SITUATIONAL ANALYSIS OF CONSUMPTION OF BAKERY FOODS AMONG UNIVERSITY RESIDENTIAL STUDENTS AND STUDY THE TRANS FAT CONTENT AND SENSORY PROPERTIES OF BAKERY PRODUCTS PREPARED WITH INTERESTERIFIED (IE) FAT

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(B.Sc. Hons. Foods and Nutrition)

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By

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A dissertation submitted in partial fulfilment of the requirements for the Degree of
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Certificate

This is to certify that the research work presented in this thesis has been carried out independently by Mr SIDDHANT NIKAM under the guidance of Dr Mini Sheth in pursuit of a Master Degree in Foods and Nutrition (DIETETICS) and this is his original work.

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*"**When you want something, all the universe conspires in helping you to achieve it"***

- SIDDHANT NIKAM

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ABSTRACT

The FSSAI initiative to eliminate industrially produced trans fat comes at a time when the burden of non-communicable diseases has risen in India and is showing an Increasing trend.

In view of paucity of data relating to trans fatty acid content of Indian bakery foods Indian , the present study was been carried out to elicit information regarding the purchasing and consumption pattern of bakery foods among the university residential students and study the sensory qualities and the trans-fat content of bakery products prepared from Regular Fat and Interesterified fat (IE).

Using a cross sectional study design, a semi structured questionnaire was administered to 160 subjects (80 males and 75 females) to elicit information with respect to Religion, Medical History, Anthropometry Parameters, Bakery foods Purchasing pattern, Knowledge Attitude and Practice Regarding Trans fat consumption. Sensory qualities of the bakery foods prepared with regular fat and Interesterified fats was carried out using 25 semi trained panellist and trans fat analysis using was done using GC technique.

The background information of the subjects revealed that their mean age of the subjects was 20.6 years and majority of them were Hindu. The medical history of students revealed that none of the subjects were obese of had diabetes. 2.4 % subjects suffered from hypertension whereas 7.7 % girls suffered from PCOS. The KAP data reveal that 58.75 % of the subjects knew about the Trans-fat and 77.5 % subjects were unaware about adverse effects of trans fat on Health. Most subjects (51.8 %) were unaware about the Difference between Natural and Industrially Produced Transfat and 65.6% of population were not aware about the PHVOS. Almost 80 % subjects did not know about the Replace Initiative taken by WHO and 86.7% of them did not know about the Interesterified Fats.

Consumption practices of bakery foods by the University residential students revealed that majority of them reported to consume Biscuits followed by Breads, Cookies at Interval an of 2-3 times a week, with female subjects consuming more than male

subjects. While a few of the subjects consumed the Biscuits on daily basis followed by Breads and Cookies.

The result on sensory evaluation of Bakery foods with respect to Colour and Appearance, Aroma, texture, taste, mouthfeel and overall acceptability showed that naan khatai and butter biscuit (Makhaniya), and cream rolls prepared from IE fats were much better than those prepared from regular fats ($p<.05$). Khari prepared from regular fat ranked higher ($P<.05$) as compared to that prepared from IE fats for all the sensory attributes. This suggests that IE fats can be used for the preparation of the four bakery foods with good sensory attributes except for Khari

The results on Trans fatty acid analysis revealed that trans fatty acid was not detected in any of the bakery products prepared from either regular fat or IE fat by the GC method when the detection limit was kept as 0.1 g per 100 g of fat. However the total fat content of all the bakery foods ranged between 40.15 g/100 gm (Butter biscuit) to 22.13 g/100 gm (Maskabun). Hence it can be concluded that although the studied bakery foods continue to remain high in fat content, they do not contain trans-fatty acid.

CHAPTER 1
INTRODUCTION

Nutrition-linked chronic disease burden has consistently been rising, particularly in the developing world due to inappropriate diet and mechanised lifestyles, and thus adding to the health-care cost. Owing to rapid transition in dietary/lifestyle practices, eating-out has become rather common which is further accelerated by the burgeoning road side vendors, restaurants, fast food joints and hotels. Majority of the commercially available snacks/fast foods are usually deep-fried containing rather high amounts of trans fatty acids (TFAs), particularly if prepared using partially hydrogenated vegetable oils (PHVO) often preferred for their low cost and better texture/longer shelf life of the food product. TFAs originate either from industrial hydrogenation of the oils (i-TFA) or through bio-hydrogenation process in ruminants (cow, sheep etc.). During partial hydrogenation of vegetable/fish oils using hydrogen gas and a metal catalyst (usually nickel), the TFAs are generated; and. reportedly, some TFAs are also formed when the fats or oils are subjected to heating/reheating for a long time. On the other hand, naturally occurring TFAs are produced in the ruminant animals by the isomerization of cis UFAs present in their feed wherein bacterial enzymes act as the catalysts. There is a mounting concern about the intake of TFA containing foods due to their deleterious health effects including non-communicable diseases (NCDs) such as cardiovascular disease (CVD), diabetes, cancer, inflammation, abnormal foetal development, hampered infant growth etc.

NON -COMMUNICABLE DIESEASES

Global health is influenced by three trends: population – ageing, rapid unplanned urbanization and globalization all of which result in a unhealthy environments and behaviours. As a result, the growing prevalence of NCDs and their risk factors has become a global issue affecting both low and middle income countries (Global status report on NCDs, 2014).

Non – communicable disease are the leading causes of death globally, almost two - thirds of all death are due to NCDs. NCDs, especially cardiovascular disease, diabetes mellitus and stroke have emerged as major public health problem in India (Mote

BN,2016). Globally NCDs cause more deaths than all other causes combined and NCDs deaths are projected to increase from 38 million in 2012 to 52 million in 2030. Four major NCDs (Cardiovascular Diseases, Cancer, chronic respiratory diseases and diabetes) are responsible for 82% of NCDs deaths (Global Status Report on NCDs 2014).

In 2005, India was reported to have experienced the " highest loss in potentially productive years of life" worldwide. In 2010, NCDs accounted for 53% of total deaths while in 2014 it raised to 60% in India. A steep rise of 7% in merely 4 years. In India, the probability of dying of dying between ages 30 – 70 years from four main NCDs is 26%. According to Non – Communicable Disease Global Survey, 1 in 4 Indians face the risk of death from NCD before they hit the age of 70 years (NCD Country Profiles 2014).

NCDs contribute to around 5.87 million deaths that account for 60% of all deaths in India. India shares more than 66% of total deaths attributed to NCDs in South East Asian region (Mote BN,2016).

TRANS FAT AND NCD.

Owing to convenience, fastness, economic viability and unique sensory attributes of fried foods, frying process is commonly employed both at the domestic and the industrial level.

Human lipase is a water-soluble enzyme which helps in the digestion, transportation and processing of dietary lipids. One of the acknowledged theory reports that while the lipase can act on cis fatty acids, it is not able to metabolize the trans isomers. As a result, TFAs remain in the bloodstream for prolonged durations and are more likely to get deposited in the arteries promoting plaque formation (Aro et al, 1997). Presence of trans fatty acids may be responsible for altered packaging of the phospholipids which might affect the membrane properties and related enzymes such as elongase, desaturase and prostaglandin synthetase (Kinsella et al, 1981). In controlled trials, consumption of trans fatty acids reduces the activity of serum paraoxonase – an enzyme closely linked with HDL-c and tissue plasminogen activator (deRoos et al, 2002; Muller et al, 2001). Mozaffarian et al (2006) have reported that compared to diets containing similar amounts of oleic/stearic/palmitic acid or the other SFAs, TFA rich diets reportedly increase plasma concentration of apolipoprotein-a (apoA).

There are mounting concerns regarding the potentially adverse health effects of TFAs. Numerous studies have reported industrial trans fat intake to be associated with various health hazards such as weight gain, inflammation, oxidative stress, cardiovascular disease, diabetes, cancer, dementia, infertility, abnormal foetal development, compromised infant growth/development etc. There is a growing body of evidence linking trans fats to heart disease; and that the effects may be much more harmful than the SFAs. Several deleterious effects of trans fat consumption have already been scientifically proven. There are several possible mechanisms whereby TFA may affect both lipid and non-lipid risk factors for cardiovascular disease. Dietary TFAs may adversely affect various hemostatic and hemato-biological properties of blood affecting the enzymatic activity and altering the composition of membrane phospholipids (Clandinin et al, 1991).

Researches indicate that even on an iso-caloric diet, trans fat may promote weight gain and abdominal fat. High levels of trans-18:2 in plasma phospholipids and erythrocyte membrane were reportedly associated with an elevated risk of fatal ischemic heart disease (IHD) and sudden cardiac death (SCD). Dietary TFAs may elevate plasma lipoprotein(a), inflammatory biomarkers and endothelial dysfunction; several studies have demonstrated elevated TFA intake to be associated with an increased risk of sudden cardiac arrest. High TFA intake.

poses detrimental effects on lipid profile – it not only raises LDL-c, TC and ApoB but also decreases HDL-c and apo-A levels (Lichtenstein et al., 2003); as a result TFAs are much more harmful than even the SFAs (Judd et al., 1994). Restrepo and Rieger (2015) demonstrated strong evidence that banning of the trans fat in restaurants can lead to a sizeable reduction in heart disease/stroke related mortality. There is a mounting concern regarding increased risk of type 2 diabetes mellitus (T2DM) associated with the trans fat consumption. TFAs can influence thrombogenesis through eicosanoid synthesis pathway and may also promote insulin resistance (Hu, Manson and Willett, 2001). Studies have reported that TFA alter gene expression associated with insulin sensitivity and T2DM risk (Poirier et al., 2006; Saravanan, 2005)

In view of the deleterious health effects of TFA, there are numerous options for reducing the TFA content of oils including modified hydrogenation process, chemical/enzymatic inter-esterification, fractionation of tropical oils, trait modification

of oils, blending of liquid and solid oil components, use of surfactants/emulsifiers, organo-gels and structured emulsions. Regulatory measures need to be accompanied by governmental support to the industry, including appropriate education and technical assistance to facilitate reformulation of oils using polyunsaturated fatty acids and monounsaturated fatty acids instead of tropical oils and animal fats. The need for using trans fat alternatives mainly emerges at the manufacturers' level for protecting community's health at large by curtailing the TFAs in their food system. In industrial frying operations, very often genetically modified vegetable oils are being used in place of PHVOs (Flickinger and Huth, 2004). Strict regulations are urgently required to be in place for monitoring oxidative degradation of unsaturated fatty acids and to curb TFA content of fried foods or the oils used for frying by adopting appropriate frying procedures. Further, it is highly imperative to establish time and temperature limits for frying operations so as to curtail trans fat content in commercially available fried foods (Wang et al., 2015)

In view of numerous adverse health effects of TFA, WHO along with the Member States have aimed at eliminating industrially produced TFA from the global food supply chain by the year 2023. WHO (2018) has therefore, planned an action package – REPLACE coupled with enactment of necessary regulations to pose strict restrictions on the amount of trans fat in fats/oils as well as the various food items. Food Safety and Standards Authority of India (FSSAI) has also launched an awareness campaign 'Heart Attack Rewind' highlighting that the trans fats consumption can result in heart attacks. Under this campaign, the masses are being educated on appropriate/proper use of edible oil since frying and repeated use of the same oil for frying increases the trans fat content in edible oils. They are also being advised for promoting safer methods of cooking like boiling, steaming, baking and grilling. Through another initiative, FSSAI has launched a trans fat free logo (TFA not exceeding 0.2g per 100 g/ml of the food product) for voluntary labelling by the food manufacturers to promote TFA-free products for attracting the health-conscious consumers. Subsidies on edible oils with healthier nutrient profiles are also being proposed to eliminate the TFA. As part of the trans fat free movement, the authorities should avoid promoting increased consumption of SFA as they are also linked to numerous health risks. Under the Food Safety and Standard Act, FSSAI aims to provide a safe, nutritious and healthy diet for school children; and thus, the foods high in fat, salt and sugar (HFSS) cannot be sold in the school canteens

or within 50 metres of the school campus or served in the mess/hostel kitchens (WHO, 2018; WHO, 2020b). Despite mounting policy implementation actions and interest towards TFA elimination, there is still a long way to attain the WHO's goal of worldwide elimination of TFA by 2023.

CHAPTER 2

REVIEW OF LITERATURE

India is a developing country with a variety of eating and living practises. As a result, in a developing country like India, the impact of NCDs is typically greater, and their mortality and morbidity rates are also higher. As a result, it is imperative to be aware of every aspect of NCDs in order to ensure the survival of the fittest (Lancet Globe Health, 2018). It has been said that "Healthy is Wealthy" and that "Life is 10% how we take it and 90% how we make it." In this kind of life, health plays a crucial role. We also need to think and act quickly in the jet age to keep up with the accelerating pace of technology. To achieve dazzling heights, man neglected his health as a result of rapid industrialization and urbanisation, and was living in a world of lifestyle diseases, often accompanied by Non-Communicable Diseases (NCDs) such as cancer, cardiovascular disease, pulmonary disease, diabetes, and mental illness, diseases that do not spread from one person to the next, but pose a significant loss on the life of mankind by disrupting the mental, physical, and economic stability.

In 2016, there were approximately 57 million deaths worldwide. NCDs were responsible for 41 million of these deaths. NCDs were responsible for 80% of deaths in low- and middle-income countries. Heart disease, stroke, cancer, chronic respiratory diseases, and diabetes are the five major NCDs (WHO,2022).

India is undergoing a rapid health transition, with an increasing burden of noncommunicable diseases (NCDs) causing significant morbidity and mortality in both rural and urban populations. India accounts for half of all deaths and 63% of total disease burden (Park's, 2021).

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. They include: 1) Coronary heart disease 2) Cerebrovascular disease 3) Peripheral arterial disease. 4) Deep vein thrombosis and pulmonary embolism 5) Rheumatic heart disease.

Cardiovascular disease (CVD) is the leading cause of death worldwide, with 17.9 million people expected to die from CVDs in 2019, accounting for 32% of all deaths worldwide. Heart failure and stroke accounted for 85% of these deaths. In 2019, 38%

of the 17 million premature deaths (age under 70) due to noncommunicable diseases were caused by CVDs (WHO, 2022).

The global prevalence of cardiovascular diseases and their risk factors is increasing. CVD cases have nearly doubled from 271 million in 1990 to 523 million in 2019, and the number of CVD deaths has nearly doubled from 12.1 million in 1990 to 18.6 million in 2019 (Global Burden of Disease 2019).

From 1990 to 2016, the India State-level Disease Burden Initiative reported overall trends in diseases, injuries, and risk factors. CVDs were responsible for 28.1% of all deaths in 2016, an increase from 15.2% in 1990. CVD prevalence was highest in states with high epidemiological transition levels, such as Kerala, Punjab, and Tamil Nadu (Sreeniwas Kumar, et al, 2020).

Coronary heart disease prevalence rates in India have increased from 1.6% to 7.4% in rural areas and from 1% to 13.2% in urban areas over the last few decades (Huffman MD et al, 2011).

The onset of the current cardiovascular disease epidemic can be linked to the industrial revolution in the seventeenth century. An increase in tobacco use, a decline in physical activity, and the adoption of a diet high in fat and cholesterol were the three factors to blame for this. The incidence of CVD kept increasing throughout the 20th century. (Eric J. Topol, Third Edition, Textbook of Cardiovascular Medicine).

According to recent studies, almost all cardiovascular risk factors are more prevalent in India than they were a few decades ago and are on the rise. The recent rise in the burden of non-communicable diseases is possibly caused by economic development, acculturation, changing dietary patterns, migration, education, change in physical activity, and change in behavioural risk factors like tobacco and alcohol use (Sreeniwas Kumar, et al, 2020).

Since these risk factors are known to be the primary contributors to chronic diseases, eliminating them would prevent at least 80% of heart disease, stroke, type 2 diabetes, and over 40% of cancer (WHO, 2022).

In order to facilitate NCD prevention and control, the Sustainable Development Goals also emphasised the need for multisectoral national policies. In addition to screening

and treating 80% of hypertensive and diabetic patients, India's national policy for 2017 aims to reduce premature CVD deaths by 25% (PAHO WHO 2022).

Early diagnosis, treatment, and management of the CVD risk factors, such as a healthy diet and exercise, will lessen the disease's toll. A healthy, balanced diet lowers the risk of NCDs by maintaining indicators of NCDs like elevated blood glucose, high blood pressure, and high body mass index at levels needed by the body.

2.1 Types of Fat

2.1.1 Monounsaturated fatty acids (MUFA)

Early diagnosis, treatment, and management of the CVD risk factors, such as a healthy diet and exercise, will lessen the disease's toll. A healthy, balanced diet lowers the risk of NCDs by maintaining indicators of NCDs like elevated blood glucose, high blood pressure, and high body mass index at levels needed by the body (Schwingshackl, L., & Hoffmann, G. (2012).

While the hydrogen atoms and the double bond are present on opposite sides in the trans-configuration, they are on the same side in the cis-configuration. The main type of MUFA found in food sources is the cis-isomers. Oleic acid (18:1 n-9) is the most prevalent cis-configured MUFA in daily nutrition, followed by palmitoleic acid (16:1 n-7) and vaccenic acid (18:1 n-7). Additionally, oleic acid is the most abundant MUFA in the diet (about 90% of all MUFAs) . Elaidic acid (trans 18:1 n-9) is the main MUFA that has been trans-configured (Schwingshackl, L., & Hoffmann, G. (2012).

Therefore, it is not surprising that the main source of MUFA depends largely on dietary habits since they are widely present in oils, nuts, seeds, fruits, and meat. MUFA are almost entirely absorbed by the intestine, just like other fatty acids, and are then either oxidised for energy production, changed into other fatty acids, or included in tissue lipids (Schwingshackl, L., & Hoffmann, G. (2012)

2.1.2 Polyunsaturated fatty acids (PUFA)

Fats are necessary for all living things. The carbon chain of fatty acid (FA) molecules is variable in length and has a methyl end and a carboxylic acid head group (1). Depending on how saturated their carbon chains are, they can be divided into different groups. Monounsaturated and polyunsaturated fatty acids (PUFAs) have one, two, or

more double bonds, whereas saturated fatty acids (FAs) have the most hydrogen atoms (Ander, B. P et al 2003).

On the basis of the first double bond's position in relation to the chain's methyl terminus, PUFAs can be further divided. For instance, the most biologically significant PUFA classes, n-3 and n-6 FAs, have their first double bonds on the third and sixth carbons, respectively, from the chain terminus. These FAs are frequently referred to as omega-3 or omega-6 PUFAs because the final carbon in the FA chain is also known as the omega carbon.

Alpha-linolenic acid (ALA) and linoleic acid, respectively, which are both essential FAs (EFAs), are used to make long-chain n-3 and n-6 PUFAs. An EFA must be obtained through dietary sources because the body is unable to produce it. EFAs can be metabolised by both animals and people into long-chain derivatives. The proportion of n-6 to n-3 PUFAs is crucial to human health because the n-6 and n-3 pathways compete with one another for enzyme activity. The metabolic production of the longer chain products from the other family will be hampered by an excess of FAs from the other family (Kaur, N et al 2014). The typical Western diet provides n-6 and n-3 PUFAs in a ratio ranging from 8:1 to 25:1 (1), values in severe contrast with the recommendations from national health agencies of approximately 4:1 (2). Lowering the n-6:n-3 ratio would reduce competition for the enzymes and facilitate the metabolism of more downstream products of ALA.

2.2 Trans fat

Different types of fats exist (Figure 1). Three fatty acids are joined to the glycerol backbone of a triglyceride molecule. These fatty acids are composed of chains with various numbers of carbon atoms joined by single or double bonds Saturated fats are fat molecules in which hydrogen atoms have taken the place of the double bonds between carbon atoms. In other words, there are four additional atoms bonded to each carbon atom.

