (Allison et al., 1999). Industrially produced TFAs are considered a major source of TFAs in the human diets in many countries.

TFAs, due to their molecular structure, possess the potential for closer packing and alignment of their acyl chains, which results in decreased molecular mobility and reduced fluidity. For these reasons, and also because of a consumer preference for vegetable fats (in comparison to animal fats), hardened vegetable fats have become widely used. Although TFAs can also be formed endogenously via oxidative stress and by free radicals (Hung et al., 2016), and are commonly formed during preparation of food, particularly during frying (Cui et al., 2017), industrially produced partly hydrogenated fats (PHFs).

Vegetable Oils in general contain mainly C18 unsaturated fatty acids (FAs), the PHFs being prepared from these oils predictably consist of mainly trans-C18 isomers (Albers et al., 2008; Alves et al., 2008).
An improved gas chromatography method for the simultaneous separation of 52 fatty acids (FAs) has been developed. For both oleic acid and linoleic acid, a good resolution was achieved for their positional and geometrical (cis/trans) isomers. This method was validated to be precise, accurate and sensitive.

Fatty acids are comprised of hydrocarbon chains terminating with carboxylic acid groups. Fatty acids and their associated derivatives are the primary components of lipids. The length and degree of saturation of the hydrocarbon chain is highly variable between each fatty acid, and dictates the associated physical properties (e.g., melting point and fluidity). Moreover, fatty acids are responsible for the hydrophobic properties (insoluble in water) exhibited by lipids. Fatty acids are composed of carbon chains containing a methyl group at one end and a carboxyl group at the other.

The methyl group is termed the omega and the carbon atom situated next to the carboxyl group is termed the α carbon, followed by the β carbon, etc. Fatty acid molecules also have two chemically distinct regions:

i. A long hydrophobic hydrocarbon chain, which is not highly reactive.

ii. A carboxyl (-COOH) group, which is hydrophilic and highly reactive.

TABLE 1.- LIST OF FATTY ACIDS

Carbon no.	Fatty acid name	Carbon no.	Fatty acid name
(C4:0)	Butyric	(C18:3)	Alpha Linolenic
(C6:0)	Caproic	(C20:0)	Arachidic
(C8:0)	Caprylic	(C20:1)	Eicosenoic
(C10:0)	Capric	(C20:2)	Eicosadienoic
(C11:0)	Undecanoic	(C21:0)	Henecosenoic
(C12:0)	Lauric	(C20:3)	Gamma ecosatetranoic
(C13:0)	Tridecanoic	(C20:4)	Arachidonic
(C14:0)	Myristic	(C20:3)	Eicostrienoic
(C14:1)	Myristoleic	(C20:5)	EPA
(C15:0)	Pentadecanoic	(C22:0)	Behenic
(C15:1)	Cis -10 Pentadecanoic	(C22:1)	Erucic
(C16:0)	Palmitic	(C22:2)	Docosadenoic
(C16:1)	Palmitoleic	(C22:4)	Docosatetranoic
(C17:0)	Heptadecanoic	(C22:5)	Docosapentanoic
(C17:1)	Cis -10 Heptadecanoic	(C23:0)	Tricosanoic

(C18:0)	Stearic	(C24:0)	Lignoceric
(C18:1 trans)	Elaidic	(C22:5 n3)	Docosapentanoic
(C18:1)	Oleic	(C22:6)	DHA
(C18:2 trans)	Linolelaidic	(C24:1)	Nervonic
(C18:2)	Linoleic		
(C18:3)	Gamma Linoleic		

GAS CHROMATOGRAPHY

Gas chromatography is a term used to describe the group of analytical separation techniques used to analyses volatile substances in the gas phase. In gas chromatography, the components of a sample are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between two phases: a stationary phase and a mobile phase. The mobile phase is a chemically inert gas that serves to carry the molecules of the analyte through the heated column. Gas chromatography is one of the sole forms of chromatography that does not utilize the mobile phase for interacting with the analyte. The stationary phase is either a solid adsorbent, termed gas-solid chromatography (GSC), or a liquid on an inert support, termed gas-liquid chromatography (GLC).

