A DISSERTATION ON

ANALYSIS OF FATTY ACID IN DIFFERENT FOOD PRODUCTS

(BAKERY AND CONFECTIONERY PRODUCTS)

SUBMITTED TO THE

DEPARTMENT OF BIOSCIENCES

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT

FOR THE

DEGREE OF MASTER OF SCIENCE

IN BIOTECHNOLOGY

BY

MD MUNSIF

M. Sc. Biotechnology (IV Semester)

Department of Biosciences

Integral University, Lucknow

UNDER THE SUPERVISION OF

Mr. Bhushan Dole

Head of Department

Gas Chromatography

Fare Lab Pvt. Ltd., Gurgaon (Haryana)

INTEGRAL UNIVERSITY

Established Under U.P. Act No 09 of 2004 by state Legislation
Approved by University Grants Commission
Phone No.: +91(0552)2890812,2890730,3296117,6451039
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

TO WHOM IT MAY CONCERN

This is to certify that **Mr. MD MUNSIF** a student of M.Sc. Biotechnology (IV Semester), Integral University has completed his four months dissertation works entitled **"ANALYSIS OF FATTY ACID IN DIFFERENT FOOD PRODUCTS (BAKERY AND CONFECTIONERY PRODUCTS)"** successfully. He has completed this work from the FARE Lab Pvt. Ltd., Gurgaon under the supervision of **MR. BHUSHAN DOLE**. The dissertation was a compulsory part for the award of his M.Sc. Degree in Biotechnology.

I wish him bright and a great future.

Dr. SNOBER S.MIR

HEAD OF THE DEPARTMENT

DEPARTMENT OF BIOSCIENCES

FARELABS
FOOD ANALYSIS & RESEARCH LABORATORY
TESTING, CALIBRATION, PROFICIENCY TESTING, R&D & TRAINING SERVICES

FARE LABS Private Limited
L-17/3, DLF Phase-II, IFFCO Chowk, M.G. Road,
Gurgaon-122002, Haryana, INDIA
Phone : +91-124-4223207, 4034205
Fax : +91-124-4036038, Cell : +91-95992 21227
E-mail : farelabs@farelabs.com
Website : www.farelabs.com

31st May 2022

TRAINING CERTIFICATE

This is to certify that **Mr. Md Munsif,** S/o Mr. Fakhre Alam from **Integral University Lucknow, MSc Biotechnology**, has successfully completed his dissertation on **"Fatty acids & trans-fatty analysis in bakery and confectionery products."** from 31st 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.

FARE LABS PVT. LTD. L-17/3 DLF Ph-II GURGAON

Kanishka Sharma
HR Department
FARE Labs Pvt. Ltd

DECLARATION

I, **MD MUNSIF**, certify that the work embodied in the training report **"ANALYSIS OF FATTY ACID IN DIFFERENT FOOD PRODUCTS (BAKERY AND CONFECTIONERY PRODUCTS)"** to be submitted to the Master of Science in Biotechnology of Integral University, Lucknow, Uttar Pradesh, India is original and is 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.

MD MUNSIF

ACKNOWLEDGEMENT

The submission of my Master's thesis gave me immense pleasure, satisfaction and unique sense of accomplishment. I convey my humble gratitude to Allah and then my parents and my other family members for their prayers, love, care and sacrifices for educating and preparing me for my future. They have made this thesis a reality by having faith in me and by instilling in me the courage to strive and achieve higher goals in life. With the help, counselling and support of many people, this report has finally completed. At this point I would like to express our sincere gratitude to **Dr. Meenakshi Tripathi (Quality Manager)** for the great supervision, for the patience she always had despite her many other duties and responsibilities, and for providing good working place.

I would like to acknowledge my profound and heartfelt gratitude and sincere thanks to my guide **Mr. Bhushan Dole (Head of Gas Chromatography Department),** FARE Labs Private Limited for his invaluable advice, continued guidance, constructive encouragement and sound support throughout the period of this research project work. And for providing me an opportunity to work on this research topic and providing facilities during the work. His erudition and rich experience shaped the course of the research and sharpened its outcome. I am sincerely thankful to him for his able guidance and pain taking effort in improving my understanding of this project.

I would like to thank **Mr. D. Mathur (Director), Mr. C. S. Joshi (Director),** FARE Labs Pvt. Ltd. for letting me take experience in Gas Chromatography Department and learn Quality and Fatty Acid Profile Analysis of various Food Products.

With immense gratitude I would thank to Integral University for giving me this opportunity to work in such a professional laboratory and of course our HOD **Dr Snober S. Mir** and course coordinator **Dr Mohd Salman Khan** for providing all facilities and helping me out whenever I required.

I gratefully acknowledge to **Dr. Lata Rana, Mr. Kunal Prakash, Mr. Rohit Malik, Akshita Arya** and **Mr. Sanjeev Sharma** who inspired and encouraged me during various steps of my work although they were busy with their daily tasks but always were available to answer my questions and clear all my doubts.

I would like to express my deep sense of gratitude to my friend Raunak for a constant source of support as a pillar throughout my work. I would extend special thanks to my friend Sugathri, Renuka, Kritika, Shabnam and my brother Pankaj, Rajesh for their kind cooperation and generous care during this research project.

Thanking everyone

Md Munsif

ABBREVIATION'S LIST

GC – Gas chromatography

FA – Fatty acid

SFA – Saturated Fatty acid

EFA – Essential Fatty acid

UFA – Unsaturated Fatty acid

TFA – Trans Fatty acid

FID – Flame Ionization detector

ECD – Electron Capture detector

MUFA – Monounsaturated Fatty acid

PUFA – Polyunsaturated Fatty acid

TCD – Thermal conductivity detector

AOAC – ASSOCIATION OF OFFICIAL ANALYTICAL COLLABORATION

MTB – Methanol, Toluene, Bromine trifluoride

TABLE OF CONTENT

ABSTRACT

Adverse health effects from the consumption of trans fatty acids (TFA) have led to efforts to decrease the consumption of foods containing these lipids. There is a need for up-to-date information on TFA levels in foods to support decision-making by regulators on labelling and health claims. Products were analyzed for fatty acid composition by using GC. Results showed that the amount of TFA, SFA, MUFA and PUFA varied considerably among the analyzed samples. Among the products analyzed, Aloo Tikki Burger showed highest fat content, coconut candy showed highest SFA content 93.469, aloo tikki burger showed highest UFA content (27%) and plane rusk showed highest TFA. The major trans forms in all samples were elaidic acid and Linoleic (C18:2, trans). Stringent regulations are required for reducing TFA content of commonly consumed foods in India.

CHAPTER I

1.INTRODUCTION

1.1 BAKERY AND CONFECTIONERY PRODUCTS

Bakery and confectionery are both food-related store products. Bakery and confectionery are highly appreciated worldwide. Due to consumer demand a wide diversity of this type of food is available all over the world (Albuquerque et al., 2017). The major distinction between a bakery and a confectionery is that a bakery sells baked goods, whereas a confectionery sells sweet goods (K Sievert et, al.2019). The bakery's offerings aren't all sweet. Similarly, not all of the confectionery's sweets are baked.

Bakery products are among the most consumed products in the world. Among them, cakes are popular and associated by the consumers as tasty products with particular sensory characteristics (Matsakidou et al., 2010). A bakery is a business or shop that makes and sells baked goods made from flour. Bakeries serve bread, bagels, buns, cakes, pastries, pies, cookies, muffins, pizza, brownies, and other baked goods (W Gisslenet.al. 2021). As you can see from the list above, bakeries make and sell both sweet and savory foods. Unlike confectionery, bakeries produce a wide range of foods. Food for main meals (bread, buns, bagels), desserts (cakes and pastries), and snacks (cookies and brownies) are available. A baker is an individual who owns and operates a bakery.

A confectionary is a store where sweets are sold. Confectionery refers to the sweets or candies made in a candy store (confectionery). The fundamental distinction between a bakery and a confectionery is that the latter sells only sweets. A bakery not only makes and sells sweet baked goods, but it also sells unsweetened foods (AF Soareset.al. 2021).