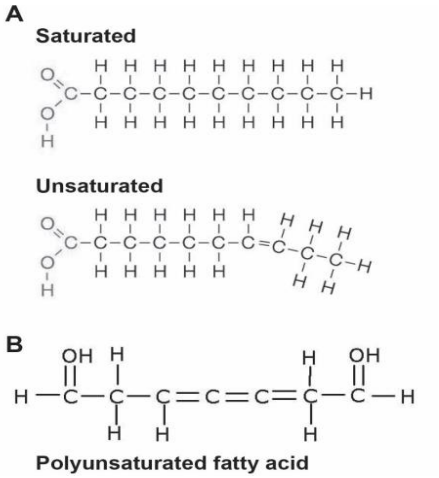


Figure 1 : chemical structures of SAFA, PUFA and MUFA.

Unsaturated fats are fat molecules that contain one or more double bonds between two carbon atoms at specific points along the chain. Unsaturated fats are classified as 'cis' or 'trans' depending on how the carbon chains are arranged across one or more double bonds (V.Marchand, 2010)

Trans fats are unsaturated fats with trans double bonds instead of cis bonds. The type of bond affects the shape of the fatty acid chain. A trans bond creates a straight chain, whereas a cis bond results in a chain that is bent. Trans fats may be monounsaturated or polyunsaturated ((V.Marchand, 2010)

Trans fats are created as a result of partial hydrogenation. Hydrogenation is the chemical addition of hydrogen atoms to cis unsaturated fat, which eliminates the double bonds between carbon atoms and makes them saturated. The food industry uses hydrogenation to extend shelf life by making fats less susceptible to rancidity, a phenomenon in which free radicals attack the double bond between carbon atoms. It also raises the melting point of the fat, making it more suitable for frying. However, during the hydrogenation process, some cis bonds in fatty acids are converted to trans bonds, resulting in trans fatty acids rather than saturated fatty acids.

Trans fats naturally occur in small amounts in the fat of certain foods such as dairy and meat. They are also present in breastmilk in concentrations directly dependent on the mothers' dietary intake of trans fats. Trans fats originating from processed foods are, by far, the largest dietary source of trans fats consumed.

2.3 Dietary Fats and Fatty acids

India has experienced a rapid change in nutrition along with an increase in the number of chronic diseases. A growing middle class now has easier access to commercially produced foods, especially baked and fried goods, thanks to the current economic expansion. The majority of these foods have fairly high trans fatty acid (TFA) content, especially if they were made with partially hydrogenated vegetable oils, which are frequently chosen because of their low price and extended shelf life (Fardet et al,2022)

TFA-containing foods are causing growing concern because to their negative health impacts, which include non - communicable diseases (NCDs) such as cardiovascular disease (CVD), diabetes, cancer, inflammation, abnormal foetal development, hampered infant growth, and so on.

India is experiencing a rapid health transition with a rising burden of NCDs causing significant morbidity and mortality both in rural and urban population. India shares fifty percent of all death and 63% of the total disease burden (Park's. 2021)

The leading cause worldwide is cardiovascular disease (CVDs), a projected 17.9 million people died from CVDs in 2019, accounting for 32% of all deaths globally. Of these, 85% were due to heart failure and stroke. Out of the 17 million premature mortalities (the age under 70) due to noncommunicable diseases in 2019, 38% were caused by CVDs (WHO, 2022)

The Global Burden of Disease Report (2016) states that NCDs are the leading cause of death worldwide, accounting for nearly 39.5 million (72%) of the 54.7 million fatalities in 2016.

The effects of TFA are greater than those of saturated fats, increasing levels of low-density lipoprotein (LDL) cholesterol while decreasing levels of high-density lipoprotein (HDL) cholesterol (Mozaffarian and Clarke, 2009).

According to the World Health Organisation (2019), partially hydrogenated vegetable oils, also known as PHVOs, are frequently used in households and businesses, especially in the unorganised sector, which includes street vendors. These oils are also present in baked and fried foods. Industrially produced TFA, also known as i-TFA, are the main dietary source of trans fat.

2.1 Dietary Fats and fatty acids

In general, fats and oils are triglycerides (glycerol and fatty acid esters) that are soluble in organic solvents but insoluble in water due to their reduced density. At room temperature, their consistency varies from liquid to solid depending on structure and makeup. Although these are commonly referred to as fats, the ones that are liquid at normal temperature are known as oils, while the solid/semi-solid ones are known as fats (Anthea, 1993).

Triacylglycerols – Most fats and oils contain mono and diacylglycerols, free fatty acids, phosphatides, sterols, fatty alcohols, and other small components. Fats and oils are regarded vital nutrients for both humans and animals since they supply not only the most concentrated sources of energy but also a variety of critical fatty acids (the precursors of crucial hormone-like compounds known as prostaglandins). They help with satiety, absorption/transport of fat-soluble vitamins, and food/diet palatability. Various amounts of fats/oils are present in almost all foods; however, their principal sources include vegetable oils, fats, meats, dairy products, chicken, fish, nuts, and oilseeds; cereals and pulses contribute very little, while vegetables/fruits provide almost microscopic amounts (Strayer, 2006).

Fatty acids (FAs) - The building blocks of lipids, account up 90-95 percent of dietary fats. The kind and amount of constituent fatty acids, as well as their position on the glycerol backbone, have a large influence on the physical and chemical properties of dietary fats. Majority The majority of fatty acids are composed of saturated/unsaturated carbon chains (typically with an odd number of carbon atoms) with a single carboxyl and a methyl group on each end. In smaller amounts, edible oils may contain branched chain/cyclic fatty acids, but animal fats often have odd carbon chain fatty acids (R.de Carvahlo et al,2018).

Fats/fatty acids perform the following functions: -

- *Most concentrated and storage form of energy (9 kcal/37kJ per g; can be stored in adipose tissues, skeletal muscles, and so on); transport energy through blood in the form of lipoproteins; aid in the absorption and transportation of fat-soluble vitamins; and provide greater satiety.*
- *These are important components of biological membranes in the form of phospholipids.*

- *Provide thermal, electrical, and mechanical insulation/protection to the body - Eicosanoids and their metabolites (fatty acid derivatives) participate in signalling cascades and are also implicated in gene regulation (FAO, 2010)*

As per Indian Council of Medical Research (2020), the Recommended Dietary Allowances (RDA) for visible fat ranges between 25-40 g/day (adult men) and 20-30g/day (adult women) depending upon the extent of their physical activity.

2.1.1 Classification of Fatty Acids

Fatty acids (FAs) are commonly classified on the basis of their carbon chain length, degree of saturation/unsaturation, position and number of double bonds, spatial arrangement and nutritional importance of FAs.

These are classified into three types based on the length of their carbon chains: short, medium, and long chain fatty acids. Although the length of a carbon chain can range from 2 to 80, the ones found in food commonly have 14, 16, 18, 20, and 22 carbon atoms (Scalfi et al., 1991).

Short chain fatty acids (SCFAs) with 2-6 carbon atoms (for example, propionic acid - C3; butyric acid - C4) are generated in the stomach by anaerobic bacterial fermentation of polysaccharides and are absorbed directly by the portal circulation (Ana Nogal et al, 2021)

Medium-chain fatty acids (MCFAs) have 6-12 carbon atoms; medium-chain triglycerides (MCTs) also passively flow across the gastrointestinal tract to the portal system without being modified or requiring bile salts. MCTs are specifically administered to patients suffering from malnutrition/malabsorption syndrome since they do not require energy for absorption, usage, or storage. Coconut oil (66% MCTs) and palm kernel oil (60% MCTs) are both good sources of MCTs (Peter Schonfeld et al,2016)

Long chain fatty acids (LCFAs) have more than 14 carbon atoms, while very long chain fatty acids (VLCFAs) have more than 20 carbon atoms, such as behenic acid (22:0), lignoceric acid (24:0), and so on (Howie et al., 2018; Clegg and Miriam, 2010; Scalfi et al., 1991). Long and very-long chain fatty acids require bile salts to emulsify and are absorbed by the lymphatic system.

Fatty acids are categorised as saturated, monounsaturated, or polyunsaturated based on their saturation/unsaturation. Because of the presence of single bonds (carbon-to-carbon), saturated fatty acids (SFAs) are the least reactive chemically. The melting point of SFAs increases with the length of the carbon chain; most SFAs are solid at room temperature (Table 2.1). These are mostly found in animal meals (meat and dairy products) and tend to boost blood levels of low-density lipoprotein cholesterol (LDLc). Unsaturated fatty acids (UFAs) have fewer hydrogen atoms because they include one or more (carbon-to-carbon) double bonds. These are primarily found in plant and sea foods.

Fatty acids with one double bond are known as Monounsaturated Fatty Acids (MUFAs), while those with more than one double bond are known as Polyunsaturated Fatty Acids (PUFAs) (PUFAs). The position of the double bond in the fatty acid has been considered from the delta end throughout; nevertheless, it is subject to alter with carbon chain elongation/shortening. PUFAs are categorised as omega 3 (n-3) or omega 6 (n-6) or omega 7 (n-7) or omega 9 (n-9) series based on the position of the first double bond from the terminal methyl end.

Unsaturated fatty acids are chemically more reactive than saturated fatty acids due to the existence of double bonds; reactivity rises with the amount of double bonds. Double bonds are most commonly found in non-conjugated PUFAs, but they can also be found in conjugated PUFAs. The presence of conjugated double bonds boosts their chemical reactivity, resulting in faster oxidation and more polymerization.

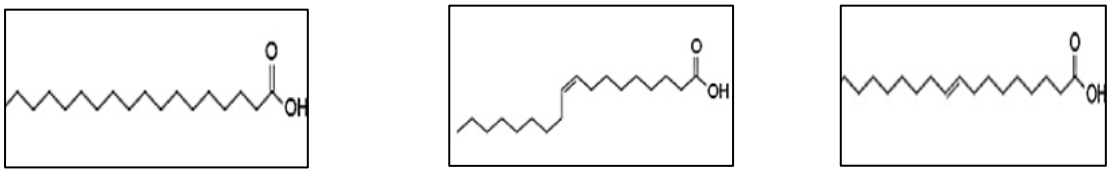
–CH=CH–CH=CH–	–CH=CH–CH2–CH=CH–
Conjugated double bond	Non-conjugated double bond

Trans fatty acids are produced either through industrial hydrogenation (i-TFA) or through ruminant bio-hydrogenation (cow, sheep etc.). Artificial TFAs are formed during the partial hydrogenation of vegetable/fish oils utilising hydrogen gas and a metal catalyst (typically nickel). Naturally occurring TFAs, on the other hand, are generated in the rumens of ruminants by the isomerization of cis-UFAs found in their feed, with bacterial enzymes acting as catalysts (Brouwer, Wanders and Katan, 2010).

The spatial arrangement of hydrogen atoms across the double bond indicates cis or trans configuration; in cis orientation, both hydrogen atoms are on the same side of the double

bond, resulting in kinked geometry, whereas in trans configuration, the hydrogen atoms are located transversely opposite to each other, resulting in a straighter chain structure.

Soybean, sunflower, safflower, mustard, olive, rice bran, and sesame oils are not only low in SFA, but the unsaturated fatty acids also exist in cis form (Kinsella et al., 1987). Conjugated linoleic acid (c9, t11 CLA) Is a geometric isomer of linoleic acid with conjugated double bonds that can be cis or trans.



Saturated (Stearic acid) **Cis (Oleic acid)** **Trans (Elaidic acid)**

Figure 2 : Geometric isomer of Stearic acid, Oleic acid, Elaidic acid with conjugated double bonds that can be cis or trans.

New PUFAs are being discovered all the time, with the most recent ones being 5,9-hexaicosadienoic acid, 5,9-docosadienoic acid, 5,9-tricosadienoic acid, 5,9,13-eicosatrienoic acid, and 5,9,13-docosatrienoic acid derived from Stoichactis helianthus. Geranoic acid, nerolic acid, and farnesoic acid are branched-chain polyunsaturated fatty acids.

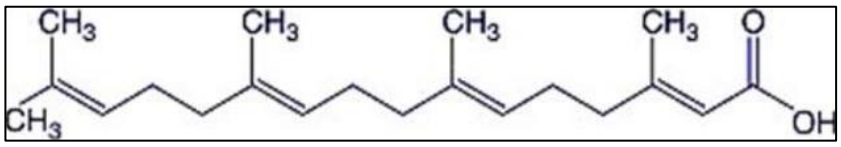


Figure 3: Structure of Geranoic acid *- 3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenoic acid*

TFAs have a greater melting point than cis-MUFA/PUFAs but a lower melting point than SFAs (stearic acid 69.6oC, oleic acid 16.3oC, and elaidic acid 43.7oC; Tables 2.1; Table 2.2).

Table 2.1 Melting Point of Saturated Fats Commonly Present in Fats/Oils

Systematic/ Common Nomenclature	Melting Point (ºC)	Typical Source	Systematic/ Common Nomenclature	Melting Point (ºC)	Typical Source
Ethanoic (C2 ; Acetic Acid)	---	---	Tetradecanoic Acid (C14 ; Myristic Acid)	54.4	Butter, coconut oil
Butanoic (C4; Butyric Acid)	(–)7.9	Butterfat	Hexadecanoic Acid (C16; Palmitic Acid)	62.9	Most fats and oils
Hexanoic (C6; Caproic Acid)	(–)3.4	Butterfat	Heptadecanoic Acid (C17; Margaric Acid)	60	Most fats and oils
Octanoic (C8; Caprylic Acid)	(–)16.7	Coconut oil	Octadecanoic Acid (C18; Stearic Acid)	69.6	Most fats and oils
Decanoic Acid (C10 ; Capric Acid)	31.6	Coconut oil	Eicosanoic Acid (C20; Arachidic Acid)	75.4	Peanut oil
Dodecanoic Acid (C12; Lauric Acid)	44.2	Coconut oil	Docosanoic Acid (C22 ; Behenic Acid)	80.0	Peanut oil

(Strayer, 2006)

From a nutritional standpoint, essential fatty acids (EFA) are fatty acids that cannot be produced in the body and must thus be obtained through diet. Omega-9 fatty acids, although required, are not essential because the body can produce them in small amounts. Our bodies are capable of denovo-synthesising the majority of fatty acids, particularly SFAs and MUFAs, but not the omega-3 and omega-6 fatty acid series. As a result, these are known as essential fatty acids (EFAs).

We cannot synthesise the omega-3 and omega-6 PUFA series, which are required for appropriate growth, reproduction, and healthy development, because our bodies lack the delta 12 and 15 desaturases required for introducing the cis-double bonds at n-6 or n-3 locations. Because of enzyme specificity, human bodies can only insert a new double bond after the seventh carbon from the methyl end; between the existing double bond and the carboxyl group; and in a non-conjugated location. Furthermore, unsaturation should be followed by elongation before the next double bond is introduced. Elongation of fatty acids requires the addition of two carbon units at the 12 carboxyl/delta end, with malonyl CoA acting as the donor of two carbon units. Linoleic acid is thus the precursor of the omega-6 PUFA series, while linolenic acid is the precursor of the omega-3 PUFA series.

Table 2.2 Melting Point of Unsaturated Fatty Acids Commonly Found in Fats/Oils

Systematic/Common nomenclature	No. of Double Bonds	Spatial configuration	Melting Point (ºC)	Typical Source
9-Decenoic (C10 ; Caproleic Acid)	1	cis	--	Butterfat
9-Dodecenoic (C12; Lauroleic Acid)	1	cis	--	Butterfat
9-Tetradecenoic (C14; Myristoleic Acid)	1	cis	18.5	Butterfat
9-Hexadecenoic (C16; Palmitoleic Acid)	1	cis	--	Some fish oils, beef fat
9-Octadecenoic (C18; Oleic Acid)	1	cis	16.3	Most fats and oils
9-Octadecenoic (C18; Elaidic Acid)*	1	trans	43.7	Partially hydrogenated oils
11-Octadecenoic (C18; Vacceinic Acid)*	1	trans	44	Ruminant fat including butter fat
9,12 Octadecadienoic (C18; Linoleic Acid)	2	cis	-6.5	Most vegetable oils
9,12,15-Octadecatrienoic Acid (C18; Linolenic Acid)	3	cis	-12.8	Soyabean oil, canola oil
9-Eicosenoic Acid (C20; Gadoleic Acid)	1	cis	--	Some fish oils
5,8,11,14-Eicosatetraaenoic (C20; Arachidonic Acid)	4	cis	-49.5	Lard
5,8,11,14,17-Eicosapentaenoic Acid (C20)	5	cis	--	Some fish oils
13-Docosenoic Acid (C22; Erucic Acid)	1	cis	33.4	Rapeseed oil
4,7,10,13,16,19-Docosahexaenoic Acid (C22)	5		--	Some fish oils

(Strayer, 2006)

The nutritionally significant omega-3 PUFAs are -linolenic acid (ALA), eicosa-pentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n3), which confer numerous health benefits related to immune response as well as the prevention/management/control of cardiovascular disease, inflammation, allergies, cancer, diabetes, hypertension, renal disorders, and so on. Linoleic (LA), gamma-linolenic (GLA), arachidonic acid (AA), and conjugated linoleic (CLA; 18:2n-6) acids are other nutritionally relevant omega-6 PUFAs.

Due to the lack of Δ12- and Δ15-desaturases, the two PUFA series with methylene interrupted cis-double bonds in omega-3 (e.g., alpha-linolenic acid, 18:3) and omega-6 (e.g., linoleic acid, 18:2) positions become required for humans (European Food Safety Authority, 2010). Long-chain unsaturated fatty acids generated from linoleic (omega-6 PUFA) and -linolenic (omega-3 PUFA) acids are thus included in EFAs; however, gamma-linolenic acid (GLA; 18:3) belongs to the omega-6 series. From a dietary standpoint, the most important -3 PUFA are 18:3 (-linolenic acid, ALA), 20:5 (eicosapentaenoic acid, EPA), 22:5 (docosapentaenoic acid, DPA), and 22:6 (docosapentaenoic acid, DPA) (docosahexaenoic acid, DHA). Conversely, dietary -6 PUFA is mostly linoleic acid (LA) and, to a lesser extent, arachidonic acid (AA); nevertheless, with doses less than 3.8 E%, the body can synthesise AA from linoleic acid (Kinsella et al., 1987).

EFAs' primary role is to produce prostaglandins, which aid in the regulation of numerous biological functions such as heart rate, blood pressure, blood coagulation, fertility, and pregnancy. Furthermore, they help to improve immunity by lowering inflammation and preparing the body to battle infections. EFAs aid in the elevation of HDLc, conferring protective effects on the cardiovascular, reproductive, and neurological systems, as well as immune function. EFAs are also required for the repair and regeneration of cell membranes, which allows cells to achieve optimal nourishment and discharge waste products. (Simpoulos, 2002).Heart disease, cancer, insulin resistance, asthma, schizophrenia, depression, postpartum depression, accelerated ageing, stroke, obesity, diabetes, arthritis, attention deficit hyperactivity disorder (ADHD), and Alzheimer's disease are among the major health risks associated with EFA insufficiency.