A typical gas chromatograph consists of an injection port, a column, carrier gas flow control equipment, ovens and heaters for maintaining temperatures of the injection port and the column, an integrator chart recorder and a detector. To separate the compounds in gas-liquid chromatography, a solution sample that contains organic compounds of interest in injected into the sample port where it will be vaporized. The vaporized samples that are injected are then carried by an inert gas, which is often used by helium or nitrogen. This inert gas goes through a glass column packed with silica that is coated with a liquid. Materials that are less soluble in the liquid will increase the result faster than the material with greater solubility.

PRINCIPLE

The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column." (Helium or nitrogen is used as carrier gas.) The various components are separated inside the column. The detector measures the quantity of the components that exit the column. To measure a sample with an unknown concentration, a standard sample with known concentration is injected into the instrument. The standard sample peak retention time (appearance time) and area are compared to the test sample to calculate the concentration.

INJECTOR

The injector can be used in one of two modes; split or split less. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode).

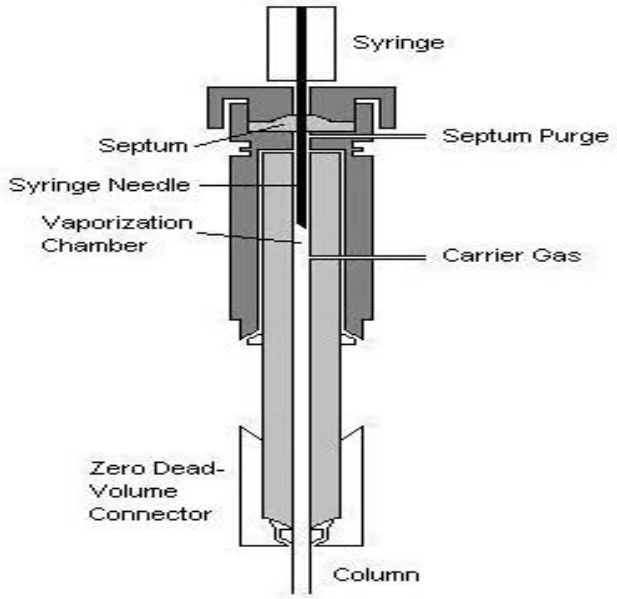


Figure 10. A cross-sectional view of a micro flash vaporizer direct injector

COLUMN OVEN

Temperature in GC is controlled via a heated oven. The oven heats rapidly to give excellent thermal control. The oven is cooled using a fan and vent arrangement usually at the rear of the oven. A hanger or cage is usually included to support the GC column and to prevent it touching the oven walls as this can damage the column.

The injector and detector connections are also contained in the GC oven. For Isothermal operation, the GC is held at a steady temperature during the analysis. In temperature programmed GC the oven temperature is increased according to the temperature program during the analysis.