Confectionery can be divided into two categories: bakers' confections and sugar confections. Sugar confections are sweet, sugar-based treats commonly consumed as snacks. Sweet confections include things like candies, chocolates, and chewing gum. Sweet baked goods, particularly those served as sweets, are included in baker's confections. Cakes, pastries, donuts, and other baked goods fall into this group (AK Naiket.al. 2015).

1.2 OILS and FATS

Oils and fats are essential components of a balanced diet. They are glycerol esters having three fatty acids in their structure (called either triacylglycerols or triglycerides) (DB Min et.al. 2010). Fats have functionality thanks to these fatty acids. Saturated, cis-monounsaturated, cis-polyunsaturated, and trans fatty acids are the four primary kinds

chemically. Saturated and trans fatty acids are solid at normal temperature, but cis-unsaturated fatty acids are liquid. Although no naturally occurring fat is 100 percent saturated or unsaturated (but rather a mixture of the two), fats are frequently referred to as ‘saturated' or 'unsaturated' due to the prevalence of one type of fatty acid over the other (JJ DiNicolantonioet.al. 2016).

Various groups of food goods will have different requirements in terms of product functionality. Bakery items (such as pastries and biscuits) require a fat that has a moderate level of solid fat (25-40%) during dough production in order to provide a light texture without excessive oil exudation in the finished product (G Talbot et.al. 2016). From a legal and functional standpoint, chocolate must be made with cocoa butter, and any fats used to substitute it must likewise comply with the law and melt and crystallise in the same way as cocoa butter does (AG Marangoniet.al. 2020). Although the terms 'oils' and 'fats' are frequently interchanged, they are normally used to distinguish triglycerides in the liquid state (oils) from those in the solid state (fats) (WC Ellefsonet.al. 2010).

They are frequently made from vegetable (palm oil, rapeseed oil, soyabean oil, olive oil, cocoa butter, etc.) or animal (pig lard, beef tallow, fish oils, etc.) sources, as well as animal milk fats (TV Akhilaet.al. 2021).

Vanaspati, butter, bakery & shortening, and other solid fats are solid at room temperature. Solid fats are primarily derived from animal foods, but they can also be made from vegetable oils through a process known as hydrogenation (A Gupta et.al. 2018).

Fats and oils are non-volatile in nature. These are also 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 (BGA Fahmyet.al. 2015)

1.3 FATTY ACID PROFILE

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 is 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 (G Hoffmann et.al. 2013). The fatty acids (FAs) composition of food is very important because lipids are one of the three major constituents of food. Their roles in biological tissues are: (1) source of energy, (2) components of biological membranes, (3) precursor for many different molecules and (4) transport vehicle for vitamin A, D, E and K. The com-position of fatty acids plays an important role in the transport of substances in and out of the cell because of their impact on the fluidity of the cell membrane (Chow, 2000; O'Keefe et.al. 2000).

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 (JK Virtanen et.al. 2014). 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 (T Fitzsimmons et.al. 2016). 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 (RK Saini et.al. 2018). Physicians should counsel cases about the importance of avoiding hydrogenated oils and foods containing trans fats because of their association with coronary heart disease patients in observational studies.

1.3.1 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) (G Talbot et.al. 2016). Saturated fat is solid at room temperature, which is why it is also known as "solid fat." (L Eyreset.al. 2012).
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) (AF Ogoriet.al. 2020).

Molecular Formula: $C_{12}H_{24}O_2$

Chemical Structure of Lauric Acid is:

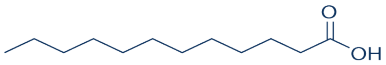


Figure 1:- Chemical structure of lauric acid

1.3.2 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)

1.3.2.1 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 (AF Ogoriet.al. 2020).

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 (DB Konuskanet.al. 2019).

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

Chemical structure of oleic acid is:

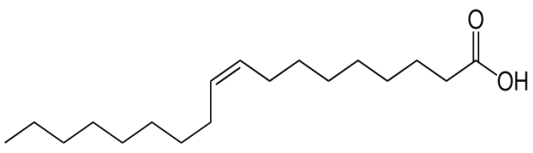


Figure 2:- Chemical structure of oleic acid

1.3.2.2 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 (RD Lanjekaret.al. 2016).

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) (L Senilaet.al. 2020).

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

Chemical structure of linoleic acid is:

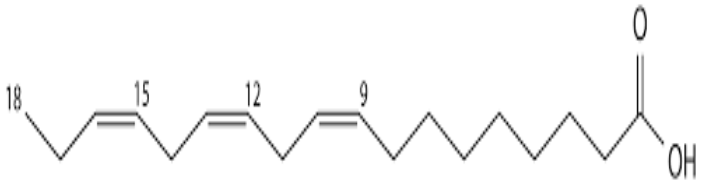


Figure 3:- Chemical structure of linoleic acid

1.3.2.3 TRANS FATTY ACIDS (TFA)

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 (N Aldaiet.al. 2013).

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 (CA Monteiro et.al. 2019). 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).

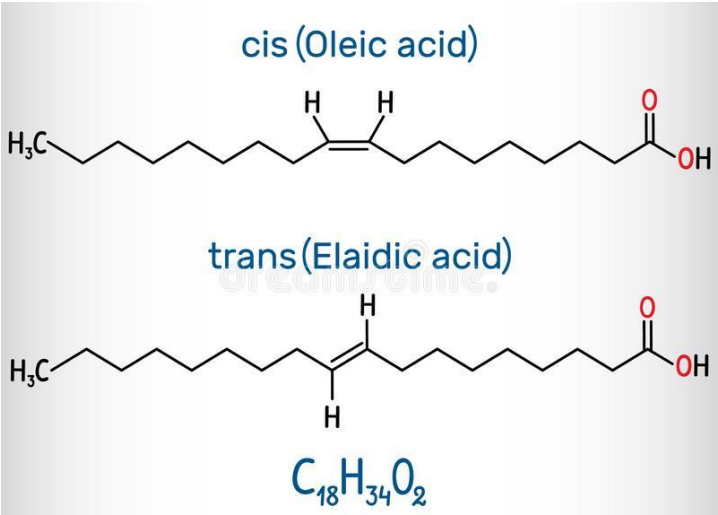
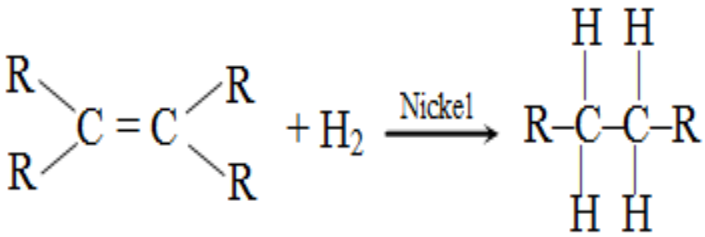


Figure 4:- Chemical structure of Trans of oleic acid

1.3.3 HYDROGENATION

The process was initially developed to make some of the unsaturated fatty acids in vegetable oils and animal fats more resistant to oxidation. The unsaturated double bonds in the fatty acids of the oil molecules react with hydrogen atoms in the presence of a catalyst in this process (as shown in the below reaction). In the commercial hydrogenation of edible oils, nickel catalyst is used. The process was initially developed to make some of the unsaturated fatty acids in vegetable oils and animal fats more resistant to oxidation (F Menaaet.al. 2013). The unsaturated double bonds in the fatty acids of the oil molecules react with hydrogen atoms in the presence of a catalyst in this process (as shown in the below reaction). Nickel



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 (M Cheet.al. 2013).

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.