The ratio of ω-3: ω-6 PUFAs in human diets has shifted dramatically throughout time. According to FSSAI (2018), the recommended SFA: MUFA: PUFA ratio is 1:1:1, and the ω 3: ω 6 ratio is 1:5 to 1:10. However, it is interesting to note that human diets developed with a 1:1 ω- 3: ω- 6 ratio, whereas our present diets may have a 1:30 ω-3: ω-6 ratio. Because -6 PUFA intakes are within the limits for a healthy diet, worries about the disrupted -3:-6 ratio are driven by low -3 intakes rather than high -6 intakes. Excess -6 PUFA and a very high -6/-3 ratio increase the pathogenesis of CVDs, cancer, inflammatory and autoimmune illnesses, whereas high amounts of -3 (a lower -6/-3 ratio) decrease the pathogenesis of these diseases (Patterson et al., 2012).

Fungi and marine microalgae are currently being exploited to generate nutraceutical oils because they are particularly rich in C20-22 PUFAs. The PUFA biosynthetic pathways in lower eukaryotes typically entail the desaturation of oleate (18:1Δ9; by Δ12 desaturase) to linoleate (18:2Δ9,12), which is then transformed to alpha-linolenate (18:3Δ9,12,15; by ω-3 desaturase). Linoleate and -linolenate are then desaturated (by a Δ6 desaturase) to get gammalinolenate (18:3Δ6,9,12) and stearidonate (18:4Δ6,9,12,15). These two fatty acids are extended to C20 PUFAs (20:3Δ8,11,14 and 20:4Δ8,11,14,17) and subsequently desaturated to arachidonic acid (AA; 20:4Δ5,8,11,14) and eicosapentaenoic acid (EPA; 20:5Δ5,8,11,14,17) by a Δ5 desaturase, respectively. PUFA de-novo synthesis has been documented in unicellular organisms; thus, microbial fermentation is another appealing alternative (Wang et al., 2016).

Marine phospholipids (produced from marine species) are said to be superior to fish oils because they include significantly more physiologically active LC ω-3 PUFAs (EPA and DHA), which can resist oxidative rancidity considerably more effectively.

Linoleic and -linolenic acids are essential components of cellular membranes and the neurological system. These important fatty acids are associated with processes such as inflammation, cardiac arrhythmia, thrombotic and vascular functioning, and so on. There is equivocal evidence that consuming an adequate amount of ω-3 fatty acids reduces the risk of cardiac arrhythmias and sudden cardiac death. Although preliminary evidence suggests that ω-6 PUFAs are a proinflammatory marker, large cross-sectional and controlled intervention trials do not support this. In a 32-year follow-up study of 1,28,000 men and women, increased linoleic acid consumption was connected to lower CHD and cancer risk, as well as total mortality (Wang et al., 2016).

Linoleic acid concentrations in blood were found to be inversely related to type 2 diabetes in prospective studies, however arachidonic acid was not (Forouhi et al., 2016; Wu et al., 2017). In a consortium of 19 studies involving 45 637 people from 16 countries, it was determined that seafood and plant-derived omega-3 fatty acids are linked to lower CHD incidence and mortality (Del Gobbo et al., 2016), whereas no such association has been reported for marine-sourced omega-3 fatty acids (Forouhi et al., 2016).

Linoleic, linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids (containing 2, 3, 4, 5, and 6 double bonds, respectively) are of greater importance among polyunsaturated fatty acids. Linoleic and linolenic acids are primarily found in vegetable oils. Lard contains trace levels of arachidonic acid as well as almost 10% linoleic acid. Fish oils include a high concentration of LCFAs with more than three double bonds, such as EPA and DHA (Strayer, 2006). In India, a vast variety of edible fats/oils are consumed, including groundnut, mustard, soybean, safflower, sunflower, sesame, rice bran, ghee/clarified butter, and vanaspati/PHVO. Vanasapti is a popular inexpensive replacement for desi ghee and is commonly used in both sweet and savoury dishes in northern India. Furthermore, it is commonly utilised in the preparation of fried, processed, ready-to-eat, packed, frozen, premixes, and road-side street foods by commercial enterprises. Table 2.3 shows the fatty acid content, including TFA concentrations, of commonly consumed dietary oils and fats.

2.2 Trans Fatty acids: Evolution, developments and food sources.

Trans-Fatty acids, whether MUFA or PUFA, have at least one or more non-conjugated double bond/s in trans orientation, resulting in a straighter chain and more rigid structure similar to that of SFAs and requiring significantly less space than their cis counterparts (Mozaffarian et al., 2006). This configuration adjustment has a significant impact on the tertiary structure. An intermediate melting point (Table 2.2) is regarded ideal in the food processing sector because it helps to give favourable food texture, flavour, and mouth feel. Unsaturated FAs naturally present in plant oils in cis-form; but, during the extraction, refining, and deodorization procedures, a small amount isomerizes to generate their corresponding TFAs. Furthermore, during industrial hydrogenation of oils or high temperature heating/frying, a substantially higher fraction of cis-fatty acids is transformed to trans isomers (Choe and Min, 2007).

2.2.1 **Trans Fatty acids** – Evolution and Developments: Although trans fats have been known since the 19th century, TFA containing PHVOs became popular among consumers and food makers due to its low cost, taste, flavour, texture, and longer shelf life (Katan, 2008). Nobel winner Paul Sabatier invented the hydrogenation technique at the end of the nineteenth century. Wilhelm Norman, a German scientist, invented the procedure for transforming liquid oils into solid fats and coined the phrase "fat-hardening" at the turn of the twentieth century. Following that, Joseph Crosfield and

Sons used the procedure in England for large-scale manufacturing of solidified oils, producing 3000 tonnes of hydrogenated fat in the first year alone. Procter & Gamble purchased the US rights to Wilhelm Normann's patent in 1909, and two years later, the first shortening (using the hydrogenation technique) dubbed Crisco was widely promoted by distributing culinary books with recipes that included Crisco shortening.

Because soybean oil was considered a by-product, it was quickly being used in the industry as shortenings in place of butter, which was in limited supply due to the expanding number of consumers. Because of its beneficial properties, the food processing sector favoured employing hydrogenated fats, increasing its frequency and utilisation. In the 1920s, butter and lard were replaced in the creation of bakery items such as breads, pies, cookies, and cakes with soybean oil-based margarines.

By the 1960s, the manufacturing of hydrogenated fats had increased fast, and the consumption of animal-based fats had been replaced by PHVOs due to their low cost, and they were regarded a healthier alternative to SFA or butter. Although researchers discovered a detrimental effect for TFA in increasing CVD risk in the mid-1950s, public awareness of the substance remained very low. It wasn't until the 1980s that animal fats (lard) became a severe health concern for the population, and TFA was gradually incorporated by food makers in the bulk of fast foods.

By the 1990s, studies have verified the negative health effects of TFAs, with 20,000 deaths per year being linked to TFA consumption in the United States alone. The US Food and Drug Administration (FDA) mandated obligatory food labelling of trans fats in food products in 2003, which was quickly followed by several other countries. The mandatory addition of TFAs on food labels required food companies to change their production techniques in order to meet the FDA mandate. *(TFA concentration should be represented as g/serving to the next 0.5g increment if <5g and to the nearest gram if ≥5g; if one serving food item contains <0.5 g, the TFA content should be noted as 'zero' g).*

The food processing industries took a little longer than expected to eliminate TFA due to hassles in re-formulating food products as well as a preference for palatable taste and flavour over the well-known health risks connected with TFA intake. During the twentieth century, TFAs were no longer limited to margarines and vegetable shortenings, but were widely used in the food processing industry due to their low cost

and full disregard for the accompanying health concerns of hydrogenated fats (Sánchez-Villegas and Sánchez-Tainta, 2018). There have been numerous advances in TFA throughout the last two decades, which are listed below (Table 2.3).

Table 2.3 Trans Fatty Acids – Evolution and Developments Over the Years.

Year	Evolution and Developments in TFAs
1890s	French chemist Paul Sabatier investigated role of nickel as a catalyst in the reaction between hydrogen and other chemical compounds during the process of hydrogenation. In 1912, he was awarded Noble prize for developing the hydrogenation process
1902	Wilhelm Normann (a German chemist/scientist) reported that liquid oils can be hydrogenated to form semi-solid fats and patented the process which as a matter of fact was changing the unsaturated fats to SFAs and TFAs. Thus, trans fat became the first man-made fat to enter the food supply.
1911	Procter & Gamble introduced Crisco vegetable shortening – an economical option for animal fats/butter in grocery stores; and it became the first TFA containing food item.
1937	During II World War, due to the shortage of butter, use of margarine (a major source of TFA) became highly popular and its demand got escalated.
1957	The American Heart Association (AHA), for the first time, proposed that curbing dietary fats, particularly SFAs present in foods like butter and beef, can reduce the risk of heart diseases.
1960	In India, PHVOs were introduced which was marketed as “Vanaspati”
1984	Consumer advocacy groups campaigned against the use of saturated fat in fast-food restaurants. In response, most fast-food companies began using PHVOs instead of beef tallow and tropical oils high in SFAs.
1993	Based on several scientific studies, health advocacy groups called for fast-food restaurants to stop using PHVOs for deep-fat frying.
1999	The U.S. government proposed a law requiring food manufacturers to list TFA content on nutrition labels; however, it was not passed.

2002	For the 1st time, U.S. government agreed with the investigators that there was no safe level for trans fat; and that people should consume as little TFA as possible
2003	Denmark became the 1st country to eliminate TFA from the food supply chain. In the same year, U.S. Food and Drug Administration (FDA) also passed a law requiring TFAs to be listed on the Nutrition Facts label of food products. The food manufacturers were given three years to comply; many of them reformulated their products to limit the TFA content.
2006	Trans fat labelling became mandatory in the United States. AHA was the first major health organization to specify a daily limit of < 1% E from TFAs. During end year, New York became the first city in U.S. to pass a regulation limiting TFAs in restaurants; other cities/states followed similar regulations.
2007	AHA launched its "Face the Fats" consumer education campaign to help the consumers better understand the type of fats including TFA and their health effects.
2010	Food Safety and Standards Authority of India (FSSAI) organized 'National Consultation on the Proposed Trans Fat Regulation' at National Institute of Nutrition (NIN).
	FSSAI invited the comments from stakeholders as well as public on the 'Revised Draft Regulation of TFAs in Partially Hydrogenated Vegetable Oils'. Based on the NIN recommendations, FSSAI intended to modify the TFA limit in PHVOs from the then existing 10% to 5% within three years.
	National Consensus dietary guidelines by Misra et al. (2011) were formulated which recommended that TFAs should be < 1 E%.
	The Indian Council of Medical Research (ICMR) Expert Group released the Nutrient requirements and recommended dietary allowances for Indians in line with FAO/WHO, it proposed TFA to be <1E% per day (ICMR 2010)
2011	Dietary guidelines for Indians also recommended that TFA intake should not exceed <1E% (NIN/ ICMR, 2011).

2013	FSSAI limited the TFA content in PHVOs, margarine, fat spreads and Interesterified vegetable fat to 10%.
2015	US FDA declared that PHVOs (primary source of i-TFA in processed foods) were no longer **'generally recognized as safe' (GRAS).**
	FSSAI reduced the maximum permissible limit of TFA to 5% (by weight) in PHVOs, margarine, fat spreads and Interesterified vegetable fat (w.e.f. Aug 2016).
	WHO launched 'REPLACE' to eliminate trans fat in foods from the global supply chain by 2023.
	FSSAI decided to bring down the TFA in vanaspati/bakery shortenings/margarine to < 2% in a phased manner by 2022.
	The European Commission (EC) proposed to limit the amount of trans fats to 2 g/100 g fat or oil.
2019	35 Member States of WHO's Regional-Office for Americas/Pan-American Health Organization (PAHO) approved a regional action plan for eliminating i-TFA by the year 2025.
	WHO released additional resources in 2019 to support country actions, including six implementation modules and a live policy tracking map – the TFA Country Score Card so as to monitor global progress towards the 2023 target.
2020	As of May 2020, best-practice TFA policies – virtual elimination of i-TFA or banning PHOs became effective for 589 million people across 14 nations (~8% of global population)
	WHO established a TFA indicator which records whether or not the various countries have adopted the WHO best-practice policies for eliminating i-TFA.

Although, many of the countries have laid down the recommendations related to TFA limits/bans, it is envisaged that the remaining nations will also take necessary steps to achieve the WHO goal of 2023.

2.2.2 Dietary sources of trans-fats.

Naturally-occurring trans fats are found in small amounts in dairy products, for example cheese and cream, and also beef, lamb and mutton, and products made from these foods. A type of trans fat occurs naturally in the milk and body fat of ruminants (such as cattle and sheep) at a level of 2–5% of total fat (Trans Fat Task Force -June 2006)

Trans fats are used in shortenings for deep-frying in restaurants, as they can be used for longer than most conventional oils before becoming rancid. In the early 21st century, non-hydrogenated vegetable oils that have life spans exceeding that of the frying shortenings became available (NYC Board of Health-2007)

By far the largest amount of trans fat consumed today is created by the processed food industry as a side effect of partially catalytic hydrogenation of unsaturated plant fats (generally vegetable oils) with cis carbon-carbon double bonds. These partially hydrogenated fats have displaced natural solid fats and liquid oils in many areas, the most notable ones being in the fast food, snack food, fried food, and baked goods industries.

Trans fats may also be produced when ordinary vegetable oils are heated to fry foods at very high temperatures and this is one reason why takeaway foods can sometimes be high in trans fats. Foods that are produced from or use hardened vegetable oils as an ingredient typically contain some trans fats (for example, biscuits, pies, cakes and fried foods). Fat spreads and margarines that have hydrogenated vegetable oil as an ingredient will usually contain some trans fats, although reformulation has led to significant reductions in recent years. Van et. al. (1998), analysed the fatty acid composition of bakery products from 14 European countries and found that proportion of trans fatty acids in cookies and biscuits ranged from <1 to 28 %. Trans fatty acids content in sweet pastry ranged from practically 0 to 33%. Croissants and doughnuts had varying amounts of trans fatty acids, with the highest value of 15% in croissants and 32% in doughnuts. The total trans fatty acid content in pizzas was mostly in the same order and varied from <1 % to 5 %. Most hydrogenated marine oil-based bakery products were found in Iceland and in Norway. In Finland, Portugal, Italy, Netherlands, and United Kingdom no bakery products based on hydrogenated marine oils could be identified. Bakery products based on hydrogenated vegetable fats were present in almost every country. It can be concluded that in bakery products it is difficult to

identify the presence of trans fatty acids. French fried potatoes contained trans fat mostly between 12 and 35%, but some products fried in animal fat or vegetable oil contained lower proportions between 0.5 and 7% total fatty acids. Deepfried croquettes were also rich in trans fatty acids. Microwave popcorn samples contained 27–34% trans fatty acids. Ready-made popcorn was low in trans fatty acids but generally contained even more saturated fatty acids. Processed foods with a long shelf life such as dry soup powders and cubes, savory snacks, and popcorn often contained relatively high proportions of trans fatty acids, although the contributions of these products to total fat intake are probably small (Aro, 1998).

Tavella et. al. (2000) determined the total fat content of 46 selected foods items in Argentina. Total fat was 2.0–3.4% in sliced bread, 2.9–25% in cookies and crackers, 50– 80% in margarines, 85% in butter, and 34–39% in snack products. In the same items, content of elaidic acid was: 2.35–27.7% in sliced bread, 2.85–28.95% in cookies and crackers, 18.15–31.84% in margarines, 4.63% in butter, and 0–10.58% in snacks. The amount of trans fats in foods exhibit great variation, due to differences in hydrogenation methods and intensity.

Similarly, Sachchidanandam (2003) determined the trans-fat content of a wide range of foods in United States prior to the effective date of the new regulation. Food products for analysis were selected on the basis of market share and data from the USDA‘s 1994–1996 continuing survey of food intake by individuals. The results showed that trans fat (g/100 g fat) ranged from 0.0 to 48.8 in bread, cake, and related products; from 14.9 to 27.7 in margarines; from 7.7 to 35.3 in cookies and crackers; from 24.7 to 38.2 in frozen potatoes; from 0.0 to 17.1 in salty snacks; from 0.0 to 13.2 in vegetable oils and shortenings; from 0.0 to 2.2 in salad dressings and mayonnaises; and from 0.0 to 2.0 in dry breakfast cereals. Serving sizes for the foods included in this survey ranged from 12 to 161 g, and trans-fat levels ranged from 0.0 to 7.2 g/serving.

Albers et. al. (2006) conducted a marketplace survey of trans-fatty acid content of margarines and butters, cookies and snack cakes, and savory snacks. Most margarines and butters (21 of 29), cookies and snack cakes (34 of 44), and savory snacks (31 of 40) were labeled as containing 0 g trans fat. However, some products contained substantial amounts of trans fat. Albers concluded that the food industry has made progress in reducing the trans-fat content in a variety of products. Nonetheless,

consumers need to read product labels because the trans-fat content of individual products can vary considerably. Products that are lower in trans and saturated fat tend to cost more, which may be a barrier to their purchase for price-conscious consumers.

Agrawal et. al. (2008), determined the trans fatty acid content in common Indian fast foods by using the food composition and analysis tables of the Indian National Institute of Nutrition to determine fatty acid composition of common nutrients. Separate tables provide nutrient composition of various Indian sweets and snacks including methods of preparation and amount of ingredients used. The nutritive values of more than 200 Indian sweets and snacks were determined and are presented. The study shows that Indian sweets and snacks are very energy-dense with calorie content varying from 136 to 494kcal/100g in sweets and 148-603kcal/100g in snacks. TFA content of sweets varies from 0.3 to 17.7g/100g and snacks from 0.1 to 19.8g/100g. This translates into 1.9-53.0 fat energy per cent in sweets and 1.8-52.0 fat energy per cent in snacks. The study consists of analysis of secondary data obtained from raw ingredients. However, many chemical changes occurring during cooking and were not estimated.

Meremae et. al (2012), determined the trans fatty acid contents in selected dietary fats in the Estonian market. Twenty-six edible fat brands, available in the Estonian retail market, were purchased and FA compositions were analyzed by gas chromatography. Saturated fatty acids (SFA) were the dominant group of FAs for all blended spreads. The total TFA for blended spreads varied from 1.18% to 9.08%, for margarines from 0.04% to 34.96% and for shortenings from 0.14% to 39.50%. Octadecenoic (C18:1) isomers were the main TFA found.

2.2.1.1 Hydrogenated/Partially hydrogenated fats and oils

Midway through the 19th century, the process of hydrogenation or partial hydrogenation was widely commercialised to create solid fats, which were then thought to be a healthier alternative to animal fats. Edible oils are commercially hydrogenated or partially hydrogenated to change their texture (solid/semi-solid) and increase oxidative stability. Margarine, frying oils, deep-fried and baked foods, confections, and a variety of processed snacks are all made primarily with PHVOs.

Not only does the number of double bonds decrease during hydrogenation, but the existing double bonds in cis-double bonds can also open up and reconfigure as trans double bonds. Furthermore, the location of double bonds along the fatty acid carbon

chain can shift. However, the formation of trans double bonds can be limited by adjusting the factors influencing the hydrogenation process, such as catalyst type/concentration, temperature, hydrogen pressure, and the degree of agitation (Marchand 2017)

Maximum TFA formation, for example, occurs at higher temperatures (200-215^{o}C), lower hydrogen pressures (100-200 kilopascal), and lower catalyst concentrations (0.005% nickel/oil). At low temperatures (165-180^{o}C), higher hydrogen pressure (300 kilopascal), and slightly higher catalyst concentration (0.008% nickel/oil), trans-isomer formation is reduced, but a greater number of double bonds are replaced by single bonds, resulting in higher SFA formation. The TFA content of PHVOs can range from 10 to 60g/100g, but it is most commonly found to be 25-45g/100g.

Non-hydrogenated refined vegetable oils, on the other hand, contain TFA up to 2g/100g and ruminant fats between 3-6g/100g (WHO, 2019; Bhardwaj et al., 2016).