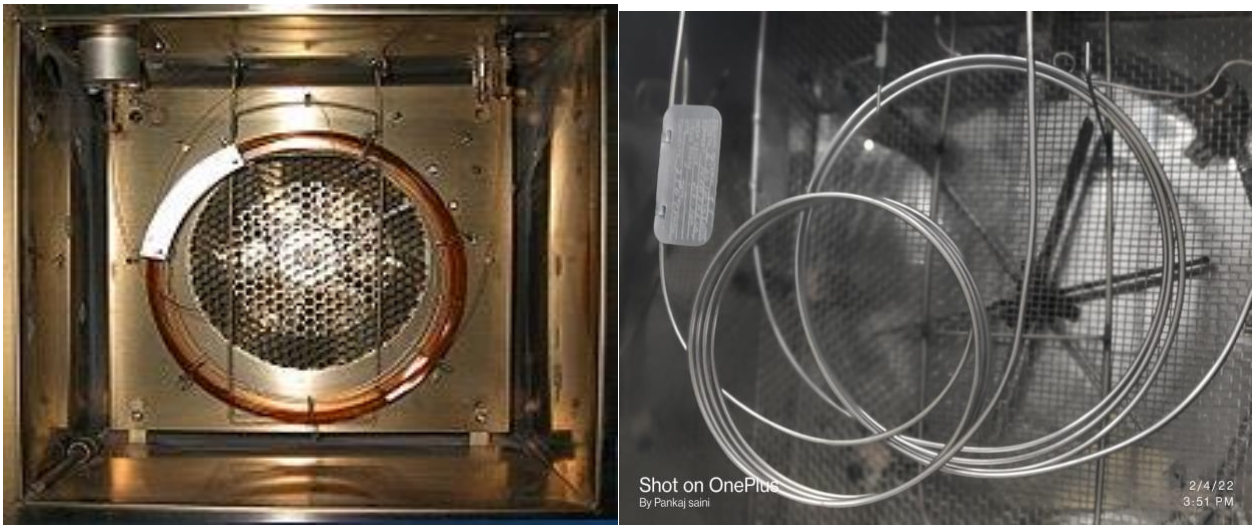


Figure 11. Column and Oven

DETECTOR

The detector responds to a physicochemical property of the analyte, amplifies this response and generates an electronic signal for the data system to produce a chromatogram. Many different detector types exist and the choice is based mainly on application, analyte chemistry and required sensitivity – also on whether quantitative or qualitative data is required. Detector choice include:

1. Flame Ionization Detector (FID)
2. Nitrogen Phosphorus Detector (NPD)
3. Electron Capture Detector (ECD)
4. Thermal Conductivity Detector (TCD)

FLAME IONISATION DETECTORS

Mechanism: Compounds are burned in a hydrogen-air flame. Carbon containing compounds produce ions that are attracted to the collector. The number of ions hitting the collector is measured and a signal is generated.

Selectivity: Compounds with C-H bonds. A poor response for some non-hydrogen containing organics (e.g., Hexachlorobenzene).
Sensitivity: 0.1-10ng
Linear range: 10^5-10^7

Gases: Combustion - hydrogen and air; Makeup - helium or nitrogen
Temperature: 250-300°C, and 400-450°C for high temperature analyses.

NITROGEN PHOSPHORUS DETECTOR (NPD)

Mechanism: Compounds are burned in a plasma surrounding a rubidium bead supplied with hydrogen and air. Nitrogen and phosphorous containing compounds produce ions that are attracted to the collector. The number of ions hitting the collector is measured and a signal is generated.

Selectivity: Nitrogen and phosphorous containing compounds
Sensitivity: 1-10 pg.
Linear range: 10^{4}-10-^{6}
Gases: Combustion - hydrogen and air; Makeup - helium
Temperature: 250-300°C

ELECTRON CAPTURE DETECTOR (ECD)

Mechanism: Electrons are supplied from a 63Ni foil lining the detector cell. A current is generated in the cell. Electronegative compounds capture electrons resulting in a reduction in the current. The amount of current loss is indirectly measured and a signal is generated.

Selectivity: Halogens, nitrates and conjugated carbonyls
Sensitivity: 0.1-10 pg. (halogenated compounds); 1-100 pg.
(nitrates); 0.1-1 ng (carbonyls)
Linear range: 10^{3}-10^{4}
Gases: Nitrogen or argon/methane
Temperature: 300-400°C

THERMAL CONDUCTIVITY DETECTOR (TCD)

Mechanism: A detector cell contains a heated filament with an applied current. As carrier gas containing solutes passes through the cell, a change in the filament current occurs. The current change is compared against the current in a reference cell. The difference is measured and a signal is generated.
Selectivity: All compounds except for the carrier gas
Sensitivity: 5-20 ng
Linear range: 10^{5}-10^{6}
Gases: Makeup - same as the carrier gas
Temperature: 150-250°

OBJECTIVES

- Analysis of fatty acid for Vanaspati by using Gas Chromatography.
- Analysis of Trans fat for Vanaspati by using Gas Chromatography.

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METHODOLOGY

The transfat analysis method utilized in this study consists of components of both AOAC 996.06 and AOAC 996.33. The GC conditions, as well as the recognition and quantification of trans fat in gas chromatography, are all subject to modifications proposed to AOAC 996.06.