1.3.4 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**

Eicosapentaenoic (EPA) (C20:5n3)
Docosahexaneoic (DHA) (C22:6n3)
Alpha-Linolenic (ALA) (C18:3n3)

- **OMEGA-6**

Linoleic Acid (C18:2n6)

- **OMEGA-9**

Oleic Acid (C18:1n9)
Erucic (C22:1n9)

1.4 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 (SK Jeburet.al. 2018).
There are three components of GC, these are:

- Injector
- Oven
- Detector

Also, there are two phases in GC:

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

Nitrogen, Hydrogen, and Zero Air are the gases used in GC. Nitrogen enters the column and uses as a carrier gas. The fuel gas is hydrogen, while zero air is used for ignition and contains 20% O2 (RB Raja et.al. 2022)

The mobile phase in gas chromatography is a carrier gas, which is typically an inert gas such as helium or an unreactive gas such as nitrogen. Although nitrogen is preferred for improved separations, helium remains the most commonly used carrier gas in approximately 90% of instruments. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support contained within a column of glass or metal tubing. A gas chromatograph is the instrument used to perform gas chromatography (M Sithersinghet.al. 2018).
The gaseous compounds being studied interact with the column's walls, which are coated with a stationary phase. This causes each compound to elute at a different time, which is known as the compound's retention time. The comparison of retention times is what makes GC useful for analysis (NF Obianaghaet.al. 2021).
The compounds in a mixture are separated using a liquid stationary phase and a gas mobile phase, with the column through which the gas phase passes 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 determined by the gas's vapour pressure. Because both processes separate the components of a mixture primarily based on boiling point differences, gas chromatography is similar to fractional distillation (H Laajimiet.al. 2022).

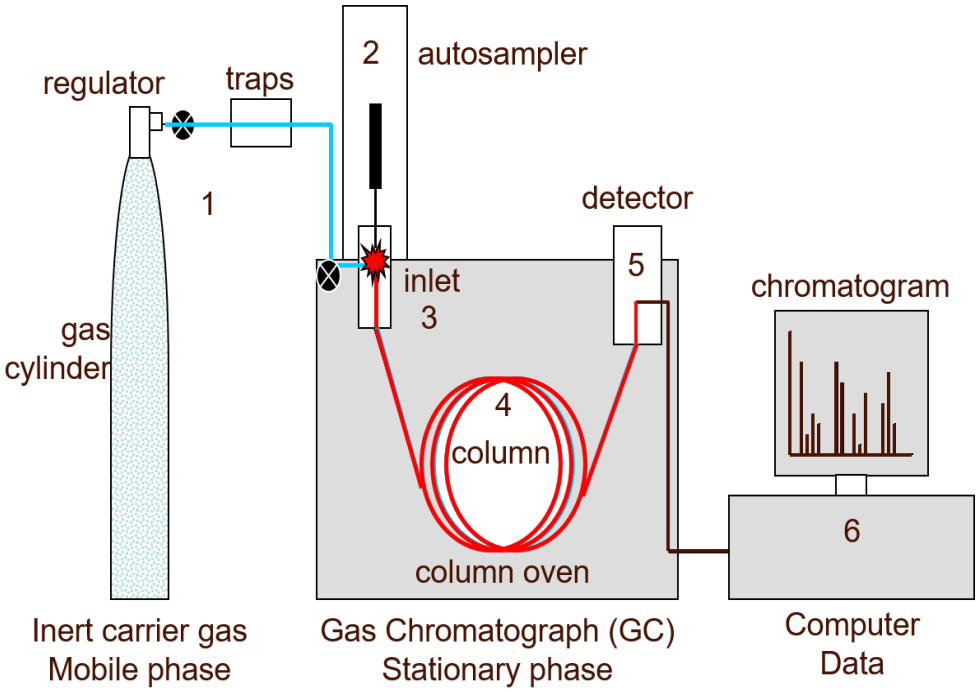


Figure 5. Diagram of Gas chromatography instrument

1.5. SAFETY PRECAUTIONS:

1.5.1 General Safety: General laboratory precautions should be followed: protective gloves, laboratory coats, and safety glasses must be worn at all times during this method's steps.

1.5.2 Chemical Hazards: All acids and bases, reagents, and organic solvents used in this analysis must be handled with extreme caution. These chemicals/reagents are toxic and/or flammable and should only be handled in well-ventilated areas or under a fume hood as needed. Before handling chemicals and reagents, obtain and review safety information such as Safety Data Sheets.

Diethyl ether: Extremely flammable and volatile. Use extreme caution when handling. The eyes and respiratory tract are irritated. Diethyl ether can be used to de-fat the skin. Under the influence of light and air, diethyl ether can form explosive peroxides. Keep away from direct sunlight and heat. Only use inside a fume hood. Keep in a tightly sealed container in a cool, dark place (preferably a refrigerator) away from light, moisture, and air.

Hexane: Eye, respiratory, and skin irritation. Hexane is a flammable and hazardous substance. Avoid eyes and skin contact. Keep the container in a flammable cabinet.

Toluene: Irritating to the eyes, respiratory system, and skin. Hazardous and flammable so avoid skin and eye contact. Keep the container in a cool and well-ventilated place.

Chloroform: Chloroform is extremely toxic and may cause cancer in humans. Handle with caution. Avoid coming into contact with your eyes and skin. Long-term exposure to chloroform may cause liver and kidney damage. When large amounts of chloroform come into contact with the skin they can cause sores. Only use inside a fume hood.

Boron trifluoride-Methanol Reagent (BF3): Avoid coming into contact with your eyes and skin. If consumed its toxic.

Petrolium ether: Flammable and vapor.

OBJECTIVES:

1.Analysis of fatty acid for bakery and confectionery Food products by using Gas Chromatography.

2. Analysis of Trans fat for Bakery and Confectionery Food products by using Gas Chromatography.

CHAPTER II

2. REVIEW OF LITERATURE

2.1 Bakery products

Fats used in bakery products, for example biscuits and pastry, need to have a certain level of solid fat present at the temperature at which the dough is mixed in order to give enough structure to hold a light aerated structure and to stop more liquid triglycerides from separating from the baked end product. Biscuit and pastry doughs are often mixed at about 25°C, which is close to the ambient temperature in many bakeries. At this temperature, the dough fat used needs to, ideally, contain between 25% and 40% solid fat. Higher solid fat levels make the dough difficult to mix; lower solid fat levels risk some of the liquid fat exuding from the final biscuit or pastry making it oily to the touch.

Historically, animal fats such as lard and beef tallow were used in many of these applications and, it has to be said, lard makes excellent pastry, largely because of the form in which it crystallizes. However, these were phased out partly for ethical and religious reasons and partly because these kinds of animal fats were considered to be 'unhealthy' due to high levels of saturated fat and, therefore, high risks of cardiovascular diseases. This is quite ironic when one considers that they were replaced by partially hydrogenated fats with high levels of *Trans* fatty acids which were subsequently found to have an even worse effect on cardiovascular disease risks. As with all hydrogenated fats, these two animal fats have now been almost completely removed from bakery products and have largely been replaced by either palm oil (Atkinson, 2011) or blends of palm oil and its fractions or with oils such as rapeseed oil. To an extent, considering the history of these changes, this is also quite ironic because palm oil contains about 50% saturates which is higher than the levels of saturates typically found in lard and beef tallow!

Bread, particularly factory produced bread, has a very specific requirement for a small amount (typically about 3%) of a very high melting fat to be present. This crystallizes around the bubbles formed during the time the bread dough is proving and rising and forms a crystal monolayer around these bubbles (Brooker, 1996). Because of the high melting point of the fat used it can retain its structure during the early stages of baking and so holds the aerated structure of the bread.

2.2 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.

LIST OF FATTY ACID PROFILE IN FOOD SAMPLES

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		

2.3 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.

2.3.1 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.

2.3.2 INJECTOR

The injector can be used in one of two modes; split or splitless. 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).