Partial hydrogenation of oils may lead to:

- The oil's melting point has been altered due to a higher amount of saturated and/or trans-monoene acids, which can affect spreadability, oral response, and baking performance (Sorangel Rodriguez-Velazquez, 2016).
- Reduced amounts of methylene-interrupted polyene acids improve oxidative stability (Yun,J.M, 2012)
- Reduced nutritional value due to lower levels of EFAs (linolenic and linoleic acids) and higher levels of trans-monoene and SFAs. It is possible, however, to replenish some of the essential fatty acids in the final product by blending with appropriate oil (Kaur N, 2014).

  - **trans-Fat generation during heating/frying** –
  - Tsuzuki, Matsuoka, and Ushida (2010) conducted extensive research indicating that subjecting unsaturated lipids to high temperatures (during oil refining/frying/baking) results in the formation of trans fats. TFA formation is a typical isomerization process that involves oxidation, thermally induced isomerization, and addition-elimination reactions of the parent cis-unsaturated fatty acids.

- Several studies have reported that TFAs are only produced under extreme heating/frying/ baking conditions (Bansal, Zhou, Tan, Neo, & Lo, 2009; Romero, Cuesta, & Sanchez-Muniz, 2000); however, the results are ambiguous in the literature.
- Karn, Abraham, and Ramakrishnan (2013) measured the TFA concentration in a variety of Indian fast foods, including fried foods, and found trans isomers of 18:1, 18:2, and 18:3 in all samples. The total trans-fat content ranged from negligible to 14.58g/100g, with elaidic acid (18:1t 9) being the most prevalent TFA. Aggarwal et al. (2008) reported a rather high trans fat content of 0.3-19.8 g/100g in Indian savouries and sweets, including fried foods, based on secondary data (Agrawal et al., 2008). In a study on the fatty acid profile of 75 Indian food samples, the highest TFA content was found to be 3.26% (Joshee, Abhang, and Kulkarni, 2019).
- Although SFA rich oils exhibit exceptional stability during frying operations, such oils are not desirable from a nutrition and health perspective. However, several studies have documented that addition of fresh oil can improve/maintain fatty acid composition of used frying oil. Fried/baked food items are bound to have significant amount of TFAs if fats/oils used in frying and baking operations contain significant concentrations of trans-fats.

2.6 Health effects of cis vs. trans fatty acids:

Long-chain PUFAs have multiple uses in promoting health and preventing disease. Due to the simultaneously effects of dietary TFA intake, it is challenging to evaluate the effects independently.

consumption of cis-UFAs and SFAs in large amounts. Based on a growing body of scientific evidence, there have been growing worries about dietary trans fats because they have many more negative health effects than cis UFAs. (peter van dael, 2021).

Health effects of cis fatty acids

Cis-unsaturated fatty acids, from the perspective of health, include MUFAs, -6 PUFAs (primarily linoleic acid) and -3 PUFAs (-linolenic acid; -3 PUFA, EPA & DHA), -7 PUFAs, and -9 PUFAs (Balić, A, et al, 2020)

Each cis-UFA has a clear nutritional function and confers a variety of positive health effects. It is well known that replacing dietary TFAs and SFAs with cis-UFAs, which are both cis-MUFAs and cis-PUFAs, lowers plasma LDL-c concentrations in addition to the preventive effects of -3 PUFAs (European Food Safety Authority, 2005).

Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) play a crucial role in the structure and function of cell membranes, including the maintenance of selective permeability. They also provide energy and act as precursors for several biologically active substances, including prostaglandins, leukotrienes, and thromboxanes (collectively known as eicosanoids) (Calder P. C. 2013).

Supplementing with long chain omega-3 PUFAs may help lower mortality rates in people with coronary heart disease and heart failure without preserved ventricular function. Therefore, omega-3 PUFAs aid in the prevention of CAD and arthritis and, as suggested by animal models, may have neuro-protective effects in Parkinson's disease (MA Gammone, et al,2019).

It is well known that long chain n-3 PUFAs are essential for pregnant women before conception, throughout pregnancy, and during lactation in order to support the infant or toddler's normal growth, neurological development, and cognitive function (Sanjay basak et al, 2020)

Because brain cells are particularly rich in certain long chain PUFAs, this points to their possible role in influencing cognitive function and human behaviour (Bentsen H. 2017)

The potential health benefits of -3 PUFAs have not only sparked the interest of nutritionists, but substantial evidence supports their role in preventing the progression of cardiovascular disease, though the mechanism is still unknown (Serini.S. et al 2020)

Similarly, the -6 PUFAs have a number of physiological functions, the most important of which are related to their role in membrane structure and the biosynthesis of short-lived derivatives (eicosanoids) that regulate various aspects of cellular activity. They are also in responsible for maintaining the skin's permeability and regulating cholesterol transport in the body (Dyall, S. C, et al 2022)

However, because there are so many ω -6 PUFA food sources in our diets, getting enough of them is easier than getting enough of the ω-3 PUFAs, which have limited dietary sources. However, increasing fish consumption (>2 servings of oil-rich fish per week) or relying on fish oil supplementation for omega-3 PUFAs is not recommended during pregnancy due to the negative health effects associated with heavy metal contamination of fish or the relatively high vitamin A concentrations in certain fish oil supplements (Meyer, B. J et al, 2003).

Health effects of trans Fatty Acids

Concerns about the potentially harmful health effects of TFAs are growing. Numerous studies have linked industrial trans fat consumption to a variety of health risks, including weight gain, inflammation, oxidative stress, cardiovascular disease, diabetes, cancer, dementia, infertility, abnormal foetal development, compromised infant growth/development, and so on (Pipoyan, D et al, 2021).

Several deleterious effects of trans fat consumption have already been scientifically proven. Increasing concerns about the consumption of TFAs is because of the related health hazards that have led to vast movements and imposing necessary regulations for minimising TFA consumption.

Consumption of processed foods increased dramatically in the latter half of the twentieth century, and PHVOs became a significant component of human diets. According to a review of 39 randomised intervention trials, all three categories - industrial TFAs, ruminant TFAs, and CLA - raise LDL:HDL and possibly the risk of CHD. The effect of ruminant trans fats and CLA on LDL:HDL was found to be less profound than that of industrial TFAs, with a non-significant difference (Brouwer, Wanders, and Katan, 2010).

An intake of 3 g CLA per day as a supplement can raise the LDL:HDL ratio by 0.050, which corresponds to a nearly 3-12% increase in CVD risk (Mozaffarian et al., 2006).

Ruminant TFAs were found to produce higher LDL and HDL levels than industrial TFAs in women but not in men (Brouwer, I. A et al 2010).

A controlled randomised single blind multiple crossover trial among adults (N = 61 healthy men/women; 18-65 years) found that after 3 weeks of consuming an i-TFA-containing diet, LDL-c concentrations were 2.6% higher than after consuming a CLA diet ((largely present in milk/meat; p = 0.049). Serum TG levels (8.9%; p = 0.004) and apo-B (2.5%; p = 0.041) were also found to be higher in the case of i-TFA versus the CLA diet (Takeuchi, H.et al., 2017).

Natural trans fats have been shown in studies to have numerous health benefits, including immune system strengthening and the prevention of overweight/obesity, atherosclerosis, cancers, and hypertension (Dhaka V ,et al 2011)

Inflammation, endothelial function and Oxidative Stress:

Systemic inflammation is a new risk factor for a variety of diseases, including coronary artery disease, insulin resistance, diabetes, dyslipidemia, and heart failure. TFA may have a negative effect on various inflammatory markers, according to epidemiological studies (Petrie, J. R. et al 2018).

TFAs influence monocyte and macrophage responses in humans, increasing monocyte production of TNF- αand interleukin-6. Trans fats have been shown to increase circulating endothelial dysfunction biomarkers and impair nitric oxide dependent arterial dilatation (Mallick, R 2022).

TFAs in the diet may have an adverse effect on various haemostatic and hematobiological properties of blood, as well as enzymatic activities and the properties of membrane phospholipids (Micha, R et al 2010).

Rats fed TFA were more susceptible to mitochondrial swelling and had lower rates of oxidative phosphorylation in an animal model (Mozaffarian et al, 2006). TFA intake was found to be positively correlated with plasma levels of tumour necrosis factor

receptor 2 (TNFR-2; P=0.002) and C-reactive protein (CRP; P=0.009) in a statistical analysis of cross-sectional data from the Nurses' Health Study (Lopez-Garcia et al., 2005).

According to a study by Smidowicz, A et al (2015), high TFA diets (8 E% TFA) resulted in significantly higher levels of CRP compared to carbohydrate diets where 8.5% fat-energy was replaced by digestible carbohydrates (P0.05).

Because trans fats are incorporated into endothelial cell membranes, they may alter cellular and macromolecular components acting at the blood vessel wall interface. This, in turn, could result in changes in anti-haemostatic properties, altered vascular tone, hyper-adhesiveness to blood leukocytes, and increased cytokine and growth factor production - all of which are known symptoms of endothelial dysfunction(Iwata et al, 2011).

Fatty acids were found to impair endothelial function as measured by flow-mediated vasodilation in a study by de Roos, Bots, and Katan (2001).

Additionally, it has been suggested that excessive TFA consumption may indirectly impair endothelial function by lowering HDLc, which may then trigger LDL oxidation (Mundi, S et al, 2018).

According to variations in hepatic peroxidative events, consumption of margarine, which has a high TFA content, may lead to oxidative stress and, in turn, liver dysfunction and disturbed hepatic lipid metabolism, which may result in non-alcoholic fatty liver disease (NAFLD), a major factor in the cardiometabolic syndrome (Dhibi et al., 2011).

Reviews by Okada et al. (2013) unequivocally show that TFA-containing diets (rich in dairy products) have pro-inflammatory effects on a variety of systemic disorders and gut inflammation.

Additionally, TFAs have been shown to have pro-inflammatory effects and to cause oxidative stress in the hypothalamus, which affects the signalling pathways that control satiety, hunger, and a number of other metabolic processes (Velloso, 2009).

Elaidic acid (tC18:1) and oleic acid produced different DNA methylation and transcriptome profiles in cultured human cells, as shown by Flores-Sierra et al. (2016). Elaidic acid also caused changes in the DNA methylome of adipose tissue.

Longhi et al. (2018) found that Wistar rats given partially hydrogenated soybean oil for 90 days had altered inflammatory markers or oxidative stress, which led to impaired cortical mitochondrial factors.

A comparable mouse model showed that a high TFA intake (5% elaidic acid) promoted oxidative stress and inflammation, which contributed to the development of atherosclerosis (Monguchi et al., 2017).

Non-communicable diseases

The leading cause of death is cardiovascular disease, followed by diabetes and cancer. High dietary TFA increases the risk of CVD by 21% and the risk of early death by almost 28% (WHO, 2020).

In India, which has a population of over 1.3 billion people, NCDs account for about 5.87 million deaths (or 60% of all-cause mortality) (Nethan, Sinha, and Mehrotra, 2017). Thus, eliminating i-TFA from the food supply chain is viewed as a globally significant and life-saving public health intervention.

Cardiovascular Disease:

According to the Seven Countries Study, countries with high TFA intake, such as Northern Europe, had higher CHD mortality rates than countries with low TFA intake, such as the Mediterranean region and Japan (Menotti, A., & Puddu, P. E. 2015). Dietary TFAs may increase inflammatory biomarkers, plasma lipoprotein(a), and endothelial dysfunction. Several studies have shown that dietary TFA intake is linked to an increased risk of sudden cardiac arrest. High TFA intake has negative effects on lipid profiles because it lowers HDL-c and apo-A levels while increasing LDL-c, TC, and Apo-B levels (Hirata, Y, et al 2017).

According to an animal study by Ibrahim and Ghafoorunissa (2001), a high TFA diet was associated with altered PUFA composition in the intestinal brush border

(membrane) and alkaline phosphatase activity. It has been proposed that TFA incorporation as a component of adipose, phospholipids, and lipoproteins in the organs and blood stream can alter the composition and activity of cell membrane receptors and enzymes (Ibrahim, Ahamed.et al, 2001)

Diabetes:

Concern over the increased risk of type 2 diabetes mellitus (T2DM) associated with trans fat consumption is growing. The Nurses' Health Study found that dietary TFA was linked to an increased risk of type 2 diabetes; for every two percent increase in TFA, the risk of developing type 2 diabetes rose by 39% (95% confidence interval: 1.15-1.67; P 0.001) over the course of the study's 14-year follow-up of 84,204 women (aged 34–59 without T2DM, CVD, or caner (Rice Bradley B. H. (2018)

After adjusting for de novo lipogenesis (16:0 and 18:0 levels), a large prospective Cardiovascular Health Study of older Americans (30,825 person-years; 287 T2DM cases) found that plasma phospholipid t16:1n-9 and t18:1 but not t18:2 TFA were significantly associated with de novo lipogenesis. Diabetes Reduces PPAR-g Expression Deficiency in Insulin Clearance By -cells, glucose induced insulin secretion According to Wang et al. (2015), isomers have a favourable correlation with the prevalence of T2DM.

TFAs only made up 0.37% of the total fatty acids in erythrocytes, according to a study conducted among Chinese (N= > 3,000 middle-aged/older subjects), according to Yu et al. (2012), and the most abundant TFA, trans-18:1, was significantly correlated with the consumption of dairy products. After taking into account a number of variables, including sociodemographic, lifestyle, BMI, and dietary intake, including other fatty acids, it was determined that subjects with the highest trans-18:1 levels had a 30% lower risk of developing type 2 diabetes.

On the other hand, elevated trans-18:2 concentrations were linked to a higher risk of dyslipidemia, independent of traditional risk factors like erythrocyte SFAs and trans-18:1 fatty acids. Phospholipid t16:1n7, which is found naturally in dairy products, has been linked in several prospective studies to a decreased risk of T2DM (Mozaffarian et al., 2010; Mozaffarian et al., 2013). Another study on animals showed that

supplementing with vaccenic acid for 8 weeks increased glucose turnover rates among those with T2DM and raised plasma C-peptide levels, which improved insulin secretion (Wang et al., 2016).

Cancer:

Slattery et al. (2001) came to this conclusion after analysing the data from a case-control study (cases: 1993; controls: 2410) carried out in Utah, Northern California, and Minnesota. They found a correlation between high TFA consumption and an elevated risk of colon cancer.

The EURAMIC study looked into the relationship between adipose tissue TFA and the occurrence of breast, prostate, and large intestinal cancers in European populations reporting wide variations in dietary TFA. The incidence of breast and large intestinal cancers was positively correlated with TFA intake, whereas breast/colon cancers showed a negative association with cis-MUFAs (Bakker et al., 1997).

TFA levels in gluteal adipose tissue were found to be positively correlated with the incidence of breast cancer in another multi-centric case-control study (Kohlmeier et al., 1997). A neutral association between dietary trans fat and pancreatic cancer was reported by prospective studies by Heinen et al. (2009) and Michaud et al. (2003).

TFAs have a variety of anti-cancer effects. Vinikoor et al. (2010) found that, compared to the lowest quartile, the highest quartile of energy-adjusted TFA consumption was positively associated with distal colorectal (sigmoid, rectosigmoid, and rectal) cancers among Whites but not African Americans in a 5-year prospective case-control study involving Whites (n=1516) and African Americans (n=392) from North Carolina.

Brain development, cognition and memory:

In younger adults age across the adjustment models, trans fat consumption negatively predicted word-recall performance (Golomb, B. A, et al., 2015). In younger adults, higher dTFA was significantly linked to worse word recall. Prospects for causality are provided by the prooxidant and energetic harms of dTFA and by triangulating with other evidence.

On brain growth and cognition, trans fat may have detrimental effects. According to animal studies (Acar et al., 2003; Larqué et al., 2003; Wauben et al., 2001), long-term TFA consumption may cause trans fat incorporation in brain membrane phospholipids, alter membrane fluidity and neurotransmission, and inhibit the activity of desaturases and elongases.

Children with ADHD have more trans-fatty acids in their RBCs than children without ADHD, according to the (Armon-Omer et al 2021)study. This study suggests that trans fatty acids and ADHD may be related. A more focused dietary intervention may result from an understanding of these findings and their clinical significance.

According to a study by Vaccarino et al. (2007), people who consumed trans fats for more than six years had a 48 percent higher risk of developing depression.

According to Pase et al. (2015), prolonged TFA consumption (10 months; starting from the post-weaning period and continuing through pregnancy and lactation across two generations) affected the animals' behavioural responses, leading to an increase in loco-motor activity and impulsive/agitated behaviour. A significant direct relationship between dietary trans fat and behavioural irritability/aggression was found in an observational study conducted among 945 adult men and women between 1999 and 2004 (Golomb et al., 2012).

Long-term TFA consumption may have a negative effect on neurotransmitters, cognition/learning ability, and memory, according to research done on Kunming mice (Zhang et al., 2018).

Consumption pattern of Bakery foods.

The food production-consumption chain is one of the most important issues of today, as food, which is the basic necessity of human life, is of critical importance in terms of community health.

Bakery products and their derivatives have an important place in the food consumption all over the world. Bread, pasta, bulgur, biscuits, cakes and breakfast cereals are the most consumed industrial cereal products. Bakery products constitute 65% of food industry in Turkey. Biscuits are a shelf-stable product because of their low moisture

content, were first used by sailors, soldiers, patients and travellers but today they are consumed almost by every age group and in every meal. Because of the increasing demand, as in many countries, there have been important developments in biscuit industry in Turkey as well, a lot of biscuit types with rich composition started to be produced. Bread with its high nutritional value, a neutral aroma, availability and cheapness is the basic food source in bakery products. Another bakery product, pasta; with its properties such as nutritious, delicious, cheap, easy to prepare, long shelf life is the most consumed food after bread. Italy is the world's largest producer with 3,326,750 tons and 32% share. Italy is followed by USA with 12%, Brazil with 11% and Russian Federation with 6%. Turkey is in the 5th place in world pasta production and its share in production is 5.1% (TURKSTAT, 2015).

A bakery product includes food items such as biscuits, cakes, pastries, flat-bread, tortilla, chapati, bun, croissant, etc. Bakery industry in India is the largest of the food industries with an annual turnover of about Rs. 3000 cr (Indian mirror, 2019). According to the latest report by IMARC the Indian bakery industry has reached a value of US$ 7.22 Billion in 2018 (Digital Journal, 2019). The Indian bakery industry is dominated by unorganized sector which comprises of around 50,000 small and medium-size producers along some organized producers (NIIR, 2019).

INDIAN BAKERY INDUSTRY -

The bakery industry in India has become very well-known as a result of the country's rapid population growth, increasing foreign influence, the rise of the working-age female population, and the nation's shifting eating habits. Due to their high nutritional value and affordability, bakery products are now well-known and widely consumed. Consumers are demanding newer options for bakery goods, and the sector has been fortifying bakery goods to sate the growing appetite of the health-conscious Indian men, women, and children. Indian food processing industry holds a significant position for the traditional activity of baking. In India's bakery industry, a number of healthy wheat-based products have been introduced and are rapidly gaining popularity. The mounting presence of bakery chains has further triggered the growth in the sector.

One of the largest segments in India's food processing industry is the bakery sector, which presents a wealth of opportunities for development, innovation, and job creation. In India, the bakery industry is divided into three subcategories: bread, biscuits, and

cakes and pastries. In 2018, the market value of the Indian bakery industry was USD 7.22 billion.