Many of the profile's minor components, however, are not available in conventional materials. As a result, the AOAC methodology is utilized to identify particular key isomers by comparing the profiles of the detected isomers. The peaks 12 trans, 18:2 (linolelaidate), 9 trans 18:1 (elaidate) and 9 trans is discovered and figured out using external standards. The AOCS approach, which quantifies the regions of 18:2 and 18:1 transfat isomers as packed peaks, with the recognition supported by retaining lengths of other identified peaks, is used to quantify additional 18:1 and 18:2 transfat isomers The activation factors of elaidate and linolelaidate are used to quantify these peaks. The peaks directly before 9 trans 18:1 elaidate (usually 4 trans–14 trans 18:1) but before 9 cis 18:1, as well as 16 trans 18:1, are trans 18:1 isomer (which elute after 9-cis 18:1). Trans 18:2 isomers are those with peaks within 15 cis 18:1 and 9 cis, 13 cis 18:2. A chromatogram of the 18:2 and 18:1 cis and trans isomers in vanaspati is shown in Figure 6. Individual isomers are discovered, but they are mixed by location to demonstrate that the method can accurately quantify 18:1 and 18:2 trans. Following that, these peak locations are examined to the external standard that is the most comparable (elaidate for 18:1 trans, and linolelaidate for 18:2 trans). The occurrence of 16 trans 18:1, which elutes between both the recognized 18:1 cis peak, is an exception to the grouping measurement. These methods can be used to report total 18:1 trans, total 18:2 trans, and total trans fatty acids. Study released in Food Chemistry backs up this approach to the identification.

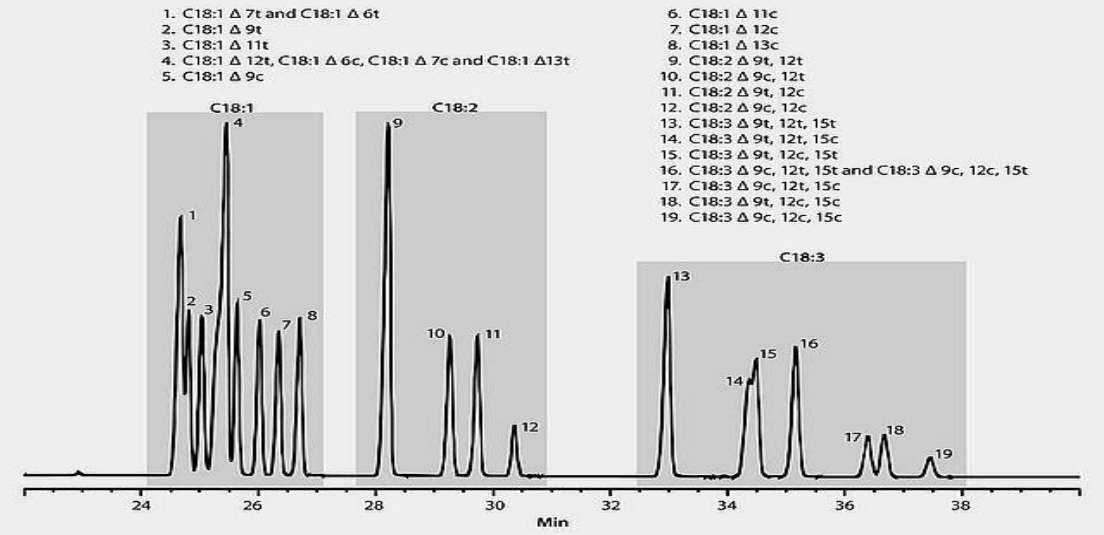

Figure 12- The cis and trans isomers of 18:1, 18:2 and 18:3

Chemicals

- Methanol- for methylation
- Toluene- as a catalyst
- Boron trifluoride – for esterification
- Hexane
- Distilled water

AOAC Official Method 996.06

Fat (Total, Saturated and unsaturated) in Food Fat and fatty acids are extracted from food by hydrolytic methods. Pyrogallic acid is added to minimize oxidative degradation of fatty acids during analysis. Triglyceride, triundecanoin (C11:0), is added as internal standard. Fat is extracted into ether, then methylated to fatty acid methyl esters (FAMEs) using BF3 in methanol. FAMEs are quantitatively measured by capillary gas chromatography (GC) against C11:0 internal standards.
Total fat is calculated as sum of individual fatty acids expressed as triglyceride equivalents. Saturated and monounsaturated fats are calculated as sum of respected fatty acids.