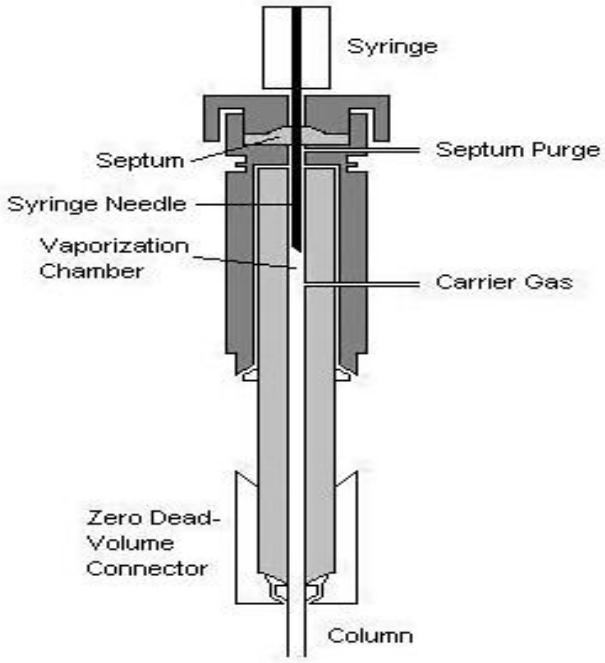


Figure 6 :- A cross-sectional view of a micro flash vaporizer direct injector

2.3.3 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.

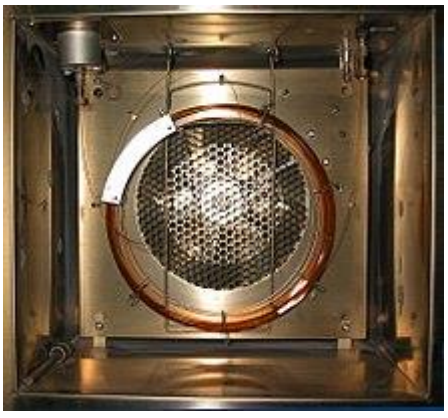


Figure 7. Column and Oven

2.3.4 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:

i. Flame Ionization Detector (FID)
ii. Nitrogen Phosphorus Detector (NPD)
iii. Electron Capture Detector (ECD)
iv. Thermal Conductivity Detector (TCD)

2.3.4.1 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.

2.3.4.2 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

2.3.4.3 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

2.3.4.4 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°

CHAPTER: III

3. METHODOLOGY

Fat extraction from food is a necessary procedure in the food industry in terms of product formulation. This is because all food labels are required to report both the saturated and unsaturated fat content of their products. In addition, consumers would also intend to determine the contents of their food, especially in terms of fat. The fat extraction experiment is a way to quantify and compare label values to experimental values for fat. Fats are commonly defined as a broad category of non-polar molecules that are sparingly soluble or insoluble in water, but soluble in benzene, chloroform, hexane, methanol, petroleum ether and diethyl ether.

A method for the quantitative measurement of total fat in foodstuffs is described. Fat is extracted by hydrolysis and inter-esterified to fatty acid methyl esters for gas chromatographic analysis. Because of commercial regulations, it is important for food producers to be able to report fat/lipids content in their products. It is also important, in several industries, to closely monitor the fat content since it affects the quality or value of the product. To be able to determine the amount of fatty acids present in fat, it is often necessary to first extract it from the material they are bound to. Fat extraction is a very useful tool for preparative purposes in which the analyte is concentrated from the matrix as a whole or separated from particular sample. Methods for extraction of fat from various food samples have been developed for decades using a wide variety of techniques. The total fat content of a food is commonly determined by its organic solvent extract. Estimations of the fat content of food are generally based on the weight of the fraction extracted by a solvent. The choice of solvents is highly critical for total fat analysis. Non-polar solvents are especially important in fat extraction given that triglycerides are non-polar molecules. The method chosen for extraction of fat depends on several factors including the types of fat in the sample (bound or unbound fat), the rest composition of the sample, cost, time constraints, and desired accuracy. The most commonly used fat extraction methods are:

- Acid Digestion Method
- Soxhlet Method
- Cold Method

3.1 Extraction of Fat

- Vegetable Oils, Cooking Oils, Ghee, and Hydrogenated Fats: - Since these are 100% pure oils/fats, there is no need to extract fat for esterification.

- In case of Margarine, other spreads, Sugar based food products, Bakery & confectionary Products fat is extracted prior to esterification.

3.1.1 REAGENTS

i. Concentrated Hydrochloric Acid
ii. Petroleum Ether (40-60 ℃)
iii. Ethanol (99.999%)
iv. Distilled Water
v. Methyl Orange Indicator

3.1.2 GC CONDITIONS

i. Column: Supelco 2560 (100m x 0.25mm x 0.20µm)
ii. Oven Temperature: 90 °C-230°C
iii. Flame Ionization Detector-260°C
iv. Flow Rate: 1.0 ml/min
v. Injection Volume: 1.0 µl
vi. Mobile phase: Carrier Gas- Nitrogen (99.999%)

3.1.3 PREPARATION OF REAGENTS

14% BF3: Take 7 ml of boron trifluoride solution in 50 ml volumetric flask and make up the volume with 43 ml methanol.

3.1.4 APPARATUS AND MATERIALS

i. Glass Beaker – 100ml, 500ml
ii. Glass Rod
iii. Weighing Balance
iv. Micropipette - 100µl-1000µl
v. Micropipette - 20µl-200µl
vi. Micropipette tips
vii. Water Bath with the temperature display
viii. Hot Plate
ix. Separating Funnel – 500ml
x. Flat Bottom Flask – 250ml
xi. Filter Paper
xii. Soxhlet Assembly
xiii. Hot Air Oven
xiv. Centrifuge
xv. Tarson Tube (Centrifuge Tube) – 50ml
xvi. Test tube

xvii. Heating Mantle
xviii. Thermometer
xix. Eppendrop
xx. Vial (if auto sampler is used)

3.2 Chemicals

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

3.3 ACID DIGESTION

Acid digestion is a method of dissolving sample into solution, by adding acids and heating, until the complete decomposition of the matrix. The acid hydrolysis method is applicable to baked products and pet foods, and facilitates the extraction of fatty acids from glycerides, that might otherwise be left un-extracted due to covalent and ionic bonding commonly bound to these fractions can be extracted. When the crude fat analysis value is lower than expected, especially for any animal food product that has been heat processed or containing ingredients that have been heat processed, acid hydrolysis should be considered as the method of choice.. The addition of hydrochloric acid breaks covalent and ionic bonds of lipids to proteins and carbohydrates, so that the lipids.

3.3.1 REAGENTS

i. Concentrated Hydrochloric Acid (For digestion)
ii. Petroleum Ether (40-60℃)
iii. Diethyl Ether
iv. Ethanol (99.999%) (For layer separation)
v. Distilled Water
vi. Methyl Orange Indicator

3.3.2 PROCEDURE

i. Take 5 gm of homogenized sample in a beaker.
ii. Add 20 ml hot distilled water and 10 ml concentrated HCl to it.
iii. Mix it well using a glass rod.

iv. Heat on hot plate at 60 ℃ until a separate colorless layer of fat appears on the top.
v. Cool the beaker at room temperature.
vi. Weigh a clean and dry flat bottom flask and note down the empty weight.
vii. Transfer the digested sample to separating funnel add 30 ml ethanol and 30 ml distilled water and shake properly.
viii. After shaking add 50 ml of petroleum ether and shake.
ix. Bottom layer is collected in the beaker and upper layer is collected in a flat bottom flask.
x. Pour the bottom layer in the separating funnel again, and repeat the extraction for 4 times.
xi. After last extraction, transfer the sample from flat bottom to the separating funnel to start washing with water.
xii. Add 30 ml distilled water and shake properly to neutralize till free from acid.
xiii. Discard the bottom layer and wash the upper layer with water for 5 times (repeat the previous step).
xiv. Confirm the water washings is acid free, using methyl orange indicator.
xv. Collect the bottom layer in the flat bottom flask.
xvi. Evaporate the solvent on water bath at 98℃.
xvii. Put the sample in oven at 105 ℃.
xviii. Cool down the flask and note down the weight.
xix. Repeat until the weight is constant.
xx. Note down the final weight.
xxi. Collect the extracted fat.

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.