Due to the entrepreneurial spirit of Indian businesses and individuals, India is one of the most exciting regions for the bakery industry. It is the third-largest producer of biscuits in the world, behind the United States and China. The Indian bakery industry is changing as a result of shifting consumer tastes, preferences, and lifestyle habits.. As part of a global trend, there is a greater demand for healthier products and alternatives, particularly when it comes to bakery goods which are now more commonly consumed daily as opposed to being a treat.

In addition to healthier options, flavour innovation is essential because millennials in particular are constantly looking for new flavours and experiences. Indian consumers prioritise convenience due to their busy lifestyles, and since loaves of bread and biscuits are fast-moving consumer goods (FMCG), bakeries are a popular choice. Although there is a demand and appetite for baked goods among Indian consumers, the country's bakery industry still faces some difficulties. With more than 2,000 organised or semi-organized bakeries and 1,000,000 unorganised bakeries, the industry can be broadly divided into organised and unorganised segments.

Today's products from bakeries are not just for the wealthy; they are also used by the average person, which is why the market for these products is rapidly expanding. Food items like buns, croissants, cakes, pastries, tortillas, chapati, and biscuits are examples of bakery products. With a yearly revenue of about Rs. 3000 cr, the bakery industry in India is the largest among the food industries (Indian Mirror, 2019). The Indian bakery industry reportedly generated US$ 7.22 billion in 2018 (Digital Journal, 2019), according to the most recent IMARC report. According to NIIR (2019), the unorganised sector, which includes some 50,000 small and medium-sized producers as well as some organised producers, dominates the Indian bakery industry. As a result, there is fierce competition in the market, and for players to survive there must be room for fluctuating customer expectations.

In 2022, the Indian bakery market was estimated to be worth USD 10.1 billion. The market is projected to expand at a CAGR of 9.6% from 2023 to 2028, growing to a value of USD 17.44 billion. The region's booming biscuit and biscuit industry supports the Indian bakery market.

Nearly 72% of sales in the Indian bakery market are attributable to the biscuit and biscuit industry. Due to their low cost and cholesterol-free composition, cookies and biscuits have a much higher market penetration in urban and rural areas than other baked FMCG products. India currently consumes the most biscuits in the world . India's East and North have the highest rates of biscuit consumption in the nation. The states with the highest rates of industrial development, West Bengal and Maharashtra, also have the highest rates of biscuit consumption. Parle Products Pvt. Ltd., Britannia Industries Ltd., ITC Limited, and Surya Food & Agro Ltd. are the top four companies in the biscuits and cookies market, and Britannia and Parle together account for a sizeable market share (Indian Bakery Market Report 2023).

Bakery consumption pattern .

Consumers of bakery products are influenced by their spouse's relatives, friends, and co-workers. According to the study, women are more likely to purchase bakery products.

In terms of consumer willingness to purchase, consumers prefer to pay lower prices for baked goods than for other alternatives, implying that pricing is an important actor influencing the purchasing decision. The study concluded that more research is needed to understand consumer purchasing behaviour at different geographical scales, including other cities, regions, and countries, because different behavioural patterns may be observed. (Gustavo Quiroga Souki 2016).

Ladislav Skoepa and Kamil Pcha investigated consumers' purchasing behaviour and decision-making process for bread as a bakery product. The most important factors influencing bread selection are freshness, appearance, and price.

According to the study, the importance of price increases with increasing age and decreases with increasing consumer income. The study emphasised the importance of brand, reference, and recommendation from family and friends in bakery brand selection (Skoepa & Pcha, 2016).

In the study, consumption, preferences, and judgements of baked goods were investigated in a sample of Isparta city centre residents. The information was gathered from 288 customers using theAccording to research findings, 2.43% of consumers did not consume biscuit products, 2.78% did not consume pasta and varieties, 9.72% did

not consume wafers, 1.39% did not consume bread and 24.31% did not consume pastries and species. It was discovered that 1.04% of those polled consumed biscuits, wafers, and bread, 0.69% consumed pasta, and 9.72% did not consume cake. The consumers polled discovered that the frequency of biscuit purchases ranged from once a week to fifteen days, the frequency of pastry purchases ranged from once a week to fifteen days, the frequency of purchasing wafers was fifteen days and the frequency of purchasing pasta was fifteen days (GUL H, et al 2017).

A study was conducted by (mayura kathane et al, 2017) to assess the purchasing habits of bakery products.

Bread, buns, biscuits, cake, and pizza bread are popular among working and non-working women in Nagpur. Bread was the most popular bakery item, followed by biscuits, cakes, and cookies. Pizza bread and buns had no significant association with women's working status (p=0.293). Working and non-working women's educational qualifications were significantly associated with the purchase of bread (p=0.000), biscuits (p=0.000), and cake (p=0.010). Age, family size, earning members, and monthly income did not show any significant associations with purchasing patterns for any of the bakery products.

Strategies for curbing the TFA content in foods.

The use of surfactants/ emulsifiers, organo-gels, structured emulsions, chemical/enzymatic inter-esterification, fractionation of tropical oils, trait modification of oils, and modified hydrogenation process are just a few options for lowering the TFA content of oils (Fattahi-far, Sahari, and Barzegar, 2006).

With mandatory TFA labelling in place, the food and oil industries have been looking into alternative strategies to reduce TFA content in their products.

Technically, it is difficult to reformulate fats to exclude TFA while retaining structural and sensory properties such as plasticity, mouth-feel, and flavour. Rather than reducing TFA to zero, some manufacturers have experimented with TFA alternatives with a targeted fatty acid profile.

The food/oil industry has developed and implemented numerous technologies for producing zero/low trans fat foods, including:

Modified hydrogenation process for obtaining low trans PHOs:

A method has been developed to selectively prevent the formation of trans stereoisomers during the hydrogenation of edible oils with less than 10% TFA by using conditioned nickel as a catalyst (Higgins, 2007). Additionally, they showed how to hydrogenate soybean oil using supercritical carbon dioxide, hydrogen, and nickel catalyst to produce a product with low TFA levels. Wright et al. (2003) used mixed metal catalysts (nickel + palladium) to hydrogenate canola oil at lower temperatures that favour the retention of cis-isomers (TFA being around 11%).

Use of tropical/fractionated tropical oils:

Fractionation of high SFA containing tropical oils (SFA ~50%; solid/semi-solid at RT) offers trans free options suitable for margarines, spreads, shortenings and confectionary fats as well as for deep-frying; these include palm, palm kernel and coconut oils (Dhaka, V, et al 2011).

Unsaturated olein and saturated stearin fractions from palm oil are two examples of application-specific fractions with varying melting points that can be separated through physical fractionation. Through fractionation, oils with hardly any impurities can be obtained.

Interesterification of mixed fats:

Interesterification, which involves repositioning fatty acids on triglyceride molecules of mixed fats/oils, is another practical method for creating customised fats with different melting points (Remig et al., 2010).

Chemical interesterification - change the triacylglycerols' physical characteristics, crystallization/melting behaviour, solid fat content, and texture; it is a tried-and-true method for obtaining low or no trans fats.

The procedure does not involve isomerization or saturation of fatty acids, in contrast to partial hydrogenation. An excellent method for obtaining zero trans fats with the desired properties is oil interesterification with hard fats. PHOs can be effectively replaced by chemical interesterification, especially in shortenings and confectionery products. However, the lack of TFAs makes it extremely difficult to obtain suitable solid fat content curves, plasticity, crystallisation, and textural properties.

Enzymatic Inter-esterification - The use of enzymes is a novel method for producing edible fats and oils. Liquid oils and fully hydrogenated vegetable fats are combined and then passed through a series of reactors that contain an immobilised, sn-1,3-specific enzyme. To achieve the desired traits, the resulting or rearranged oil can be used alone or combined with other liquid oils or hydrogenated fats (List, 2014). In a study, trans-selective Candida antarctica lipase A was used as the biocatalyst for lipase-catalyzed selective hydrolysis, which purportedly removed nearly 73.3 percent of the total TFAs at 46.5 degrees of hydrolysis to produce low/zero trans products from partially hydrogenated soybean oil (Jala, Xu, and Guo, 2013).

trans Fat related Regulations and Recommendations –

Trans-fatty acids are undesirable, is harmful to our health, and are frequently associated with a wide range of illnesses and disorders. By 2023, WHO and its Member States hope to have eradicated industrially produced TFA from the world's food supply chain.

According to most recent WHO (2018) estimates, TFA intake is linked to an annual global mortality rate of more than 5,00,000 people from CVDs.

2.4 billion people in 32 countries are currently subject to mandatory TFA policies, which will cover 32% of the world's population by 2020. Two of these, covering 36 million people, recently passed best-practice policies that will go into effect over the next two years. Of these, 14 countries (covering 589 million people, or nearly 8% of the world's population) have best-practice policies in place.

Best-practice TFA laws have also been adopted by 26 additional nations, covering 779 million people, and they will go into effect in the following two years. Furthermore, 62 countries have national policies, strategies, or action plans in place that express a commitment to reducing TFA in the food supply, while 27 countries have additional complementary measures already in place. For the remaining 64 nations, either no action has been taken to end TFA or the situation is unknown (WHO, 2020)

WHO identified TFA elimination as one of the priority targets in its 13th General Programme of Work (GPW13). In 2018, WHO called for the global elimination of industrially produced TFA by 2023.

In light of the numerous harmful health effects of TFA, WHO (2018) decided to remove it from the global food supply chain. As a result, they planned an action package called

REPLACE and the adoption of necessary laws to impose strict limitations on the amount of trans fat in oils, fats, and other food products. This six-step action plan, called REPLACE, mentions

Review sources of industrially produced TFA and the landscape needed for necessary changes in the policy.

Promote substitution of industrially produced trans fat with healthier fat/oil options

Legislate or enact regulatory actions to eliminate industrially-produced trans fat.

Assess and monitor trans fat content in the food-supply chain and bring about reduction in TFA consumption by the populations.

Create awareness regarding the adverse health effects of trans fat among policymakers, manufacturers, suppliers and the general public.

Enforce compliance with effective policies and strict regulation.

By 2023, the WHO plans to completely remove synthetic TFA from the world's food supply. The "Generally Recognised as Safe" (GRAS) designation of PHOs has already been revoked by the US Food and Drug Administration (2015).

CHAPTER 3

SCOPE OF INVESTIGATION

Based on literature survey, the present study entitled "Situational analysis of consumption of bakery foods among university residential students and study the trans fat content and sensory properties of bakery products prepared with Interesterified (IE) fat". was undertaken with the following scope of investigation.

- To determine the Knowledge, attitude and practices related to bakery foods amongst Residential University Students
- To procure 5 bakery foods prepared with IE fats and regular shortening from 2 selected bakeries
- To conduct comparative sensory trials of selected bakery foods prepared with regular shortening and Interesterified Fat (IE) fats.
- To analyse the trans-fat content of the commonly consumed bakery foods prepared with regular shortening

Phase 1 - Situational analysis on KAP (Knowledge, attitude and practices) related to bakery foods amongst Residential University Students.

1) To determine the KAP (Knowledge, attitude and practices) related to trans fat and bakery foods amongst Residential University Students.

2) To collect detailed information on Background of the subjects, Medical History, frequency of Consumption of bakery products by means of semi structured questionnaire (Google Form).

Phase II - Trans-fat Analysis of the commonly consumed bakery foods.

1) 5 most commonly consumed bakery foods from questionnaire responses were selected.

2) Trans Fat analysis using Gas Chromatography GC technique (Talal & Jumat, 2013).

Phase III - Product Development of selected bakery foods with IE fats Replacing regular shortening and its Trans Fat Determination.

1) Two bakery were identified who volunteered to prepare the regular and modified selected bakery products

2) Trans Fat analysis using GC technique (Talal & Jumat, 2013).

Phase IV - Comparative sensory trials of selected bakery foods prepared with IE and regular shortening.

1) Sensory trials and Physico chemical properties of bakery foods with regular shortening and IE fats was conducted using Semi-trained panel members (n = 25) using a 9 point hedonic rating scale.

CHAPTER 4

METHODS AND MATERIALS

In the year 2023, the goal was to reduce consumption and usage of trans fat with the help of the REPLACE plan, which was launched by the WHO and the Food Safety Standards Authority of India (FSSAI). As bakery industry and its consumption is growing at very rapid pace, there is a need to investigate alternative, healthier approaches to lower the trans-fat level of baked goods.

The present study's goal is to find out how frequently consumers eat baked goods made with bakery shortenings heavy in total fats and study their sensory properties prepared with regular shortening and IE fat. The various methods and materials required to fulfill these objectives are presented under the following Heads.

Section 4.1.1 Statutory Clearance of the Study.

Section 4.1.2 Type of Study and Determining Sample Size.

Section 4.1.3 Situational Analysis on Knowledge, Attitude and Practice of University Residential Students towards Bakery Products.

Section 4.1.4 Exclusion and Inclusion Criteria to Participate in Study.

Section 4.1.5 Statistical Analysis.

Section 4.2.1 Procurement of Bakery food products from regular bakery shortening and Interesterified fat (IE).

Section 4.2.2 Trans Fat Analysis of bakery products Prepared from Regular Fat Shortening and Interesterified fat (IE) using Gas Chromatography (GC)

Section 4.3.1 Selection and Training of the Panelist for Organoleptic Evaluation.

Section 4.3.2 Conducting sensory evaluation of selected bakery foods.

Section 4.3.3 Statistical Analysis.

4.1.1 Statutory Clearance of the Study.

Ethical Clearance was obtained from the Ethical Committee – Institutional Ethics Committee for the Human Research (IECHR), Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda, Vadodara. Ethical Approval number IECHR/FCSc/MSc/2022/43 was obtained for conducting this study.

4.1.2 Type of Study and Determining Sample Size.

Study Design used in this study is a cross sectional in nature and sampling technique applied here is Random sampling/ Purposive Sampling.

The sample size calculation is based on the study (Gažarová M et.al 2018) on consumption pattern of bakery foods & this study had a sample size of 120.

Considering 20% Non- response Rate, the final sample size was estimated as 150

4.1.3 Situational Analysis on Knowledge, Attitude and Practice of University Residential Students towards Bakery Products.

Total of 160 University Students comprising of Males (n =83) and Females (n =77) Residing in M.S.University Hostels of age group 18 to 28 years old were screened for Knowledge, Attitude and Practice of University Residential Students towards Bakery Products using Semi structured Questionnaire through Google form. (Annexure 1 & 2)

- **4.1.3.1 Administration of Interviewer – Based Questionnaire.**
  Respondents were Briefed about the Objective of the Study and were motivated to Participate in the study. Questionnaire were administered to the Respondents and information was obtained.
  General information regarding age, gender, Presence of Diseases was collected using Google form.

- **4.1.3.3 Consumption Pattern Specific Questionnaire**
  A detailed Questionnaire was developed to gather information upon Frequency of Consumption of Bakery Foods on Daily, Weekly, 2-3 times a week, Fortnightly, Rarely and Never was collected (Annexure 2).

4.1.4 Exclusion and Inclusion Criteria to Participate in Study.

- **4.1.4.1 Exclusion Criteria**

  Students not willing to participate.

- **4.1.4.2 Inclusion Criteria**

  The students should be part of the University Hostel.

4.1.5 Statistical Analysis

Collected data were entered into a Microsoft excel sheet. Data analysis was performed using Statistical Package of social Sciences (IBM SPSS Statistics 25). Results were expressed in terms of mean standard deviation and Student t test was used to determine the difference between the Consumption Pattern of Bakery foods in males and females.

4.2.1 Procurement of Bakery food products from Regular bakery shortening and Interesterified fat (IE).

Preparation of Two Set of Bakery Products, one from Normal Fat Shortening and one with Interesterified Fat. These include Maskabun, Khari, Cream Roll, Naan-Khatai and Butter biscuit (Makhaniya).

With the help of the Vadodara Municipal Corporation and Food Inspector. Two bakeries from Baroda city (Goodies and Muffins) were approached for preparing total of five bakery foods using regular bakery shortening (ALFA) and Interesterified vegan fat (EMKAY) procured from amazon.

Plate 1

Interesterified fat (IE) used for making Bakery Products

Plate 2

Regular fat used for making Bakery Products

Plate 3

khari prepared from IE fat (A) and regular fat (B)

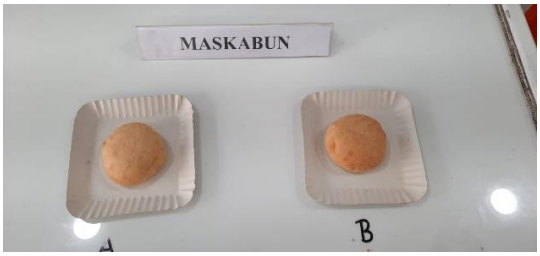


Plate 4

Maskabun prepared from IE Fat (A) and regular fat (B)

Plate 5

Naan Khatai prepared from IE fat (A) and and regular fat (B)

Plate 6

Cream roll prepared from Regular Fat (A) IE fat (B)

Plate 7

Butter biscuit prepared from IE fat (A) and regular fat (B)

Section 4.2.2 Trans Fat Analysis of bakery products Prepared from Regular Fat Shortening and Interesterified fat (IE).

Fat extraction and determination of total fat content of the Bakery foods.

Analysis of the Trans Fat used for Baking/extracted from food items for various sensory parameters:

The fat samples used for Baking Bakery products under varying conditions were analysed for the fatty acid profile including TFA content and total fat content for bakery foods Maskabun, Khari, Naan khatai, Cream roll, Butter biscuit.

Estimation of fatty acid profile including TFA content: The samples of fat used for frying or the oil extracted from the food items were methylated and were derivatized into Fatty Acid Methyl Esters (FAMEs).

Preparation of FAMEs was carried out because:

It is difficult to analyse fatty acids in their free/non-derivatized form as well as to assess as these highly polar compounds which tend to form hydrogen bonds resulting in adsorption.

• FAMEs are prepared to differentiate even minor changes in UFAs; thus, the polar carboxyl functional groups must be neutral.

• FAMEs improve sample stability and speed up quantification for further analysis. The esterification reaction involves the condensation of the carboxyl group of an acid and the hydroxyl group of an alcohol, and it works best in the presence of a catalyst (such as boron trichloride). The catalyst protonates an oxygen atom in the carboxyl group, increasing the acid's reactivity. With the loss of a water molecule, an alcohol reacts with the protonated acid to form an ester. The length of the alkyl chain in the resulting esters is determined by the alcohol used (methanol produces methyl esters, while ethanol produces ethyl esters).

• Methyl esters are the most commonly studied methylation derivatives; during this step, the fatty acids to be derivatized become sufficiently volatile to elute at reasonable temperatures without thermal decomposition. The pre-existing ester bonds in complex lipids are hydrolyzed to release free fatty acids, which are trans-methylated to form fatty acid methyl esters. (Detaillats and Cruz Hernandez), 2016).

The most precise and practical method that makes use of fatty acid methyl esters is gas chromatography. With IR and FTIR spectroscopy techniques, it is rather challenging to distinguish the trans-forms from their cis-counterparts. Highly polar stationary phase, such as SP-2560, SP-2340, OV-275, BPX-70, and CP-SIL-88, are required to separate trans- and cis-forms. A capillary column with a longer length (100 or 120 metres) is used to effectively isolate the cis and trans isomers with better resolution. The application of GC is limited to volatile substances, which makes it unsuitable for samples that are thermally labile. Additionally, a suitable detector is needed to identify the peaks, and some samples need to be meticulously prepared.