APPARATUS AND MATERIALS

i. Glass Beaker – 100ml, 500ml
ii. Weighing Balance
iii. Micropipette - 100µl-1000µl
iv. Micropipette-20µl-200µl
v. Micropipette tips
vi. Water Bath with the temperature display
vii. Test tube
viii. Heating Mantle
ix. Thermometer
x. 10.Vial (if auto sampler is used)

REAGENTS

i. Methane – methylation
ii. Toluene – as a catalyst
iii. Boron Triflorate– ester form
iv. Hexane – volatile dissolve
v. Water – impurity settle down

FAME PREPERATION METHODS

Fatty Acid Methyl Esters (FAME) is the procedure of fatty acid profile sample preparation for **Gas Chromatography**. The FAME preparation methods are based on (**AOAC-996.06**) International standard.

REAGENT USED

In FAME preparation methods we use 14% methanolic BF3 (Boron Trifluoride), Methanol & Toluene in 1:1:1 ratio i.e., if we want to make 60ml M.T.B (Methanol, Boron Trifluoride, Toluene) so we mix in 20:20 ratio. In this reagent we use BF3 for esterification of oil, Methanol for methylation of oils and Toluene as a catalyst.

PROCEDURE:

- Take 200 mg oil sample in test tubes.
- Add 2ml MTB (Methyl, Toluene, and BF3) in ratio of1:1:1.
- Then the sample is kept in heater bath at 60°-70° C for 1hours.
- At 15 min interval, have to shake well.
- After completing 1hr, the sample is kept at room temperature for 5-10min.
- Add 1ml hexane and 1ml water in sample.
- Vortex it to shake well.
- Take 1ml sample from upper layer of sample in vial/Eppendorf with the help of micro pipette.
- Inject 10 Micro Litre in GC.
- Analysis of fatty acids by graph.
- Calculate SFA, MUFA, PUFA & *Trans*.

PRECAUTIONS

i. Glassware should be properly cleaned and rinsed with the solvent.
ii. BF3 is a carcinogen, use mask to avoid inhaling it directly.
iii. While shaking of separating funnel, release air pressure to avoid bumping and loss of sample.
iv. Potassium hydroxide is extremely caustic and can cause severe burns, therefore protect skin and eyes while performing the test.
v. Use fume removal device (spot extractors) to remove flammable vapors produced.

WATER BATH

For Our sample preparation we need a water bath at **65℃**. So, we make it on Heating Mantle with Beaker and filled half with water. In this way we make our water bath on heating mantle.