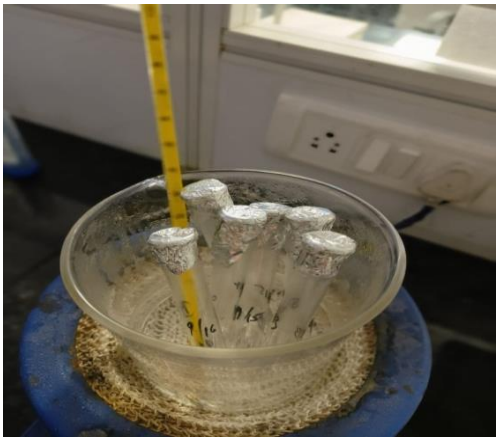


Fig.11 **–** WATER BATH

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.12**–**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.13 – 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.

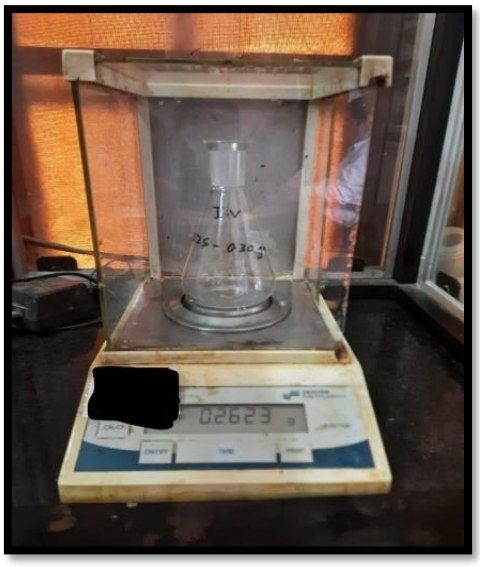


Fig.14 **–** WEIGHING BALANCE

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*cis*ion. 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.15– VORTEX

3.3.1 ASSEMBLY

1. The source material containing the compound to be extracted is placed inside the thimble.
2. The thimble is loaded into the main chamber of the Soxhlet extractor.
3. The extraction solvent to be used is placed in a distillation flask.
4. The flask is placed on the heating element.
5. The Soxhlet extractor is placed atop the flask.
6. A reflux condenser is placed atop the extractor.

3.3.2 OPERATION

The solvent is heated to reflux. The solvent vapor travels up a distillation arm, and floods into the chamber having thimble containing the sample. The condenser ensures that any solvent vapor cools, and drips back down into the extraction chamber. The chamber containing the sample slowly fills with warm solvent. Some of the fat dissolves in the warm solvent. When the Soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. The thimble

ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours or days.

3.3.3REAGENTS

i. Petroleum Ether (40-60 ℃)
ii. Diethyl Ether

3.3.4 PROCEDURE

i. Weigh a clean and dry flat bottom flask and note down the empty weight.
ii. Take 5 gm sample in a thimble.
iii. Add 2ml TAG standard to it using a micropipette.
iv. Plug the thimble using cotton.
v. Put the thimble in the Soxhlet Apparatus.
vi. Add the petroleum ether to the flask & connect the Soxhlet apparatus
vii. Reflux at 60-80 ℃ for 16 hours.
viii. Disconnect the Soxhlet assembly.
ix. Evaporate the solvent on water bath at 98℃.
x. Put the sample in oven at 105 ℃.
xi. Cool down the flask and note down the weight.
xii. Repeat until the weight is constant.
xiii. Note down the final weight.
xiv. Collect the extracted fat.

3.4 SOXHLET METHOD

Soxhlet Extraction is a Continuous solid/liquid extraction. Soxhlet Method is required where the desired compound has a limited solubility in a solvent. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

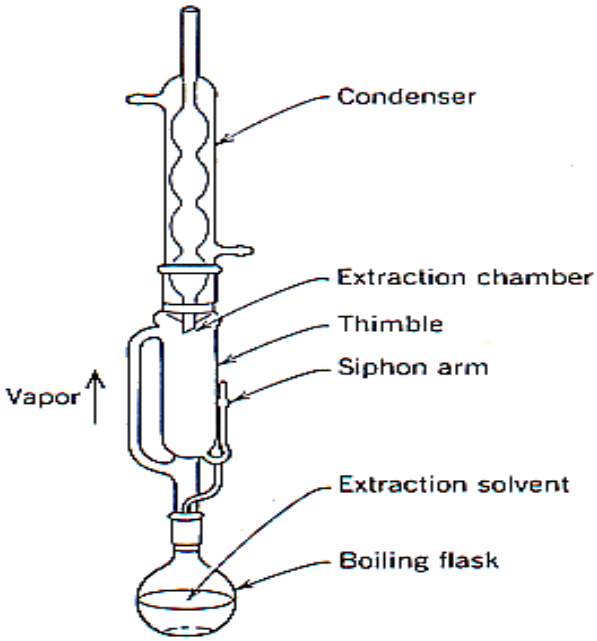


Figure 8. Diagram of Soxhlet

A Soxhlet extractor has three main sections:

A percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a siphon mechanism, which periodically empties the thimble.

3.5 COLD METHOD

To prevent the degradation/decomposition of polyunsaturated fatty acids due to prolong heating and to prevent generation of Trans fatty acids, lipid extraction should be done cold. A “new-method” for cold lipid extraction has been developed, which uses low toxicity solvents like petroleum ether employing the new method, which uses petroleum ether thrice the amount of sample containing fat, fat is extracted from the sample and the mono-and polyunsaturated fatty acids do not suffer degradation. Although, cold method is less preferred for determination of total fat present in the sample, but can be used to determine the fatty acid profile.

3.5.1 REAGENTS

i. Methanol(methylation)
ii. Toluene (used as catalyst)
iii. Boron trifluoride (BF3-used as esterification)
iv. Petroleum ether (fat extract)
v. Hexane
vi. Distilled water

3.5.2 PROCEDURE

i. Take 10-15 gm of sample in a centrifuge tube.
ii. Add petroleum ether (40-60 ℃) thrice the volume of sample taken.
iii. Shake well for 5 minutes.
iv. Place the sample for 15 minutes at 2000 rpm in centrifuge. The upper layer of petroleum ether will have dissolved fat while the residue will get settled down as bottom layer.
v. Take out the upper layer in a 100 ml beaker.
vi. Put the beaker on the water bath at 60 ℃ so that the ether gets evaporated and we get fat sample.

3.6 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, triundecanoic (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 C21:0 internal standard. 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.

3.7 Methylation of Extracted Fat

3.7.1 Preparation

i. Take 200 mg of extracted fat/oil sample in test tube and add 2 ml toluene and 2ml of 14% BF3.
ii. Seal the mouth of the test tube using cotton plug so that the toluene doesn't evaporate.
iii. Shake the test tube properly and place it in the water bath at 100 ℃ for 45 minutes.
iv. Shake the sample test tube in intervals of 15 minutes.
v. Take out the test tube from the water bath, and allow to cool to room temperature.
vi. Add 1g Na_2SO_4, 5ml distilled water and 2ml hexane to it.
vii. Vortex until two clearly distinct transparent layers appear.

viii. Transfer the 1ml of upper layer to the vial using a micropipette.

ix. Analyze the above esterified sample immediately on GC.

3.8 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.

3.9 INSTRUMENTATION

3.9.1 AIM: To study the gas chromatograph of different samples.

3.9.2 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.

3.9.3 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 ℃.