The GC technique has been used in the current study to assess the fatty acid profile and estimate the TFA content of the chosen bakery foods in order to achieve greater accuracy in trans fatty acid isolation. Chromatography of Gas The distribution of various molecules of the compounds being separated between the mobile gas phase and the stationary phase is the basis for compound separation in gas chromatography (GC). The direct separation and analysis of gaseous samples, liquid solutions, and volatile solids that sublime can all be done using this innovative and adaptable technique. The techniques of derivatization in GC can be used if the samples to be analysed are non-volatile. The following are the distinctive qualities of gas chromatography:

• **Analytical Technique**: It is used for both quantitative and qualitative evaluations of the various components in a sample.

• **Physical research technique**: It can be used to investigate a variety of GC-related topics, including the determination of partition coefficients, the operation of thermodynamics, and isothermal adsorption.

• **Preparative Technique**: The GC system can be scaled-up to isolate and quantify various fatty acids after the identification of harsh analytical conditions.

• **Online Monitoring Probe**: A gas chromatograph can be fixed in the system, allowing for daily monitoring of the processing-stream.

• **Automated System:** The auto-sampler GC can be connected to a computer to enable overnight operation of the GC. The estimation of fatty acids, triglycerides, cholesterol/other sterols, alcohols, gases, solvent analysis, water, simple sugars, oligosaccharides, amino acids, pesticides, herbicides, food additives, antioxidants, nitrosamines, drugs, flavour compounds, and many other substances has been done using gas chromatography. Hardware for gas chromatography: The gas supply system, regulators, injection port, oven, column, detector, electronics, and recorder/data handling system are the main components of a GC.

• Gas supply systems: A source of carrier gas is necessary for the gas chromatograph. High purity gases must be used, and regulators, gas lines and fittings must all be of good quality. Regulators should have stainless steel diaphragms rather than polymer ones because polymers emit volatiles that could cause peaks to appear in the analytical

run. To filter out any moisture or contaminants from the incoming gases, there should be traps in the gas lines.

Injection port: It serves as a location for the introduction of the sample, its vaporisation, and possibly some dilution and splitting. The majority of materials analysed by GC are liquid samples, and these are always injected using a syringe (either manually or automatically). A soft septum in the injection port creates a gas-tight seal but is also permeable to a syringe needle for sample introduction. For the sample to pass through the column and undergo separation, it must be vaporised in the injection port. The injection port might also split the injection so that only a portion of the analyte enters the column as well. In order to enable effective chromatography, the injection volume might need to be decreased due to the capillary columns' limited capacity. Using a manual syringe technique or an automated sampling system, solution or gas may be injected into the injection port for the sample. The most common single source of error or low precision in GC analysis is manual sample injection. Since ten-microliter syringes are more robust than micro syringes, they are frequently used, and sample injection volumes typically range from 1 to 3 µl.

Section 4.3.1 Selection and Training of the Panelist for Organoleptic Evaluation.

a. Screening of semi trained panelist: In this section panel members were selected and trained further. Threshold test was conducted to select the panel members. Threshold test was conducted among the Students from the Department of Foods and Nutrition, From Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda, Vadodara to determine the Panelists. Threshold is defined as a point on the stimulus scale at which transition in a series of Sensations or Judgements occurs. It measures an Individual ability to smell, taste or feel specific characteristics which is required for evaluating food products during Research and Development. In this study, while conducting Threshold test, panel members were given different degree of three types of Solutions namely – Sweet, Sour and Salty. Individual were asked to taste it and Determine its Strength in Varying Degree (Mild, Strong, Very Strong etc.) and mention in the form (Annexure 4) given to them. Subjects who passed the Threshold test were included to conduct the Organoleptic Evaluations of Bakery Food Products Developed from Normal Fat and Interesterified fat.

b. Development of Score Card for Organoleptic Evaluation – Hedonic Scale was used to score Bakery food products developed from both Normal fat and Interesterified Fat (IE) criteria such as Colour and Appearance, Aroma, Texture, Aftertaste taste, Taste, Mouthfeel, Overall acceptability.

Section 4.3.2 Conducting sensory evaluation of selected bakery foods.

Five bakery foods namely (Khari, Butter biscuit (Makhaniya), Maskabun, Cream Roll, Naan khatai) thus, each prepared from Interesterified fat (IE) and Regular Fat were presented to 25 semi-trained Panel members. Samples were coded before the presentation and panelist were asked to rate each sample using the 9-scale hedonic scale for 6 sensory attributes. A sample of score card is presented in (Annexure 5). The plates sharing the evaluation of the bakery foods prepared for Regular Fats and Interesterified Fats (IE) is shared in plates.


Plate 8 - Panelist performing Sensory Evaluation.

Section 4.3.3 Statistical Analysis.

Collected data were entered into a Microsoft excel sheet. Data analysis was performed using Statistical Package of social Sciences (IBM SPSS Statistics 25). Results were expressed in terms of mean standard deviation. Students t test was performed to find the difference KAP , various sensory attributes.

CHAPTER 5

RESULTS

Presently, urban Indian population is going through a phase of dietary transition; people have started opting for commercially available packaged foods or quick homemade foods (Misra et al, 2009a). These snacks often regarded as "comfort/ convenience/ ready to eat foods" are quickly prepared or are available commercially anywhere anytime. In view of paucity of data relating to trans fatty acid in Bakery foods , the present study has been carried out to elicit information regarding the consumption of bakery foods among students residing in the M.S. University hostel and replace the fat used to prepare these products with Interesterified (IE) fat and study their sensory properties and finally determine their trans fat content and total fat content.

For the study, the subjects were enrolled from the University Residential Population. The Detailed Methodology regarding the selection of subjects is given in the methods and material chapter. The Results of this Section are discussed under the following broad Heads.

5.1. Background Information, Medical History of the Subjects.

5.2. Knowledge of the Students Regarding the Trans - fat.

5.3. Attitude & Practice of the Students Regarding Trans Fat Consumption.

5.4. Consumption Pattern of the of Bakery foods among the university Residential Students.

- 5.4.1. Types of Bakery foods Consumption
- 5.4.2. Frequency of Consumption of Bakery Foods

5.5. Sensory Evaluation

5.6. Trans Fat analysis and Total fat content.

1. Background Information, and Medical History of the Subjects.

Background Information of the subjects was collected using a pre – tested Questionnaire through Google Form and included information on Religion, Anthropometric Measurements, Name of the Hostel Residing. Table 5.1 Shows the Background information of the Subjects.

The Mean age of the subjects was about 20.6 years. Majority of the Study population (88.1%) followed Hinduism while (5 %) of the subjects followed Muslim Religion. Among all the Subjects level of illiteracy was nil.

The Self-reported medical history of the subjects given in Table 5.1. showed that the prevalence of PCOD/PCOS among the Female respondents was 3.7% followed by 1.2% of Hypertension and 5% Others.

Table 5.1 Background Information of the subjects (N, %)

(Religion, Medical Conditions)

Variables	**Males N = 83 (51.8)**	**Females N = 77 (48.1)**	**Total N = 160**	**%**
Mean age Years	20.5	20.6		
Religion				
Hindu	74 (89.1)	67 (87.1)	141	88.1
Muslim	1 (1.2)	7 (9.9)	8	5
Christian	1 (1.2)	0	1	0.6
Jain	1 (1.2)	3 (3.8)	4	2.5
Others	5 (6.02)	1 (1.2)	6	3.7

Medical Condition				
Diabetes	0	0	0	
Hypertension	1	1	2	1.2
	(1.2)	(1.2)		
PCOD/PCOS	0	6	6	3.7
		(7.7)		
Others	5	3	8	5
	(6.02)	(3.8)		

5.2 Knowledge of the Students Regarding the Transfat.

The knowledge of the students regarding the transfat was assessed using the structured questionnaire is shown in table 5.2.

Table 5.2: Knowledge of the Students Regarding the Transfat.

5.3. Attitude & Practice of the Students Regarding Trans Fat Consumption.

Sr. no	Knowledge of Students	Male N = 83			Female N = 77		
		Yes	No	Maybe	Yes	No	Maybe
1	Do you know the term "trans fat"?	41 (49.3)	42 (50.6)		53 (68.8)	24 (31.1)	
2	Do you know that trans - fats has severe adverse effect on health?	37 (44.5)	46 (55.4)	44 (53.1)	43 (55.8)	34 (44.1)	
3	Is there any difference between natural and artificial/industrial produced trans fats?	39 (46.9)	7 (8.4)	37 (44.5)	38 (49.3)	6 (7)	33 (42.8)
4	Are industrial produced trans fats hazardous?	34 (40.9)	9 (10.8)	40 (48.1)	45 (58.4)	4 (5.1)	28 (36.3)
5	Do you know about partially hydrogenated vegetable oils (PHVO)?	20 (24.9)	54 (65.6)	9 (10.8)	34 (44.1)	39 (50.6)	4 (5.1)
6	Do you know about REPLACE initiative taken by WHO?	17 (20.4)	66 (79.5)		17 (22.07)	60 (77.9)	
7	Do you have any idea about FSSAI initiative to eliminate trans fats in India?	17 (20.04)	66 (79.5)		21 (27.2)	56 (72.7)	
8	Do you know about Interesterified Fat?	11 (13.2)	72 (86.7)		11 (14.2)	66 (85.7)	

Table 5.3 shows that Majority of the students (53.1%) check labels on the packaged products and about 46.8% of the population do not check label on packaged Product. When asked about purchase of packaged food items, 77.5% of the subjects said that they do not buy the unpackaged food items. About 33.3 % of the Subjects Reported that

they will appreciate the Bakery products made from IE Fats while 44.3% of the Subjects will may or may not Appreciate the Bakery Products have made from IE fats. About 47.5% Reported that they will accept the Bakery products made from IE fats if the product cost 10 – 15 Rs Higher as taking Health as major concern.

Table 5.2: Knowledge of the Students Regarding the Transfat.

Table 5.3 – Attitude & Practice of Students Regarding Bakery Products

Sr. no	Attitudes & Practice of Students	Male N = 83			Female N = 77		
		Yes	No	Maybe	Yes	No	Maybe
1	Do you check label on packaged products?	51 (61.4)	32 (38.5)		34 (44.1)	43 (55.8)	
2	Do you buy unpackaged food items?	24 (28.9)	59 (71.08)		12 (15.5)	65 (84.4)	
3	Would you Appreciate if Bakery Products are made From IE fats?	34 (40.9)	13 (15.6)	36 (43.3)	28 (36.3)	14 (18.1)	35 (45.4)
4	Will you prefer to buy Bakery Products made with Interesterified Fat (IE) if the price is higher than the regular Bakery Products by 10 - 15 Rupees?	44 (53.01)	13 (15.6)	26 (31.3)	34 (44.1)	10 (12.9)	33 (42.8)
5	Will you prefer to buy bakery products made with IE fats if the taste is altered than the regular bakery products?	39 (46.9)	17 (20.4)	27 (32.5)	27 (35.06)	16 (20.7)	34 (44.1)

5.4 Consumption Pattern of the of Bakery foods among the university Residential Students.

The Dietary and Purchasing practices of the subjects were assessed using structured questionnaire.

Table 5.4 shows the dietary practices followed by the subjects. The Information Revealed that majority of the subjects Reported to consume Biscuits (38.1%) Followed by Breads (22.5%), Cookies (21.8%) at Interval of 2-3 times a week. While some Subjects (8.7%) Consumed the Biscuits on daily basis Followed by Breads (5.6%), Cookies (5.1%). It was also Observed that weekly consumption of Flaky pastry/ Indian Khari among the subjects was 23.1%.

Table – 5.4 -Frequency of consumption Pattern of the of Bakery foods among the university Residential Students.

Sr no	Types of food	Daily			2 – 3 times a week			Weekly			Fortnightly			Rarely			Never		
	Types of foods	Male N=83	Female N=77	Total	Male N=83	Female N=77	Total	Male N=83	Female N=77	Total	Male N=83	Female N=77	Total	Male N=83	Female N=77	Total	Male N=83	Female N=77	Total
1	Breads	7	2	9 (5.6)	16	20	36 (22.5)	22	4	26 (16.2)	0	8	8 (5)	18	15	33 (20.6)	2	1	3 (1.8)
2	Biscuits	10	4	14 (8.7)	30	31	61 (38.1)	13	20	33 (20.6)	6	7	13 (8.1)	10	8	18 (11.2)	0	2	2 (1.2)
3	Cookies	4	1	5 (3.1)	18	17	35 (21.8)	18	15	33 (20.6)	9	8	17 (10.6)	18	27	45 (28.1)	2	4	6 (3.7)
4	Puff / Flaky Pastry	1	0	1 (0.6)	8	4	12 (7.5)	16	21	37 (23.1)	7	15	22 (13.7)	33	25	58 (36.2)	4	7	11 (6.8)
5	Indian Khari	2	0	2 (1.25)	8	6	14 (8.75)	17	9	26 (16.2)	6	18	24 (15)	27	24	51 (31.8)	9	15	24 (15)
6	Toast	0	1	1 (0.6)	7	8	15 (9.3)	17	11	28 (17.5)	10	14	24 (15)	17	28	45 (28.1)	5	10	15 (9.3)
7	Benne Biscuits/ Butter	2	3	5 (3.1)	4	3	7 (4.3)	15	9	24 (15)	13	13	26 (16.2)	25	25	50 (31.2)	10	19	29 (18.1)
8	Pizza	0	0	0	2	5	7 (4.3)	13	7	20 (12.5)	13	16	29 (18.1)	34	38	72 (45)	7	6	13 (8.1)
9	Cake	0	0	0	3	2	5 (3.1)	13	8	21 (13.1)	7	14	21 (13.1)	42	47	89 (55.6)	4	1	5 (3.1)

5.5. Sensory evaluation of bakery foods made from Regular fat and Interesterified fat (IE).

5.5.1 Color and Appearance:

As seen in (table 5.5) no significant difference was seen in any of bakery products prepared from regular Fat and with Interesterified fat (IE) with respect to Color and Appearance except Naan khatai and Butter biscuits Prepared from IE fat which were better than those prepared from regular fat and this difference was statistically significant. ($p<0.5$).

Table 5.5 – Colour & Appearance of Bakery Products Prepared from IE fats and Regular fats.

Sr. no		IE Fats	Regular Fats	% Difference	t-test
1	Maskabun	7.53 ± 1.14	7.0 ± 1.89	7.03 ↑	0.26 ^{NS}
2	Khari	7.2 ± 1.6	7.8 ± 1.22	7.69 ↑	0.11 ^{NS}
3	Naan khatai	7.96 ± 0.98	7.16 ± 1.29	10.05 ↓	0.01**
4	Cream roll	8.06 ± 1.06	7.66 ± 1.19	4.96 ↑	0.18 ^{NS}
5	Butter Biscuit (Makhaniya)	8 ± 0.85	7.33 ± 1.13	8.37 ↓	0.01**

Note : NS = non – significant,*= <0.05**,** = <0.01.

5.2 Aroma:

As seen in (table 5.6) no significant difference seen in any of bakery products prepared from regular Fat and with Interesterified fat (IE) with respect to Aroma.

The aroma of all the Bakery foods except Khari Prepared from IE fat was slightly better than those prepared from regular fat. However, difference was statistically significant. ($p<0.5$).

Table 5.6 – Aroma of Bakery Products Prepared from IE fats and Regular fats.

Sr. no		IE Fats	Regular Fats	% Difference	t-test
1	Maskabun	7.13 ± 1.05	6.8 ± 1.28	4.62 ↑	0.29 ^{NS}
2	Khari	6.63 ± 1.88	7.56 ± 1.37	12.3 ↓	0.029*
3	Naan khatai	7.86 ± 1.08	7.66 ± 1.27	2.54 ↑	0.52 ^{NS}
4	Cream roll	7.6 ± 1.30	7.1 ± 1.22	6.57 ↑	0.16 ^{NS}
5	Butter Biscuit (Makhaniya)	7.56 ± 1.11	7.46 ± 1.32	1.32 ↑	0.75 ^{NS}

Note : NS = non – significant,*= <0.05**,** = <0.01.

5.5.3 Texture.

As seen in table 5.7, no significant difference was seen in most of bakery products prepared from regular Fat and with Interesterified fat (IE) except butter biscuits whose texture was significantly better for those prepared from IE fat. Khari prepared from regular fat was significantly better than that prepared from IE fat.

Table 5.7 – Texture of Bakery Products Prepared from IE fats and Regular fats.

Sr. no		IE Fats	Regular Fats	% Difference	t-test
1	Maskabun	7.6 ± 1.14	7.1 ± 1.27	6.57 ↑	0.12 ^{NS}
2	Khari	6.46 ± 1.87	8.33 ± 0.74	1.87 ↓	1.43 ^{NS}
3	Naan khatai	7.73 ± 1.12	7.6 ± 1.22	1.68 ↑	0.66 ^{NS}
4	Cream roll	7.93 ± 1.23	7.6 ± 1.22	4.16 ↑	0.30 ^{NS}
5	Butter Biscuit (Makhaniya)	8.36 ± 0.79	7.73 ± 1.03	7.53 ↑	0.011***

Note: NS=non-significant; *= <0.05,**= <0.01, ***=<0.001

5.5.4 Aftertaste

As seen in (table 5.8) except for khari, no significant difference seen in any of bakery products prepared from regular Fat and with Interesterified fat (IE) with respect to Aftertaste.

The Aftertaste of all the Bakery foods except Khari Prepared from IE fat was slightly better than those prepared from regular fat. However, difference was statistically significant. ($p<0.5$)

Table 5 .8 – After taste of Bakery Products Prepared from IE fats and Regular fats.

Sr. no		IE Fats	Regular Fats	% Difference	t-test	P value
1	Maskabun	7.4 ± 1.17	7 ± 1.52	5.4 ↑	0.26 ^{NS}	
2	Khari	6.16 ± 2.03	7.83 ± 0.96	21.3 ↓	0.0001***	
3	Naan khatai	7.63 ± 1.13	7.7 ± 1.39	0.9 ↑	0.84 ^{NS}	<0.001
4	Cream roll	7.7 ± 1.36	7.2 ± 1.49	6.4 ↑	0.22 ^{NS}	
5	Butter Biscuit (Makhaniya)	8.03 ± 0.83	7.4 ± 1.2	7.84 ↑	0.02 ^{NS}	

Note: NS=non-significant; *= <0.05,**= <0.01, ***=<0.001

5.5.5 Taste

The t-test revealed that there was a Significant difference in Taste (Organoleptic Properties) of Khari prepared from Regular fat and Interesterified fat (IE). Panelist preferred Khari made from Regular Fat rather than Khari prepared from Interesterified Fat**.**

As seen in (table 5.9) no significant difference seen in any of bakery products prepared from regular Fat and with Interesterified fat (IE) with respect to **Taste**.

The **Taste** of all the Bakery foods except Khari Prepared from IE fat was slightly better than those prepared from regular fat. However, difference was statistically significant. ($p<0.5$)

Table 5.9 – Taste of Bakery Products Prepared from IE fats and Regular fats.

Sr. no		IE Fats	Regular Fats	% Difference	t- test
1	Maskabun	7.46 ± 1.14	7.13 ± 1.40	4.52 ↑	0.32 ^{NS}
2	Khari	6.43 ± 2.24	7.76 ± 1.28	17.1 ↓	0.007**
3	Naan khatai	7.76 ± 1.14	7.86 ± 1.28	1.27 ↓	0.75 ^{NS}
4	Cream roll	8.03 ± 1.16	7.46 ± 1.52	7.09 ↑	0.11 ^{NS}
5	Butter Biscuit (Makhaniya)	8.23 ± 0.88	7.63 ± 1.30	7.29 ↑	0.04 ^{NS}

Note: NS=non-significant; *= <0.05,**= <0.01, ***=<0.001

5.5.6 Mouthfeel

As seen in (table 5.10) no significant difference seen in any of bakery products prepared from regular Fat and with Interesterified fat (IE) with respect to **Mouthfeel** for Maskabun and Nankhatai.