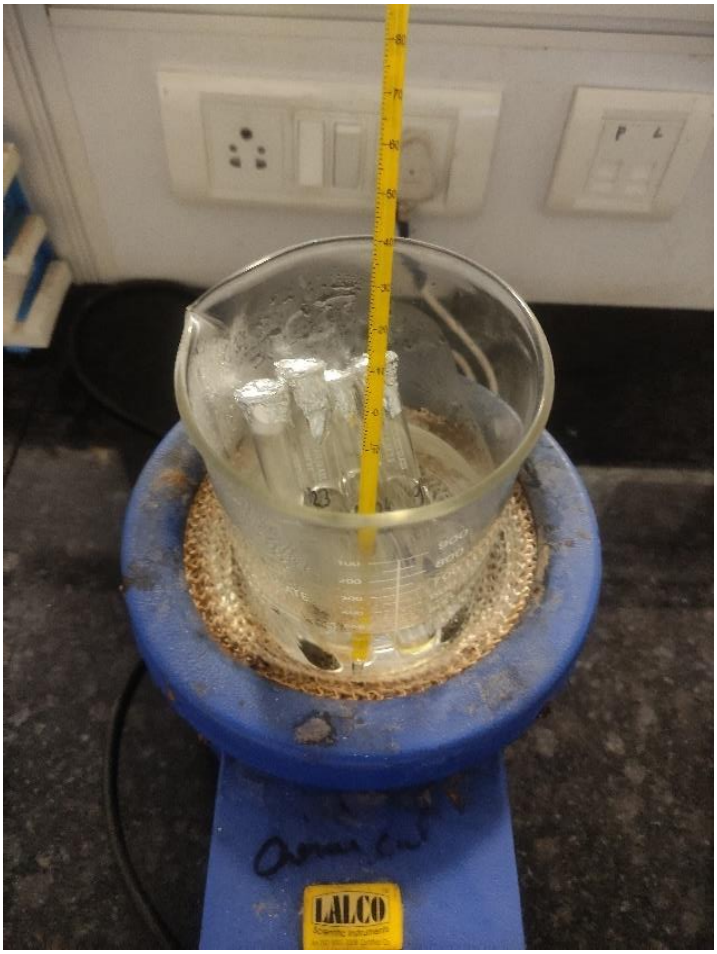

Fig.13 – WATER BATH

THERMOMETER

General purpose laboratory glass **thermometers Mercury** liquid filling Packaged in square plastic tubes for storage Vertical numbers for easy reading Permanent markings, numbers and lines Partial immersion. **Thermometers** are designed to be used with laboratory equipment. Calibrated against NIST standards to assure accuracy and reproducibility.

Fig.14 – THERMOMETER

VIALS

A **vial** is a small glass bottle, often used to store medication as liquids, powders or capsules. They can be used as scientific sample vessels; for instance, in autosampler devices in analytical chromatography. There are different types of vials such as a single dose vial and multi-dose vials often used for medications. The single dose vial is only used once whereas a multi-dose vial can be used more than once. The CDC sets specific guidelines on multi-dose vials.

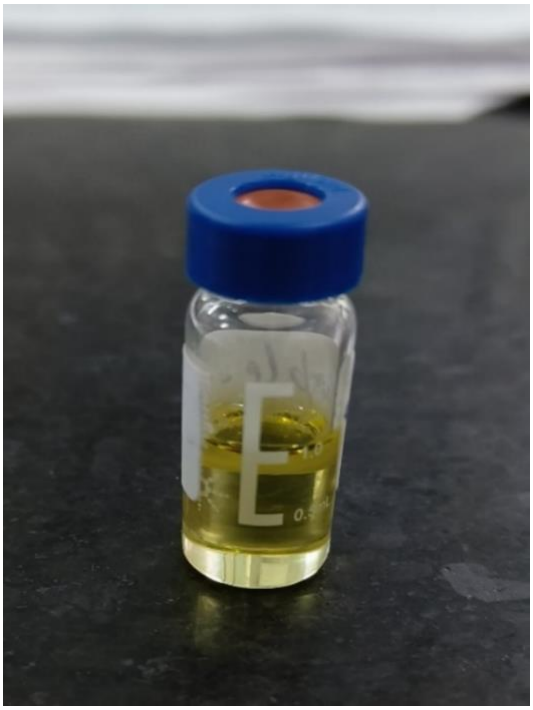

FIG.15 - VIALS

WEIGHING BALANCE

A weighing balance is an instrument which is used to determine the weight or mass of an object. Available in a wide range of sizes with multiple weighing capacities they are essential tools in laboratories.