3.9.4 OVEN PROGRAMMING

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

Figure 7: Example of a typical gas chromatograph

CHAPTER- IV

4. RESULT AND DISCUSSION

4.1 CALCULATION

For Food Samples

- **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 bond
- **Trans Fatty Acids**- Unsaturated fatty acid with one or more double bond in the trans configuration

- **SFA (%wt.) = Area%*Fat%**
- **MUFA (%wt.) = Area%*Fat%**
- **PUFA (%wt.) = Area%*Fat%**
- **Trans fat (%wt.) = Area%*Fat%**

4.2 Analysis of Fatty Acid profile of Food Samples

Fatty Acid Profile of cookies		
Fatty acid	**Choco Cookies**	**Butter Cookies**
Butyric(C4:0)	4.869	0.5
Caproic(C6:0)	0.299	0.309
Caprylic(C8:0)	1.171	0.109
Capric(C10:0)	2.033	0.189
Lauric(C12:0)	2.313	0.897
Myristic(C14:0)	9.426	1.187
myristioleic(C14:1)	0.906	ND
Pentadecanoic(C15:0)	1.012	ND
Cis-10 Pentadecanoic(C15:1)	0.219	ND
Palmitic(C16:0)	35.134	47.204
palmitoleic(C16:1)	0.108	0.083
Heptadecanoic(C17:0)	0.844	ND
cis-10 Heptadecanoic(C17:1)	0.622	ND
Stearic(C18:0)	0.259	5.521
Elaidic(C18:1,trans)	ND(DL-0.005)	2.053
Oleic(C18:1)	14.488	35.129
C-9,T-12 Linoleic(C18:2,trans)	0.834	0.131
T-9,C-12 Linoleic(C18:2,trans)	0.334	0.256
Linoleic(C18:2)	21.494	6.286
Aracidic(C20:0)	3.398	0.062
Linolenic(C18:3)	0.237	0.084
SFA	60.758	55.978
MUFA	16.343	37.265
PUFA	22.899	6.757
TRANS	1.168	2.44
FAT%	28	19
SFA g/100	1.7	1.06
MUFA g/100	0.46	0.71
PUFA g/100	0.64	0.13
TRANS g/100	0.03	0.05

Fatty Acid Profile of Cake		
Fatty acid	**Dry fruit Cake**	**Butterscotch Cake**
Caprylic(C8:0)	ND(DL-0.005)	0.076
Capric(C10:0)	ND(DL-0.005)	0.108
Lauric(C12:0)	0.152	0.52
Myristic(C14:0)	0.336	1.46
Palmitic(C16:0)	17.951	45.41
palmitoleic(C16:1)	0.309	0.137
Stearic(C18:0)	6.937	8.572
Oleic(C18:1)	66.696	32.88
C-9,T-12 Linoleic(C18:2,trans)	ND(DL-0.005)	0.127
T-9,C-12 Linoleic(C18:2,trans)	ND(DL-0.005)	0.124
Linoleic(C18:2)	5.747	9.931
Aracidic(C20:0)	0.45	0.283
Linolenic(C18:3)	0.311	0.372
Eicosenoic(C20:1)	0.939	ND
Behenic(C22:0)	0.172	ND
SFA	25.998	56.429
MUFA	67.944	33.017
PUFA	6.058	10.554
TRANS	ND(DL-0.005)	0.251
FAT%	9	6.50
SFA g/100	2.34	3.67
MUFA g/100	6.11	2.15
PUFA g/100	0.55	0.69
TRANS g/100	ND(DL-0.005)	0.02

Fatty Acid Profile of Rusk		
Fatty acid	**Sesame Rusk**	**Plain Rusk**
Lauric(C12:0)	0.188	0.191
Myristic(C14:0)	1.068	0.957
Palmitic(C16:0)	45.711	49.526
palmitoleic(C16:1)	0.183	0.077
Stearic(C18:0)	4.658	4.59
Elaidic(C18:1,trans)	2.823	3.036
Oleic(C18:1)	33.968	33.025
C-9,T-12 Linoleic(C18:2,trans)	0.6	0.599
T-9,C-12 Linoleic(C18:2,trans)	0.913	0.78
Linoleic(C18:2)	9.208	6.922
Aracidic(C20:0)	0.356	0.118
Linolenic(C18:3)	0.153	0.179
Behenic(C22:0)	0.127	ND
Lignoceric(C24:0)	0.044	ND
SFA	52.152	55.382
MUFA	36.974	36.138
PUFA	10.874	8.48
TRANS	4.336	10.738
FAT%	14	7.00
SFA g/100	0.73	0.39
MUFA g/100	0.52	0.25
PUFA g/100	0.15	0.06
TRANS g/100	0.06	0.08

Fatty Acid Profile of Candies		
Fatty acid	**Coconut Candy**	**TuttiFruiti Candy**
Butyric(C4:0)	0.181	ND
Caproic(C6:0)	0.687	ND
Caprylic(C8:0)	8.975	ND
Capric(C10:0)	6.133	ND
Lauric(C12:0)	49.035	0.687
Myristic(C14:0)	18.327	1.244
Palmitic(C16:0)	7.523	45.296
palmitoleic(C16:1)	ND(DL-0.005)	0.092
Stearic(C18:0)	2.557	14.603
Elaidic(C18:1,trans)	ND(DL-0.005)	ND
Oleic(C18:1)	5.407	27.56
Linoleic(C18:2)	1.093	9.839
Aracidic(C20:0)	0.051	0.342
Linolenic(C18:3)	0.031	0.224
Eicosenoic(C20:1)	ND(DL-0.005)	0.113
SFA	93.469	62.172
MUFA	5.407	27.765
PUFA	1.124	10.063
FAT%	9.30	2.30
SFA g/100	8.69	1.43
MUFA g/100	0.5	0.64
PUFA g/100	0.1	0.23

Fatty Acid Profile of Bread		
Fatty acid	**Multi Grain Bread**	**Garlic Bread**
Capric(C10:0)	ND(DL-0.005)	0.112
Lauric(C12:0)	0.353	0.225
Myristic(C14:0)	0.491	0.752
Palmitic(C16:0)	22.789	15.965
palmitoleic(C16:1)	ND(DL-0.005)	0.178
Stearic(C18:0)	4.121	6.001
Elaidic(C18:1,trans)	ND(DL-0.005)	0.224
Oleic(C18:1)	35.698	24.585
C-9,T-12 Linoleic(C18:2,trans)	0.614	0.848
T-9,C-12 Linoleic(C18:2,trans)	0.8	0.8
Linoleic(C18:2)	31.612	45.35
Aracidic(C20:0)	2.313	0.699
Eicosenoic(C20:1)	ND(DL-0.005)	1.043
Linolenic(C18:3)	1.209	2.89
Behenic(C22:0)		0.328
SFA	30.067	24.082
MUFA	35.698	26.03
PUFA	34.235	49.888
TRANS	1.414	1.872
FAT%	3.1	17
SFA g/100	0.93	4.09
MUFA g/100	1.11	4.43
PUFA g/100	1.06	8.48
TRANS g/100	0.04	0.32

Fatty Acid Profile of Burger and Pizza		
Fatty acid	**AalooTikki Burger**	**Veg Pizza**
Butyric(C4:0)	ND(DL-0.005)	2.765
Caproic(C6:0)	ND(DL-0.005)	1.742
Caprylic(C8:0)	ND(DL-0.005)	1.37
Capric(C10:0)	0.124	2.362
Lauric(C12:0)	0.17	6.735
Myristic(C14:0)	1.018	10.423
myristioleic(C14:1)	ND(DL-0.005)	1.163
Pentadecanoic(C15:0)	ND(DL-0.005)	1.042
Cis-10 Pentadecanoic(C15:1)	ND(DL-0.005)	0.219
Palmitic(C16:0)	14.527	29.967
palmitoleic(C16:1)	0.131	0.782
Heptadecanoic(C17:0)	ND(DL-0.005)	0.502
cis-10 Heptadecanoic(C17:1)	ND(DL-0.005)	0.226
Stearic(C18:0)	5.375	7.687
Elaidic(C18:1,trans)	ND(DL-0.005)	1.172
Oleic(C18:1)	21.504	19.658
C-9,T-12 Linoleic(C18:2,trans)	0.35	0.045
T-9,C-12 Linoleic(C18:2,trans)	0.354	ND(DL-0.005)
Linoleic(C18:2)	51.559	10.788
Aracidic(C20:0)	0.842	0.157
Eicosenoic(C20:0)	0.412	1.014
Linolenic(C18:3)	4.652	0.181
SFA	21.45	65.766
MUFA	21.635	23.22
PUFA	56.915	11.014
TRANS	0.704	1.217
FAT%	34.4	13.4
SFA g/100	7.38	8.81
MUFA g/100	7.44	3.11
PUFA g/100	19.58	1.48
TRANS g/100	0.24	0.16

DISCUSSION

Various kind of food products including cookies, bread, cake, rusk, candy, burger, pizza were analyzed for total fat, trans fat and complete fatty acid composition. Among the total food products 12 were samples.