Except Khari the **Mouthfeel** of cream roll and Butter biscuit Prepared from IE fat was significantly better than those prepared from regular fat. And this difference was statistically significant. ($p<0.5$)

Table 5.10 – Mouthfeel of Bakery Products Prepared from IE fats and Regular fats.

Sr. no		IE Fats	Regular Fats	% Difference	t- test
1	Maskabun	7.26 ± 1.43	7 ± 1.43	3.58 ↑	0.47^{NS}
2	Khari	6.53 ± 2.12	7.8 ± 1.13	16.28 ↓	0.006***
3	Naan khatai	7.56 ± 1.25	7.73 ± 1.23	2.24 ↓	0.61^{NS}
4	Cream roll	8.03 ± 1.11	7.36 ± 1.47	8.34 ↑	0.05*
5	Butter Biscuit (Makhaniya)	7.7 ± 1.06	7.1 ± 1.35	7.79 ↑	0.05*

Note: NS=non-significant; *= <0.05,**= <0.01, ***=<0.001

5.5.7 Overall acceptability

As seen in table 5.11, except Khari, no significant difference seen in any of bakery products prepared from regular Fat and with Interesterified fat (IE) with respect to Overall acceptability.

And this difference for Khari was statistically significant. ($p<0.01$)

Table 5.11 – Overall Acceptability of Bakery Products Prepared from IE fats and Regular fats

Sr. no		IE Fats	Regular Fats	% Difference	T- test
1	Maskabun	7.4 ± 1.40	7.3 ± 1.57	1.35 ↑	0.86^{NS}
2	Khari	6.6 ± 2.14	8 ± 0.85	17.5 ↓	0.002**
3	Naan khatai	8 ± 0.96	7.9 ± 1.35	1.25 ↑	0.91^{NS}
4	Cream roll	8.06 ± 1.15	7.56 ± 1.43	6.20 ↑	0.14^{NS}
5	Butter Biscuit (Makhaniya)	8.16 ± 1.00	7.66 ± 1.27	0.04 ↑	0.10^{NS}

Note: NS=non-significant; *= <0.05,**= <0.01, ***=<0.001

5.6 Trans Fatty Acid Analysis (TFA) and total fat content (Per 100 gm) of Bakery foods prepared from Interesterified fats (IE) from and Regular Fats.

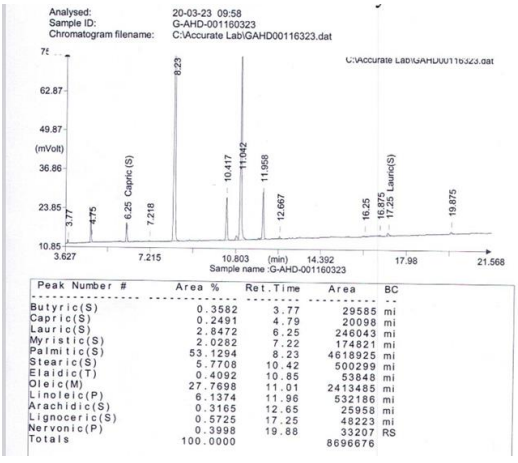
All five 5 foods were subjected to determination of total fat content and trans fat content. (Table 5.12) As can be seen, trans fatty acids was not detected in any of the bakery products prepared from either regular shortening or IE fat. The fat content of the bakery foods range between 40.15 to 22.13 gm/100 gm.

5.12 Trans Fatty Acid Analysis (TFA) and total fat content (Per 100 gm) of Bakery foods prepared from Interesterified fats (IE) from and Regular Fats.

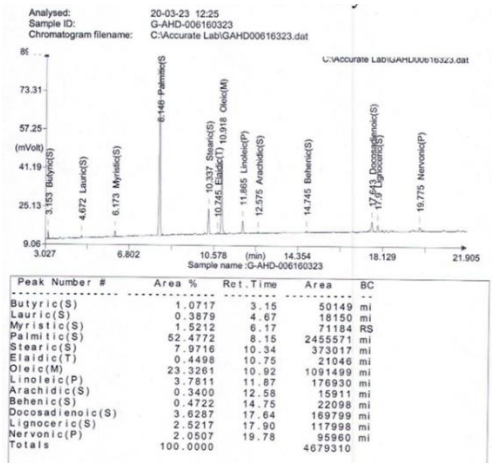
Sr No.	Bakery Products	Interesterified fats		Regular Fats	
		Trans fat gm/100 gm	Trans fat gm/100 gm	Trans fat gm/100 gm	Total fat gm/100 gm
1	Maskabun	N.D.(D.L =0.1)	22.48	N.D.(D.L =0.1)	20.13
2	Khari	N.D.(D.L =0.1)	22.80	N.D.(D.L =0.1)	22.70
3	Naan khatai	N.D.(D.L =0.1)	27.49	N.D.(D.L =0.1)	25..48
4	Cream Roll	N.D.(D.L =0.1)	32.41	N.D.(D.L =0.1)	30.25
5	Butter Biscuit (Makhaniya)	N.D.(D.L =0.1)	40.15	N.D.(D.L =0.1)	39.47

Trans fatty acids (Elidiac Acid) were determined using GC method whose detectable limit was <0.1 . The chromatograms obtained for all the 10 bakery foods are presented below.

Chromatogram obtained for Maskabun Prepared with Interesterified Fat (IE) and Regular Fat. (Plate 9)

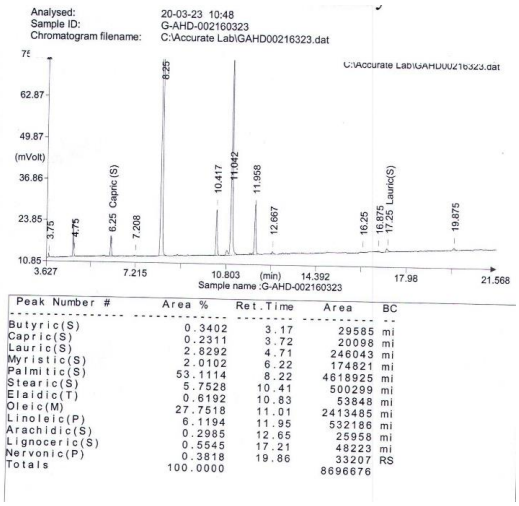


Maskabun (Normal Fat)

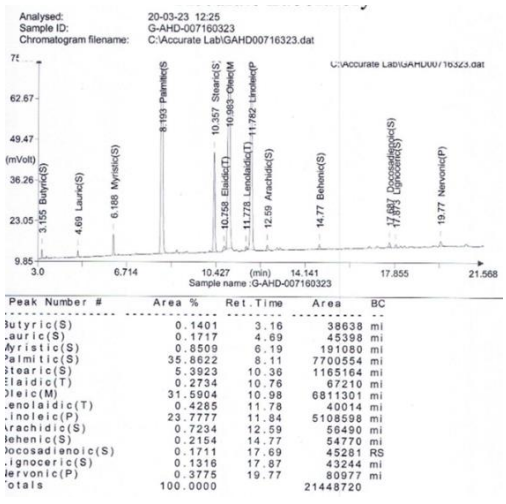


Maskabun (IE) Fat.

Chromatogram obtained for Khari Prepared with Interesterified Fat (IE) and Regular Fat. (Plate 10)



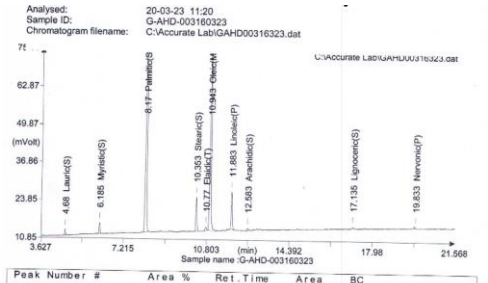
Peak Number #	Area %	Ret.Time	Area	BC
Butyric(S)	0.3402	3.17	29585	mi
Capric(S)	0.2311	3.72	20098	mi
Lauric(S)	2.8292	4.71	246043	mi
Myristic(S)	2.0102	6.22	174821	mi
Palmitic(S)	53.1114	8.22	4618925	mi
Stearic(S)	5.7528	10.41	500299	mi
Elaidic(T)	0.6192	10.83	53848	mi
Oleic(M)	27.7518	11.01	2413485	mi
Linoleic(P)	6.1194	11.95	532186	mi
Arachidic(S)	0.2985	12.65	25958	mi
Lignoceric(S)	0.5545	17.21	48223	mi
Nervonic(P)	0.3818	19.86	33207	RS
Totals	100.0000		8696676	



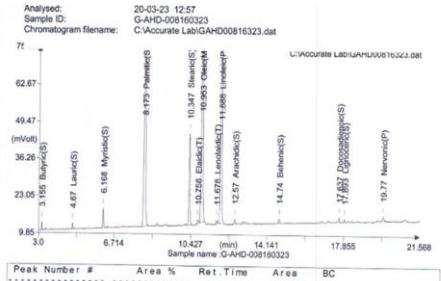
Peak Number #	Area %	Ret.Time	Area	BC
Butyric(S)	0.1401	3.16	38638	mi
Lauric(S)	0.1717	4.69	45398	mi
Myristic(S)	0.8509	6.19	191080	mi
Palmitic(S)	35.8622	8.11	7700554	mi
Stearic(S)	5.3923	10.36	1165164	mi
Elaidic(T)	0.2734	10.76	67210	mi
Oleic(M)	31.5904	10.98	6811301	mi
Lenolaidic(T)	0.4285	11.78	40014	mi
Linoleic(P)	23.7777	11.84	5108598	mi
Arachidic(S)	0.7234	12.59	56490	mi
Behenic(S)	0.2154	14.77	54770	mi
Docosadienoic(S)	0.1711	17.69	45281	RS
Lignoceric(S)	0.1316	17.87	43244	mi
Nervonic(P)	0.3775	19.77	80977	mi
Totals	100.0000		21448720	

Khari (Normal Fat) **Khari (IE Fat)**

Chromatogram obtained for Butter Biscuit (Makhaniya) Prepared with Interesterified Fat (IE) and Regular Fat. (Plate 11)



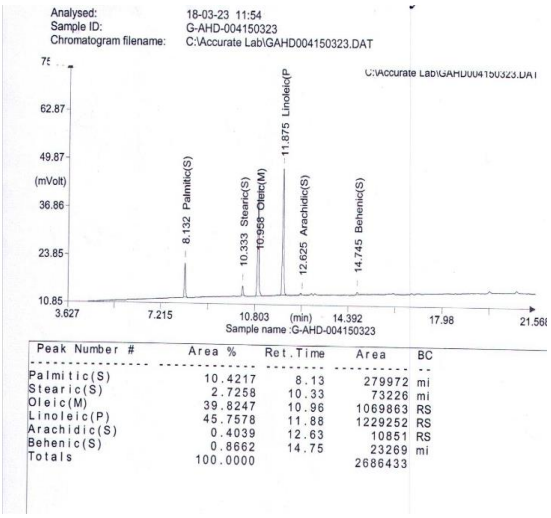
Peak Number #	Area %	Ret.Time	Area	BC
Butyric(S)	0.1010	2.85	6984	mi
Capric(S)	0.0870	3.16	6012	mi
Lauric(S)	0.6144	4.68	42481	mi
Myristic(S)	1.4667	6.19	101404	mi
Palmitic(S)	51.8109	8.17	3582063	mi
Stearic(S)	5.6342	10.35	389533	mi
Elaidic(T)	0.7211	10.77	49856	mi
Oleic(M)	32.3994	10.94	2240009	RS
Linoleic(P)	6.2399	11.88	431408	RS
Arachidic(S)	0.3118	12.58	21556	RS
Lignoceric(S)	0.3522	17.14	24348	RS
Nervonic(P)	0.2615	19.83	18078	RS
Totals	100.0000		6913732	



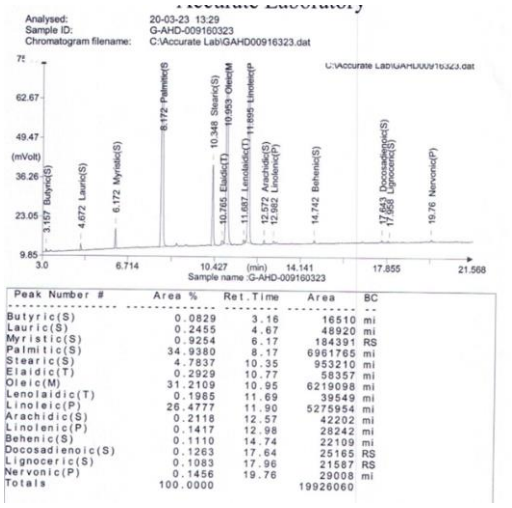
Peak Number #	Area %	Ret.Time	Area	BC
Butyric(S)	0.1801	3.16	38638	mi
Lauric(S)	0.2117	4.67	45398	mi
Myristic(S)	0.8909	6.17	191080	mi
Palmitic(S)	35.9022	8.17	7700554	mi
Stearic(S)	5.4323	10.35	1165164	mi
Elaidic(T)	0.3134	10.76	67210	mi
Oleic(M)	31.7562	10.95	6811301	mi
Lenolaidic(T)	0.1866	11.68	40014	mi
Linoleic(P)	23.8177	11.89	5108598	mi
Arachidic(S)	0.2634	12.57	56490	mi
Behenic(S)	0.2554	14.74	54770	mi
Docosadienoic(S)	0.2111	17.64	45281	RS
Lignoceric(S)	0.2016	17.89	43244	mi
Nervonic(P)	0.3775	19.77	80977	mi
Totals	100.0000		21448720	

Makhaniya (Normal Fat) **Makhaniya (Normal Fat)**

Chromatogram obtained for Creamroll Prepared with Interesterified Fat (IE) and Regular Fat. (Plate 12)



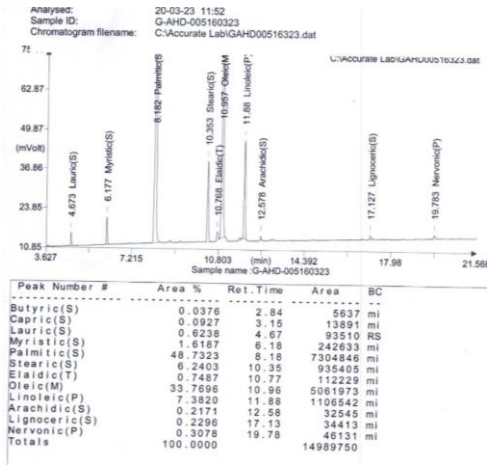
Peak Number #	Area %	Ret.Time	Area	BC
Palmitic(S)	10.4217	8.13	279972	mi
Stearic(S)	2.7258	10.33	73226	mi
Oleic(M)	39.8247	10.96	1069863	RS
Linoleic(P)	45.7578	11.88	1229252	RS
Arachidic(S)	0.4039	12.63	10851	RS
Behenic(S)	0.8662	14.75	23269	mi
Totals	100.0000		2686433	



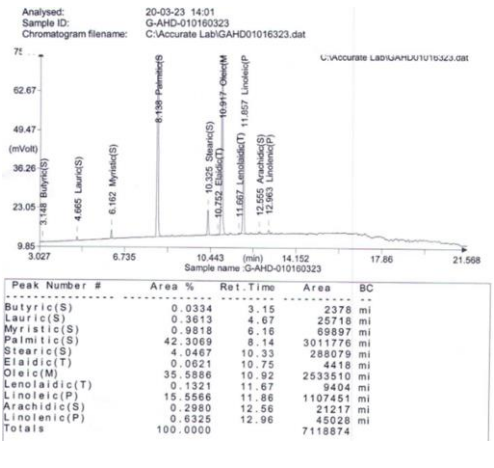
Peak Number #	Area %	Ret.Time	Area	BC
Butyric(S)	0.0829	3.16	16510	mi
Lauric(S)	0.2455	4.67	48920	mi
Myristic(S)	0.9254	6.17	184391	RS
Palmitic(S)	34.9380	8.17	6961765	mi
Stearic(S)	4.7837	10.35	953210	mi
Elaidic(T)	0.2929	10.77	58357	mi
Oleic(M)	31.2109	10.95	6219098	mi
Lenolaidic(T)	0.1985	11.69	39549	mi
Linoleic(P)	26.4777	11.90	5275954	mi
Arachidic(S)	0.2118	12.57	42202	mi
Linolenic(P)	0.1417	12.98	28242	mi
Behenic(S)	0.1110	14.74	22109	mi
Docosadienoic(S)	0.1263	17.64	25165	RS
Lignoceric(S)	0.1083	17.96	21587	RS
Nervonic(P)	0.1456	19.76	29008	mi
Totals	100.0000		19926060	

Creamroll (Normal Fat) **Creamroll (IE Fat)**

Chromatogram obtained for Naan Khatai Prepared with Interesterified Fat (IE) and Regular Fat. (Plate 13)



Peak Number #	Area %	Ret.Time	Area	BC
Butyric(S)	0.0376	2.84	5637	mi
Capric(S)	0.0927	3.15	13891	mi
Lauric(S)	0.6238	4.67	93510	RS
Myristic(S)	1.6187	6.18	242633	mi
Palmitic(S)	48.7323	8.18	7304846	mi
Stearic(S)	6.2403	10.35	935405	mi
Elaidic(T)	0.7487	10.77	112229	mi
Oleic(M)	33.7696	10.96	5061973	mi
Linoleic(P)	7.3820	11.88	1106542	mi
Arachidic(S)	0.2171	12.58	32545	mi
Lignoceric(S)	0.2296	17.13	34413	mi
Nervonic(P)	0.3078	19.78	46131	mi
Totals	100.0000		14989750	



Peak Number #	Area %	Ret.Time	Area	BC
Butyric(S)	0.0334	3.15	2376	mi
Lauric(S)	0.3613	4.67	25718	mi
Myristic(S)	0.9818	6.16	69897	mi
Palmitic(S)	42.3069	8.14	3011776	mi
Stearic(S)	4.0467	10.33	288079	mi
Elaidic(T)	0.0621	10.75	4418	mi
Oleic(M)	35.5886	10.92	2533510	mi
Lenolaidic(T)	0.1321	11.67	9404	mi
Linoleic(P)	15.5566	11.86	1107451	mi
Arachidic(S)	0.2980	12.56	21217	mi
Linolenic(P)	0.6325	12.96	45028	mi
Totals	100.0000		7118874	

Naan Khatai (Normal Fat) **Naan Khatai (IE Fat)**

CHAPTER 6
DISSCUSSION

Global energy imbalances and obesity levels are growing substantially, resulting in an increase in the prevalence of NCDs. Most chronic diseases are common and frequently coexist. Chronic illness risk factors are extremely common among South Asians, particularly the Indian population. Unhealthy diets are one of the most major and modifiable risk factors. While food habits are culturally based, there has been a global shift towards processed meals in recent decades.

Bakery products and their derivatives play an important role in global food consumption. The most popular industrial cereal products are bread, pasta, bulgur, biscuits, cakes, and breakfast cereals. In Turkey, bakery products account for 65% of the food industry .Because of their low moisture content, biscuits are a shelf-stable product that was originally used by sailors, soldiers, patients, and travellers, but they are now consumed by almost every age group and in almost every meal.. The consumption practices of bakery food among the university students in the present study did not reveal high intake as reported by other countries. This could be because of high literacy levels and less pay pockets of the university students who do not spend much on the bakery foods.