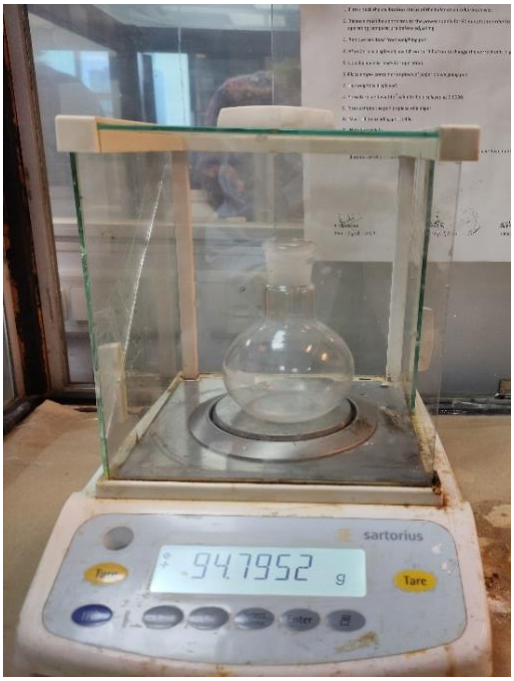

Fig.16 – WEIGHING BALANCE

VORTEX

Vortex mixers are one of the primary technologies for mixing laboratory samples in test tubes, well plates, or flasks. They use a fairly simple mechanism to agitate samples and encourage reactions or homogenization with high degrees of pre*ci*sion. Motorized drive shafts beneath the sample platform oscillate rapidly and *trans*fer orbital motion to sample containers loaded into the mixer. This causes sample fluids to circulate and undergo turbulent flow, otherwise known as a vortex.

Fig.17– VORTEX

INSTRUMENTATION

AIM: To study the gas chromatograph of different samples.

WORKING PRINCIPLE:

1 µL of sample is injected by a hypodermic needle through a self-sealing silicon rubber septum into a heated metal block into the head of the column. The temperature of the sample port is such that the sample is rapidly vaporized without decomposing the sample. The carrier gas entering the sample injector sweeps off the vaporized sample and passes down the temperature programmed column.

GC PROGRAMMING

GC system with FID detector and packed inlet fixed with capillary column SUPELCO-2560 having the dimension 100m length,0.250mm diameter and 0.20mm inner diameter. 1ml/min Nitrogen used as carrier gas. While the inlet temperature is kept 230 ℃ and detector temperature is 240 ℃.

OVEN PROGRAMMING

Keep the oven temperature at 140 ℃ for 5min raise to 240 ℃ at the rate of 4 ℃ /min for 15minutes.

Figure 18: Example of a typical gas chromatograph with insets showing the heated injection ports—note the symbol indicating that it is hot—and the oven containing the column. This particular instrument is equipped with an autosampler for injecting samples, a capillary column, and a mass spectrometer (MS) as the detector. Note that the carrier gas is supplied by a tank of compressed gas.

RESULT AND DISCUSSION

RESULT:

CALCULATION

- **Saturated Fatty Acid**- Sum of all saturates without double bonds
- **Monounsaturated Fatty Acid**- Sum of all fatty acid with one cis double bond
- **Polyunsaturated fatty acid** - Sum of all fatty acid with two & more than two cis double bonds.
- **Trans Fatty Acids**- Unsaturated fatty acid with one or more double bond in the trans configuration.

- **SFA (%wt.) =** $\frac{\textbf{Area \%} * \textbf{Fat \%}}{\textbf{100}}$
- **MUFA (%wt.) =** $\frac{\textbf{Area \%} * \textbf{Fat \%}}{\textbf{100}}$
- **PUFA (%wt.) =** $\frac{\textbf{Area \%} * \textbf{Fat \%}}{\textbf{100}}$
- **Trans fat (%wt.) =** $\frac{\textbf{Area \%} * \textbf{Fat \%}}{\textbf{100}}$

Analysis of Fatty Acid profile of Different Vanaspati Sample

Fatty acid	Vanaspati Sample 1 20210826-003-004	Vanaspati Sample 2 20210726-002-004	Vanaspati Sample 3 20210726-008-015
Lauric(C12:0)	0.515	0.294	
Myristic(C14:0)	1.306	1.297	1.162
Palmitic(C16:0)	46.114	53.604	46.426
Palmitoleic(C16:1)	0.152		
Stearic(C18:0)	4.443	4.555	7.356
Oleic(C18:1)	35.688	31.429	33.332
Linoleic(C18:2)	6.737	7.249	8.728
Linolenic(C18:3)	0.223	0.335	0.312
Aracidic(C20:0)	0.5		
Eladic(C18:1) Trans	3.231	0.344	2.271
C9 T12 Linolenic trans	0.819	0.434	0.212
T9 C12 Linolenic trans	0.723	0.459	0.2
SFA	52.43	59.75	54.94
MUFA	35.84	31.43	33.33
PUFA	6.96	7.58	9.04
TRANS	4.77	1.24	2.68
TOTAL	100.00	100.00	100.00

DISCUSSION

Within the different vanaspati samples, there was a greater difference in the FA component's material, which might be different due to variances between the origins of vegetable oils and the degree to which they have been hydrogenated.