Total fat content of food products:

The total fat (g/100 g food) contents of 12 samples are given in the Table 1, 2, 3 and 4, respectively. Total fat content in the branded samples analyzed was in the order: Aloo Tikki burger > choco cookies > butter cookies > garlic bread > sesame rusk > veg pizza > coconut candy > Dry fruit cake > Plain rusk > Butterscotch cake > multi grain bread > Tuttifruiti candy. The type of fatty acids present in any food product will attain significance with respect to health implications, only if the total fat content is higher.

The higher the fat content, intakes will be more, and simultaneously, the presence of unhealthy fatty acids like SFA and TFA will attain significance with respect to pre-disposing to heart diseases. Foods that are high in trans or saturated fatty acids are associated with an increased risk of cardiovascular disease and diabetes (Mozaffarian et al., 2006)

Saturated fatty acid content in various related food products:

The above table shows saturated fatty acid content of various food products. The SFA content of the 12 food products in the ranged from 0.051% to 49.526%. The highest amount of saturated fat (49.526%) was found in plain rusk while it was lowest (0.051%) in coconut candy. The predominant fatty acids present in these items which had high fat content as well as high SFA was palmitic acid, which ranged from 7.523 to 49.526%. The highest amount of palmitic acid was found in plain rusk while it was lowest in coconut candy. Stearic acid (C18:0) was the second dominant saturated fatty acid present in all food samples. The highest amount of stearic acid was found in tuttifruiti candy (14.603%) while it was lowest in coconut candy (2.557%). Other SFA like butyric acid (C4:0), caprylic acid (C6:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), arachidic acid (C20:0) and behenic acid (C22:0) were also determined in lower concentrations in other foods.

The dominant fatty acid among the saturated group was palmitic acid (C16:0), which ranged from 7.523% in coconut candy to 49.26% in trans-esterified fat. In unbranded food items also, palmitic acid was the predominant fatty acid. This indicates that a greater amount of fats rich in palmitic acid is used in food preparation or due to repeated reuse of oil for frying. Reheating of oils increases SFA and may be the reason for higher SFA in food samples.

Unsaturated fatty acid content of several food products:

The total amount of unsaturated fat in food samples ranged from 6.5% in coconut candy to 74% in Dry fruit cake. The types of fatty acids present in food items attains significance only if the total fat content in the foods is present in higher amounts like in, garlic bread, plain rusk, butter cookies, coconut candy, butterscotch cake, veg pizza and dry fruit cake. In these food items, the total unsaturated fatty acid content ranged from 6.53 to 74%. Among all these 12 food items, the predominant unsaturated fatty acid was oleic acid which ranged from 5.4% in coconut candy to 66.696% in dry fruit cake. Linoleic acid, which is an n-3 PUFA, was the other fatty acid present in amounts ranging from0.031 in coconut candy to 4.652% in aloo tikka. In food products with fat content higher than 14%, around 10% of linoleic acid was present. Among the above food items, total UFA ranged from 34.234% in veg pizza to 74.002% in Dry fruit cake. Cis-10 Pentadecanoic(C15:1) was detected only in veg pizza (0.219%) also cis-10 Heptadecanoic(C17:1) was detected only in veg pizza (0.226%).

Among these products, those with fat content more than 14% and UFA content more than 40% could be of significance in terms of their beneficial effects on health. Among the MUFA, oleic acid was the major fatty acid present. The amount of oleic acid ranged from 5.407% in coconut candy to 66.696% in dry fruit cake. Linoleic acid content was also significantly high ranging from 31.612 to 51.559% in fried items like multi grain bread, garlic bread and aloo Tikki burger among samples.

Trans fatty acid content of different food products:

In bakery samples the amount of total TFA ranged from 0.05 in veg pizza to 3.036% in plain rusk. Total TFA was higher in plain rusk (3.036%), sesame rusk (2.823%), among the food products having higher fat content. Trans fatty acids are high as observed in rusks when compared to other products. The severe difference was found in trans fatty acids as they were found from 0.02 to 0.32 g/100g.

The TFA content assumes significance in terms of their ill effects on the health of consumers, only if fat content is also high. Hence, consumption of the above listed products might prove to be harmful if consumed in large amounts and at higher frequencies. The major TFA observed in all samples was elaidic acid (C18:1 trans-9) and C-9,T-12 Linoleic(C18:2,trans) in the range of 0.045- 0.6%. Out of 12 samples, three samples contained elaidic acid (C18:1 trans) at more than 1% (1.172, 2.823, 3.036%), one samples had less than 1% and two samples did not contain any TFA. C-9,T-12 Linoleic(C18:2,trans) was present in seven sample at less than 1% (0.127, 0.045, 0.599, 0.6, 0.35, 0.614, 0.848).T-9,C-12 Linoleic(C18:2,trans) was present in two samples (multi grain bread and garlic bread) at0.8% and four samples has less than 0.8% (0.124, 0.913, 0.78, 0.354). Two samples did not contain any TFA.

CHAPTER: V

CONCLUSION

The above study was carried on the bakery products for detailed analysis on fatty acid composition in various related products.
The practical and analytical work done during me thesis submission reveals various profiling including saturated, monounsaturated, polyunsaturated and trans fatty acids. Trans fatty acids are high as observed in rusks when compared to other products.
The range of saturated were from 0.051 to 49.526. The range of monounsaturated were from 0.077 to 66.696. Similarly, the range of polyunsaturated were found to be from 0.181 to 51.559. The severe difference was found in trans fatty acids as they were found from 0.02 to 0.32 g/100g.
As per FSSR the Trans percentage is 2% of food fat.

Comparatively gas chromatography is more sensitive and efficient with respect to determination of fatty acid methyl ester of edible oil, bakery products, confectionery as compare to other forms of chromatography.

The four types have different chemical structures and physical properties. The bad fats, saturated and Transfats, tend to be more solid at room temperature (like a stick of butter), while monounsaturated and polyunsaturated fats tend to be more liquid (like liquid vegetable oil).
Fats can also have different effects on the cholesterol levels in your body. The bad fats, saturated fats and Trans fats raise bad cholesterol (LDL) levels in your blood. Monounsaturated fats and polyunsaturated fats can lower bad cholesterol levels and are beneficial when consumed as part of a healthy dietary pattern.

As we analyze the sample of bakery products and confectionery products in the GC. we found the trans-fat limit of ranging from 0.5% to 2 % which is in the limit of FSSAI. As a result, the products will be allowed on the market. If the limit is more than 2% than it is not allow for market.

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.

REFERENCES

- Atkinson G (2011). Saturated fat reduction in biscuits' in *Reducing Saturated Fats in Foods* ed Talbot G. Woodhead Publishing, Cambridge.

- Brooker BE (1996). The Role of Fat in the Stabilization of Gas Cells in Bread Dough. J. Cereal Sci. 24(3), 187-198.

- AOAC Official Method 969.33, "Fatty acids in food samples and fats Preparation of Methyl Esters Boron Trifluoride Method".

- AOAC Official Method 996.06, "Fat (Total Saturated and Unsaturated) in Foods. Hydrolytic Gas extraction gas Chromatographic method".

- AOAC Official Method 994.10, "Fat (Cholesterol) in Foods. Hydrolytic Gas extraction gas Chromatographic method.

- Bhanger MI and Anwar F., "Fatty acid (FA) composition and contents of trans unsaturated FA in milk and dairy products" 2004.

- Pavia, Donald L., Gary M. Lampman, George S. Kritz, Randall G. Engel (2006). Introduction to organic Laboratory Techniques (4th Ed.). Thomson Brooks/Cole.pp. 797-817.

- Albuquerque, T. G., Santos, J., Silva, M. A., Oliveira, M., & Costa, H. S. (2017). Multivariate characterization of salt and fat content, and the fatty acid profile of pastry and bakery products. *Food & function*, *8*(11), 4170–4178.

- Matsakidou, A., Blekas, G., &Paraskevopoulou, A. (2010). Aroma and physic characteristics of cakes prepared by replacing margarine with extra virgen olive oil. *Food Science and Technology, 43*, 949-957.