. The KAP data in the present study revealed that 58.75 % of the subjects knew about the Trans-fat. While 77.5 % subjects were unaware about adverse effects of trans fat on Health Similar findings were reported by Poonam Khanna et al, 2022 in which it was reported that 92% of participants did not know about the trans fat. They also reported that in a urban commuter college, out of 222 college students 37% of participants reported never checking trans fat information on a food label while consuming which supports our survey, in which 10.5% did not check food labels.

As per the Indian bakery market report, 72% of the sales on Indian bakery market attributed to the biscuit and biscuit industry.

The present study revealed improved sensory characteristics of the bakery products when prepared from IE fats as compared to those prepared from Regular. Similar findings have been reported by (Zhen Zhang et al, 2018 about improved physical-

characteristics of bakery products prepared with IE fat. Improved cake volume with fine structure and clear lifting properties, affirmed the potential for its application in bakery fats.

In the present study, the trans fat content of the bakery foods as analysed by GC method was below the detectable limits of 0.1 gm/100 of fat. However, the trans fat content in many bakery foods across the European countries was found to be in wide ranges. Van et. al. (1998), analysed the fatty acid composition of bakery products from 14 European countries and found that proportion of trans fatty acids in cookies and biscuits ranged from <1 to 28%. Trans fatty acids content in sweet pastry ranged from practically
0 to 33%. Croissants and doughnuts had varying amounts of trans fatty acids, with the highest value of 15% in croissants and 32% in doughnuts. the amount of trans fats in foods exhibited great variation, due to differences in hydrogenation
methods and intensity. In US, the results showed in
that trans fat (g/100 g fat) ranged from 0.0 to 48.8 in bread, cake, and related products; prior to the effective date of the new regulation (Sachchidanandam 2003).

The current study leads us to the conclusion that the trend of low bakery food consumption among university students living on campus is not very high, which is a positive development given the rise in NCD incidences in the world's populations. This was also supported by our study, which found that there was a very low prevalence of hypertension and no incidence of diabetes or heart disease. Additionally, less than <0.1 gm of trans fat per 100 gm of fat are present in bakery products made with regular shortening (palmolein oil). We can therefore draw the conclusion that bakery products made in Vadodara do not require the use of IE fat and can continue to use regular shortening without posing any risk associated with trans fat consumption provided the shortening used is prepared from very low trans fat palmolein oil.

CHAPTER 7
SUMMARY AND CONCLUSION

Rapid economic, demographic and lifestyle transitions have resulted in increased prevalence of lifestyle related non-communicable diseases (NCD) in the developing countries including India. Coupled with strong genetic and environmental influences, imbalanced dietary profile (characterized by high energy, low fibre, high fat - particularly trans fatty acids-TFA; coming from partially hydrogenated vegetable oils) has emerged as an independent risk factor (TFA contain at least one non-conjugated double bond in the trans configuration) (Edward M joy et al 2017) .Owing to their strong association with serious health problems like obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular diseases, complications during pregnancy, hindrance in foetal/ infant growth etc (Emylin U Alejandro et al 2020) ,TFA have been removed from the food supply by many of the developed nations. However, in the developing world, including India, not much work has been done in this regard; and as a result, TFA containing fats (vanaspati/ margarine) continue to exist in the food supply leading to an increased burden of lifestyle related NCD (Shauna M Downs et al 2017).

Of all the countries, India is one of the largest snack markets and people consume more than 400,000 tonnes of snacks every year. These so called “comfort foods” include fried foods and bakery foods that are high in energy (particularly fats) and low in other nutrients (Agrawal et al, 2008).

The FSSAI initiative to eliminate industrially produced trans fat comes at a time when the burden of non-communicable diseases has risen in India and is showing an Increasing trend.

The REPLACE action package provides a strategic approach to eliminating industrially-produced trans fat from national food supplies, with the goal of global elimination by 2023 (WHO 2023).

IE fats were introduced as a suitable replacement as they can provide desirable functional properties, related to the product's melting point, texture and shelf stability. Without such fats, these products would need to be made from high-saturated fat products, such as butter or lard, otherwise they would have very different textural

characteristics; consider the textural and stability challenges in manufacturing biscuits made from rapeseed oil – a softer, less shelf-stable product would result (C.E Mills *et al*, 2017).

The drive for a reduction in industrial *trans* fats in food products was prompted by evidence indicating associations between consumption and undesirable cardiovascular health effects (Mozaffarian *et al*. 2006).

However very few literatures are available upon Trans fat content and food products developed from Interesterified fat in Indian bakery food context.

This study titled **"Situational analysis of consumption of bakery foods among university residential students and study the trans-fat content and sensory properties of bakery products prepared with Interesterified (IE) fat"** is been divided into four phases.

Phase I - Situational analysis on KAP (Knowledge, attitude and practices) related to bakery foods amongst Residential University Students.

Phase II - Trans-fat Analysis of the commonly consumed bakery foods.

Phase III - Product Development of selected bakery foods with IE fats Replacing regular shortening and its Trans Fat Determination using Gas Chromatography (GC) technique.

Phase IV - Comparative sensory trials of selected bakery foods prepared with IE and regular shortening.

And the present study was conducted in collaboration with the Vadodara Municipal Corporation, Gujarat, India.

The results and major highlights of all the phases of the study are summarized below –

6.1. Phase I

This phase of the research was designed to obtain data on Consumption pattern of bakery foods among the University residential students. Which was further divided into Daily, 2-3 times a week, weekly, fortnightly, rarely and never.

Salient features of Phases I

6.1.1 Knowledge, Attitude and Practice of Students Regarding Bakery Products and Trans fat.

- In this phase, semi structured questionnaire was developed to screen individuals for Knowledge, Attitude and Practice Regarding Bakery Products and Trans fat. In which Bakery food frequency consumption pattern was assessed.
- The mean age of the subjects was 20.6 years. Majority (88.1%) of the hostel inmates followed Hinduism while 5 % of the subjects followed Muslim religion. Literacy level of the subjects was 100% None of the subjects suffer from diabetes only 2.4 % subjects suffered from hypertension whereas 7.7 % girls suffered from PCOS.
- It was observed that half of the population know about what the trans fats are. And about 77.5% of subjects were unaware about the adverse effects of trans fat on health.

  It was also noted that 65.6% of population are not aware about the PHVOS.

  About 79.5 % of population don't know about the REPLACE Initiative taken by WHO.

  It was also observed that 86.7% of population don't know about the Interesterified Fat.
- Attitude and Practice of the Students regarding the Bakery Products is shown which reported that majority of the subjects (53.1%) check label on the packaged products and when asked about whether they would prefer to purchase bakery foods made from IE fats, 33.3 % of them reported that they will purchase the bakery products made from IE fats.

  About 47.5% reported that they will purchase the bakery products made from IE fats even if the product cost is 10 – 15 Rs higher, considering health as major concern.
- It was observed that Majority of them reported to consume Biscuits (38.1%) followed by Breads (22.5%), Cookies (21.8%) at Interval of 2-3 times a week. While a few of the subjects (8.7%) Consumed the Biscuits on daily basis followed by Breads 5.6% and Cookies 5.1%, It was on also observed that consumption of Flaky pastry/ Indian Khari among the subjects was 23.1% was on weekly basis

6.2 Phase II

In this phase Trans fat analysis of 5 most commonly consumed bakery foods from questionnaire responses was conducted. And Trans fat analysis was done using GC Technique.

6.3 Phase III

In this phase of the study Procurement of selected bakery foods with IE fats Replacing regular shortening and its trans fat determination using Gas Chromatography (GC) technique was conducted.

Salient features of Phases III

Identification of the Bakeries

- Two bakeries (Goodies and Muffins, Vadodara, Gujarat) were selected by the Food Safety Officer (VMC) which voluntarily participated in preparation and procurement of the bakery products
- Five different bakery products were developed in total. One that contains Interesterified fat (IE) and one with regular fat shortening.
- Bakery products developed were khari, maskabun, naan khatai, cream roll and butter biscuit (Makhaniya).

Phase IV

In this phase of the study, organoleptic evaluation was performed using nine point hedonic scale by 25 semi-trained panel members who qualified the threshold test.

The result on sensory evaluation of Bakery foods with respect to Colour and Appearance, Aroma, texture, taste, mouthfeel and overall acceptability showed that naan khatai and butter biscuit (Makhaniya), and cream rolls prepared from IE fats were much better than those prepared from regular fats ($p<.05$). Khari prepared from regular fat ranked higher ($P<.05$) as compared to that prepared from IE fats for all the sensory attributes. This suggests that IE fats can be used for the preparation of the four bakery foods with good sensory attributes except for Khari.

In this phase of the study, Trans fat analysis was conducted using the Gas Chromatography (GC) technique. It was observed that Trans fatty acids was not detected in any of the bakery products prepared from either regular fat or IE fat.

Total fat content of all the bakery foods ranged between 40.15 g/100 gm (Butter biscuit) to 22.13 g/100 gm (Maskabun).

Hence, null hypothesis was rejected and following alternative hypothesis was accepted.

1. It is possible to prepare selected Bakery foods using IE fats without significant Changes in Sensorial Properties.
2. There will be significant difference in trans fat content of bakery foods prepared with regular shortening and IE Fats.

CHAPTER 8

RECOMMENDATIONS AND FUTURE SCOPE OF INVESTIGATION

The current study is one of the few to evaluate the impact of various bakery food consumption patterns among university residential students and to examine the sensory properties of bakery products made with interesterified (IE) FAT. The study will assist in building a comprehensive database on the habits of consumption of various bakery foods among university residential students.

The laboratory results can assist the government and the industry in developing healthier, less expensive trans-free options for consumers to prepare bakery foods. They can also assist in assessing the quality of bakery foods made by commercial establishments, including those in the unregulated sector and roadside vendors, among others.

Future Scope –

1. To determine the trans fat content of fried foods sold in urban Vadodara.
2. To Determine the trans fat content present in Indian Breads (Naan , Tandoori Roti, Roti, laccha paratha etc)
3. To Determine the trans fat content of Indian fried Snacks.
4. Trans fat content from local bakeries
5. To undertake research on effectiveness of educational modules to generate awareness of trans fat.

CHAPTER 9

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Annexures I

CONSENT FORM

Dear Students,

Greetings of the Day!!!

I, Siddhant Nikam, MSc student working in the Department of Foods and Nutrition, Faculty of Family and Community Sciences, The M. S. University of Baroda. I am conducting my MSc research study under the guidance of Prof. Mini Sheth entitled "**SITUATIONAL ANALYSIS OF CONSUMPTION OF BAKERY FOODS AMONG UNIVERSITY RESIDENTIAL STUDENTS AND STUDY THE TRANS FAT CONTENT AND SENSORY PROERTIES OF BAKERY PRODUCTS PREPARED WITH INTERESTERIFIED (IE) FAT**.".

Abiding by the laws of research ethics, I declare that your answers will be treated with absolute confidentiality only for my research study. The data obtained will not be shared with any third party. If you have any questions or want to receive more detailed information about the survey, please contact me by email at siddhantnikam0311@gmail.com

I understand your time is precious; It will take 15-20 minutes to complete the survey. Therefore, to make this study successful, I humbly request you to please provide your valuable response.

Please help us to circulate the survey link with Friends of your respective Department who are Residing in MS University Hostel.

Information about the Study

The study is based on Trans fat consumption in the University Residential Students. Trans fat is which is present in Bakery food items which we consume on daily basis. The trans-fat is also called as a silent killer present in food which cause several NCD's like CVD, Obesity, cancer and many more Diseases

The study is divided into 4 phases.

For Phase1, You will be required to fill up the questionnaire regarding your eating habits, eating pattern, and dietary intake in a google form after giving your consent to be a part of this study.

In Phase 2, In this, the Pre tested Questionnaire will be analysing top 5 consumed Bakery items.

Which are consuming on Daily, weekly, monthly basis.

The expected outcome of the study includes the improvement in the Bakery items by replacing the trans-fat by IE fat.

Please find the link for the form

https://forms.gle/uMHu1K2zLCYrqai98

Kindly give your consent for participation.

Your participation is essential for the success of the study.

Thank you so much for your efforts and answers!

Sincerely,

Siddhant Nikam — Research Scholar

Prof.Mini Sheth — Guide

Dept. of Foods and Nutrition

Annexures II

Questionnaire Regarding Knowledge, Attitude and Practices with Respect to consumption pattern of Bakery foods among University Residential Students.

Name –

Age –

Sex – Male ☐ Female ☐

Religion –

Hindu ☐

Muslim ☐

Jain ☐

Christian ☐

Other ☐

Phone number –

Department –

Name the Hostel in which you are Residing?

Email Address?

Do you consume bakery products?

YES ☐ NO ☐

If yes, how frequent do you consume them?

- Daily
- Alternate day
- Weekly
- Once in a month
- Occasionally
- Never

Which type of bakery item do you consume?

- Indian Khari
- Toast
- Benne Biscuits (Commonly called as Butter)
- Biscuits
- Cookies
- Pizza Base
- Cream Rolls
- Puffs
- Cakes
- Other

If Other Please Specify?

Do you know the term "trans fat"?

Yes

No

Do you know that transfats has severe adverse effect on health?

Yes

No

Maybe

Is there any difference between natural and artificial/industrial produced trans fats?

Yes

No

Maybe

Are industrial produced trans fats hazardous?

Yes

No

Maybe

Do you know about partially hydrogenated vegetable oils (PHVO)?

Yes

No

Maybe

Do you check label on packaged products?

Yes

No

Sometimes

Do you buy unpackaged food items?

Yes

No

Maybe

Do you know about REPLACE initiative taken by WHO?

Yes

No

Presently, what is the trans fats limit in India?

Do you have any idea about FSSAI initiative to eliminate trans fats in India?

Yes

No

Do you know about Interesterified Fat?

Yes

No

Maybe

Would you Appreciate if Bakery Products are made From IE fats?

Yes

No

Maybe

Will you prefer to buy bakery products made with IE fats if the taste is altered than the regular bakery products?

Yes

No

Maybe

Will you prefer to buy Bakery Products made with Interesterified Fat (IE) if the price is higher than the regular Bakery Products by 10 - 15 Rupees?

Yes

No

Maybe

Will you prefer to buy bakery products made with IE fats if the taste is altered than the regular bakery products?

Yes

No

Annexures III

INFORMED CONSENT

Information on the Study (Sensory Evaluation)

I, Siddhant Nikam, pursuing my M.Sc. from the Department of Foods and Nutrition, Faculty of Family and Community Sciences, The M.S. University of Baroda, I am conducting a Research study on the "**SITUATIONAL ANALYSIS OF CONSUMPTION OF BAKERY FOODS AMONG UNIVERSITY RESIDENTIAL STUDENTS AND STUDY THE TRANS FAT CONTENT AND SENSORY PROPERTIES OF BAKERY PRODUCTS PREPARED WITH INTERESTERIFIED (IE) FAT."**

Trans fat which is present in Bakery food items that we consume on daily basis, is also called as a silent killer present in food which cause several NCD's like CVD, Obesity, cancer and many more Diseases. To combat ill-effects of trans fatty acid consumption, immediate recommendations are required to limit TFA in diet and processed foods. For this we are developing healthier Bakery products with Interesterified Fat (IE) fats.

These products like (Khari, Cream Roll, Butter biscuit, Maska bun, Naan-khatai) are safe to consume and do not pose any harmful after effects. Interesterified Fat (IE) also has established safety levels in many recent research studies and its consumption is safe.

Two sets of products will be given for Tasting to the panel for evaluation of its Sensory Parameters. One set of products will be prepared with Regular fats and other set will be prepared with IE fats.

I request you to Perform Threshold test which will be conducted on **1st February 2023**.

Once you are selected as a panel member (which will be notified to you), you will need to Taste the given products and give feedback for its quality parameters.

In case you are allergic/ intolerant to any of the above-mentioned Products, please do not participate in the study.

Venue – Food Lab 2 Department of Foods and Nutrition, Faculty of Family and Community Sciences, The MS University of Baroda

Note - In case you are allergic/ intolerant to any of the above-mentioned Products, please do not participate in the study.

Prof. Mini Sheth
Research Study Supervisor
Ph: 09879359229

Siddhant Nikam
MSc Research Scholar
Ph: 8999680099

SUBJECT CONSENT FORM

Name: Age: Sex:

Phone number:

Email Id:

I confirm that I have read and understood the above information and have/had the opportunity to ask questions.

I understand that my participation in this study is voluntary and I am free to reject being a part of this study, without giving a reason.

I understand that the ethics committee and the regulatory authorities will not need my permission to look at my health records and my identity will not be revealed in any information released to third parties or published.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I understand the study will involve multiple times of collection of data, in terms of tasting the product and give marks for its quality.

I agree to consume the Bakery Products made from Interesterified (IE) fat and Regular fat.

I voluntarily agree to participate in this community health study conducted by the Department of Foods and Nutrition, Faculty of Family and Community Sciences, The MS University of Baroda.

_____

Date: Signature of the Participant

Annexures IV

Threshold Test

Sensitivity Threshold Test

Name – Date –

You are Provided with a series of Containers having solutions with increasing concentration of one of the taste Qualities (Sweet, Salty, Sour). Please start with Sr. No. and Continue with the rest. The samples are not allowed to be retested. Please describe the taste or give intensity scores using the scoring pattern shown Separately here below.

Intensity Scores

Set No	Description of the Taste and Feeling factors					
	A1	A2	A3	A4	A5	
A						

Set No	Description of the Taste and Feeling factors					
	B1	B2	B3	B4	B5	
B						

Set No	Description of the Taste and Feeling factors					
	C1	C2	C3	C4	C5	
C						

Scale

While awarding the intensity scores take the following basis into account

0 – None or Taste of pure water

? – Different from water but taste quality not identifiable

X – Threshold very weak (Taste Identifiable)

1 – Weak Taste

2 – Medium

3 – Strong

4 – Very Strong

5 – Extremely Strong.

Annexures V

HEDONIC SCORE CARD THE SENSORY EVALUATION SEMI TRAINED PANEL

Research Topic – SITUATIONAL ANALYSIS OF CONSUMPTION OF BAKERY FOODS AMONG UNIVERSITY RESIDENTIAL STUDENTS AND STUDY THE TRANS FAT CONTENT AND SENSORY PROPERTIES OF BAKERY PRODUCTS PREPARED WITH INTERESTERIFIED (IE) FAT.

NAME: DATE:

PRODUCT NAME:

You are given a Bakery Product One of the Bakery products being sensory evaluated is created with Normal fat, while the other is made with Interesterified fat (IE). Taste the Product and check (√) How much you like or dislike each of characteristic of the Product.

Degree of Preference	Colour and Appearance		Aroma		Texture		Aftertaste taste		Taste		Mouthfeel		Overall acceptability	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Like extremely														
Like very much														
Like moderately														
Like slightly														
Neither like or dislike														
Dislike slightly														
Dislike moderately														
Dislike very much														
Dislike extremely														

Annexures VI

Ethical Certificate

Institutional Ethics
Committee for Human
Research
(IECHR)

FACULTY OF FAMILY AND COMMUNITY SCIENCES
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

Ethical Compliance Certificate 2022 – 2023

This is to certify that Mr. Siddhant Nikam's study titled, "**Situational analysis of consumption of bakery foods among university residential students and study the trans-fat content and sensory properties of bakery products prepared with Interesterified (IE) fat**" from Department of Foods and Nutrition has been approved by the Institutional Ethics Committee for Human Research (IECHR), Faculty of Family and Community Science, The Maharaja Sayajirao University of Baroda. The study has been allotted the ethical approval number **IECHR/FCSc/MSc/2022/43.**

Prof Mini Sheth
Member Secretary
IECHR

Prof Shagufa Kapadia
Chairperson
IECHR