For specific purposes, Vanaspati samples were divided into three major categories depending on their palmitic and stearic acid levels, which was investigated by a number of researchers. Here we categories the different vanaspati sample based on their trans fatty acid (C18:1 9T Elaidic, c-9, t-12 linoleice (C18:2), t-9, c12 linoleic (C18:2)). In vanaspati sample A we observed that trans is 2.049, in vanaspati sample B, trans is 0.47 and in bakery margarine, trans is 0.044.

According to the FSSAI, the TFA of edible oil cannot exceed 3%, which is effective only until 2021, after which the restrictions will drop to 2%. As a result, the first vanaspati sample has more than 2% TFA, while the other two samples contain less than 2% TFA.

As a result, vanaspati will be allowed on the market for the first time in 2021, after which they would become unhealthy, according to the FSSAI. But other two vanaspati sample B and bakery margarine have less % of TFA 2.049,0.044% respectively, so these types of vanaspati will be allowed on the market in 2022 as well.

Because TFA are unhealthy, we should limit our intake of them in our diet.
Low fat and oil consumption (less than 20% of energy ingestion) Vitamin E deficiency is more likely as a result of this and essential fatty acid deficit, both of which can cause changes in HDL and triglycerides. Usage of trans fats has been connected to the following risks to human health.
Cardiovascular diseases Breast cancer, Colon cancer, Diabetes, Obesity.

SIGNIFICANCE

Vanaspati, a form of vegetable ghee that is a Partially Hydrogenated Vegetable Oil (PHVO). Vanaspati has an important role in our edible oil economy. Its production is about 1.2 million tons annually. It has around 10% share of the edible oil market. Newer oils like soyabean, sunflower, rice bran and cottonseed and oils from oilseeds of tree and forest origin had found their way to the edible pool largely through vanaspati route.

VANASPATI PROVIDES ENERGY**,** The Energy value is the number of calories which body obtains from the foods. Vanaspati ghee is high in calories. One tablespoon of vanaspati ghee contains 122.4 calories as compared to 85.6 calories in desi ghee.

All the vanaspati brands were manufactured from PALM/ PALMOLEIN OILS, as declared by them. Remember to check the nutrition facts label on packaged food items for their trans-fat content.

CONCLUSION

Many baked items employ hydrogenated vegetable oils to improve taste and texture. Furthermore, these oils are more stable and resistant to oxidation, which occurs when fats are exposed to heat and causes them to break down.

Because of their unique properties, trans fatty acids provide various advantages for processed foods. Trans fatty acids have been related to the development of a a variety of health problems, notably cardiovascular disease, fetal and new-born brain development and growth, childhood infections, and so on, due to their different structures. Zero- and low-trans fats are becoming more popular among food manufacturers, and their consumption is increasing. However, removing all TFA elimination would be detrimental to one's health because it would eliminate nutrition trans fats like vaccenic acid. Calcium, protein, and iron are all difficult to obtain from plants or other sources, are ample in dairy animal products like meat and dairy. The restriction of these foods will hurt the general public, with the worst implications for babies, for survival and prosperity, they require a diversity of fatty acids. To reduce transfat intake, four different measures are required. Providers of healthcare, for example, could educate their patients on ways to limit transfat consumption. Consumers should be aware of trans fat-containing products and avoid them. Alternative fats should be used in preparing food and manufacture by eateries producers, and local, state, and governmental institutions should support these attempts by enacting enacted regulations prohibits the use of trans fats. These methods should aid in the reduction of trans fatty acids ingestion, which will have considerable health benefits. Furthermore, more research is required to determine the health-related the results of new TFA- free items, as replacing TFA with goods that are just as dangerous, if not worse, is impractical.

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