- Chen, Y., Yang, Y., Nie, S., Yang, X., Wang, Y., Yang, M., … Xie, M. (2014). The analysis of trans fatty acid profiles in deep frying palm oil and chicken fillets with an improved gas chromatography method. *Food Control*, *44*, 191–197.

- Kohn, A., & Mitchell, B. (2006) Lipid Technol. 18, 279–282

- Aggarwal A, Gupta R, Varma K, Mathur B. High trans fatty acid content in common Indian fast foods. *Nutr Food Sci.* 2008;38(6):564–569. doi:
  .
- Chow, C. K. (2000). Fatty acid in foods and their health implications (2nd ed.). NewYork: Marcel Dekker.
- O'Keefe, S. F. (2002). Nomenclature and classification of lipids. In C. C. Akoh, D. B. Min (Eds.), Food lipids: chemistry, nutrition and biotechnology (2nd ed.). New York: Marcel Dekker.
- alonso, l., m.j. fraga, m. juarez, and p. carmona, fatty acid composition of spanish shortenings with special emphasis on trans unsaturation content as determined by fourier transform infrared spectroscopy and gas chromatography, 79:1–6 (2002).
- bayard, c.c., and r.l. wolff, trans-18:1 acids in french tub margarine and shortenings: recent trends, 72:1485–1489 (1995).
- aro, a., j.m. antoine, l. pizzoferrato, o. reykdal, and g. van poppel, trans fatty acids in dairy and meat products from 14 european countries: the transfair study, j. food compos. anal. 11:130–160 (1998).
- alonso, l., m.j. fraga, and m. juarez, determination of trans fatty acids and fatty acid profile in margarines marketed in spain, j. am. oil chem. soc. 77:31–136 (2000).
- Soares, A.F., Honorio, A.R., de Lima, D.C.N. and Tribst, A.A., 2021. Sweet processed foods in Brazil: use of sugar and sweeteners, inclusion of sugar claims and impact on nutritional profile. International Journal of Food Science & Technology, 56(9), pp.4428-4433.
- Sievert, K., Lawrence, M., Naika, A. and Baker, P., 2019. Processed foods and nutrition transition in the Pacific: Regional trends, patterns and food system drivers. Nutrients, 11(6), p.1328.
- Gisslen, W. (2021). Professional baking. John Wiley & Sons.
- Naik, A. K. (2015). Empirical Study on Packaging Factor Influencing Consumer Buying Decision in Confectionery Item (Doctoral dissertation).
- Min, D. B., &Ellefson, W. C. (2010). Fat analysis. In Food analysis (pp. 117-132). Springer, Boston, MA.
- DiNicolantonio, J. J., Lucan, S. C., & O'Keefe, J. H. (2016). The evidence for saturated fat and for sugar related to coronary heart disease. Progress in cardiovascular diseases, 58(5), 464-472.

- Talbot, G. (2016). Developing food products for customers with low fat and low saturated fat requirements: processed foods. In Developing Food Products for Consumers with Specific Dietary Needs (pp. 129-154). Woodhead Publishing.
- Marangoni, A. G., Van Duynhoven, J. P., Acevedo, N. C., Nicholson, R. A., & Patel, A. R. (2020). Advances in our understanding of the structure and functionality of edible fats and fat mimetics. Soft Matter, 16(2), 289-306.
- Akhila, T. V., &Swarnalatha, A. (2021). A Study on Fats and Oils Consumption Pattern Among Selected Households in Kerala. International Journal of Recent Advances in Multidisciplinary Topics, 2(4), 164-167.
- Gupta, A., Sharma, R., Sharma, S., & Singh, B. (2018). Oilseed as potential functional food Ingredient. Trends & Prospects in Food Technology, Processing and Preservation, 1st ed.; Prodyut Kumar, P., Mahawar, MK, Abobatta, W., Panja, P., Eds, 25-58.
- Fahmy, B. G. (2015). Fatty acids profile in the Camel. Egypt. J. Chem. Environ. Health, 1(1), 244-273.
- Hoffmann, G. (2013). The chemistry and technology of edible oils and fats and their high fat products. Academic Press.
- Virtanen, J. K., Mursu, J., Tuomainen, T. P., &Voutilainen, S. (2014). Dietary fatty acids and risk of coronary heart disease in men: the Kuopio Ischemic Heart Disease Risk Factor Study. Arteriosclerosis, thrombosis, and vascular biology, 34(12), 2679-2687.
- Chee, B., Park, B., Fitzsimmons, T., Coates, A. M., &Bartold, P. M. (2016). Omega-3 fatty acids as an adjunct for periodontal therapy—a review. Clinical oral investigations, 20(5), 879-894.
- Saini, R. K., &Keum, Y. S. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review. Life sciences, 203, 255-267.
- Talbot, G. (2016). The stability and shelf life of fats and oils. In The stability and shelf life of food (pp. 461-503). Woodhead Publishing.
- Eyres, L. (2012). 25.10 Fats and oils. Essentials of Human Nutrition, 428.
- Ogori, A. F. (2020). Source, extraction and constituents of fats and oils. Journal of Food Science and Nutrition, 6(2), 100060.
- Konuskan, D. B., Arslan, M., &Oksuz, A. (2019). Physicochemical properties of cold pressed sunflower, peanut, rapeseed, mustard and olive oils grown in the Eastern Mediterranean region. Saudi Journal of Biological Sciences, 26(2), 340-344.
- Lanjekar, R. D., &Deshmukh, D. (2016). A review of the effect of the composition of biodiesel on NOx emission, oxidative stability and cold flow properties. Renewable and Sustainable Energy Reviews, 54, 1401-1411.
- Senila, L., Neag, E., Cadar, O., Kovacs, M. H., Becze, A., &Senila, M. (2020). Chemical, nutritional and antioxidant characteristics of different food seeds. Applied Sciences, 10(5), 1589.

- Aldai, N., de Renobales, M., Barron, L. J. R., & Kramer, J. K. (2013). What are the trans fatty acids issues in foods after discontinuation of industrially produced trans fats? Ruminant products, vegetable oils, and synthetic supplements. European Journal of Lipid Science and Technology, 115(12), 1378-1401.
- Monteiro, C. A., Cannon, G., Lawrence, M., Costa Louzada, M. D., & Pereira Machado, P. (2019). Ultra-processed foods, diet quality, and health using the NOVA classification system. Rome: FAO, 49.
- Menaa, F., Menaa, A., Tréton, J., &Menaa, B. (2013). Technological approaches to minimize industrial trans fatty acids in foods. Journal of food science, 78(3), R377-R386.
- Che, M. (2013). Nobel Prize in chemistry 1912 to Sabatier: Organic chemistry or catalysis? Catalysis today, 218, 162-171.
- Jebur, S. K. (2018). Carbon black production, analyzing and characterization. Missouri University of Science and Technology.
- Raja, R. B., Sarathi, R., &Vinu, R. (2022). Selective Production of Hydrogen and Solid Carbon via Methane Pyrolysis Using a Swirl-Induced Point–Plane Non-thermal Plasma Reactor. Energy & Fuels, 36(2), 826-836.
- Sithersingh, M. (2018). Determination of polar solvents by static headspace extraction–gas chromatography (SHE-GC).
- Obianagha, N. F., Okafor, C. J., Chukwuani, U., Ogundahunsi, O. A., Ogbonnia, S. O., Obeagu, E. I., ... & Said, O. H. (2021). Evaluation of the Phytochemical Constituents of Extracts of Kigeliaafricana Fruit and Sorghum bicolor, Stalk in Lagos Nigeria. Journal of Pharmaceutical Research International, 49-58.
- Laajimi, H., Galli, F., Patience, G. S., &Schieppati, D. (2022). Experimental methods in chemical engineering: Gas chromatography—GC. The Canadian Journal of Chemical Engineering.
- Mozaffarian D, Katan M B, Ascherio A, Stampfer M J and Willett W C 2006. Trans fatty acids and cardiovascular disease. New England Journal of Medicine, 354: 1601-1613.