INVESTIGATION ON SATURATED AND TRANS FAT CONTENT IN POPULAR POULTRY FOOD PRODUCTS

FOR THE DEGREE OF MASTER OF SCIENCE (M. Sc.) IN CHEMISTRY

SUBMITTED BY

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Dedicated

To

My Parents

&

Honorable Supervisor

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Abstract

Foods are prepared and served in many different ways to fulfil their purposes. Since nature is perfect, food sources in their original states are generally less harmful to human. However, due to the ways these foods are handled especially during processing or storage, can lead to some chemical transformations of their major ingredients like protein, lipid, antioxidants or other additives. The extent of these alterations can be as serious as changing the taste or flavor of the food or in most cases can just be a mere changes in the structural components of the major ingredients thereby producing an unnoticeable effect that may not be compatible with the normal physiochemical processes of the human system. This causes an abnormal cellular function which is prerequisite to some health complications like cancer, diabetes, cardio vascular diseases and other foodborne health problems. Dietary fats are a major energy source for the body. Fatty acids are also involved in many other vital processes in the body (e.g. structural components of cell membranes, precursors for bioactive molecules, regulators of enzyme activities, regulation of gene expression).There are lack of information on the fatty acid content in Bangladeshi food. Unsaturated fatty acid more than one quarter of total daily calories by fatty acids. The objective of the study is to investigated on saturated and unsaturated fatty acids content in poultry based fast food products available in Bangladeshi market. The content of fatty acids is most commonly determined by gas chromatography & ATR-FTIR. Total fat, as well as the type of fat, determine the effect of their consumption on health. Fatty acids can be divided into several groups with respect to their structure, physiological role and biological effects. Our study on the fast food samples collected from around the Dhaka city have provided some vital information about the fatty acid contains of these foods. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) have been found in the fast food samples. None of the fast food samples that have been analyzed contain any trans fatty acids. The results of ATR-FTIR spectroscopy were found in good agreement with the results of GC-FID. All fast food samples contained omega-6 (linoleic acid) fatty acids in various amounts ranging from 8.30% to 18.90%. The ratio of omega-6 and omega-3 in Chicken Botik (D) was 2.32:1. This ratio is in the acceptable range. Except sample D, none of the fast food sample contains linolenic acid (omega-3). Chicken Winglet (A) and Chicken Hot Wings (B) of KFC have higher amount of saturated fatty acids which are 28.73% and 25.92% respectively. Intake of saturated fat is known to increase low density lipoprotein (LDL) cholesterol, and therefore has been associated with increased risk of cardiovascular disease (CVD)

Chapter 1

Introduction

1.1General

Fats are essential part for living organism [1]. Fats and oils are bio-products found in plants and animals as energy reserves. These biomolecules in combination with other molecules play a very important role in maintaining the healthy life of organisms[2].In recent years increase to interest in ways to manipulate the fatty acid composition of meat & the use of fatty acid with food composition of meat basically poultry food product. Industrial food processing has been acting on this objective over many years to diminish fats, particularly saturated and industrially produced transfats, in foods. For this reason meat is take to be a major source of fat in the diet and especially of fatty acids, which have been implicated in diseases associated with our modern life, especially in developing countries. These causes various cancers, diabetes and especially coronary heart disease [3]. For example in the UK, the Department of Health (1994) recommended that fat intake be reduced to 30% of total energy intake (from about 40%) with a figure of 10% of energy intake for saturated fatty acids (from 15%). Now, the recommended ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (P: S) should be increased to above 0.4. Since some meats naturally have a P:S ratio of around 0.1, meat has been implicated in causing the imbalanced fatty acid intake of today's consumers[4].Altering fat can have important consequences to overall [5].This is cause meat is major source of fat in the diet and specially trans fatty acid. Fatty acids are essential contribution of all body tissues, its urgent necessary part of phospholipid component of cell membranes [6]. Dietary fat is an essential part of our lives [6]

Fatty acids are organic compounds that can be saturated or unsaturated. Unsaturated fatty acids can be monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA). Fatty acid is a carboxylic acid with a typical RCOOH structure, containing of a long aliphatic hydrocarbon chain and a terminal carboxyl group which either saturated or unsaturated. Naturally occurring fatty acid contain unbranched chain of an even number of carbon atom 4 to 28.Fatty acid that have contain one or more double bond is known as unsaturated and without double bond is known as saturated[1]. In the chain the behavior of two carbon atoms that are bound next to either side of the double bond can occur in cis or trans configuration. The cis configuration has adjacent two hydrogen atoms to the double bond on the same side of the chain. The cis configuration of double bond force the chain to bend consequently to limiting the choice of conformation (Figure1.1 &

1.2). Trans fatty acid (TFA) is defined as unsaturated fatty acids containing one or more isolated double bonds in a trans configuration [7]. The differences in physical and chemical properties of fatty acids lead food products prepared by using different fats [8]. For some, it's the lifestyle that has a long history of consumption of certain types of food and food preparation methods. However, there is insufficient data or scientific findings to the varieties of food preferences.

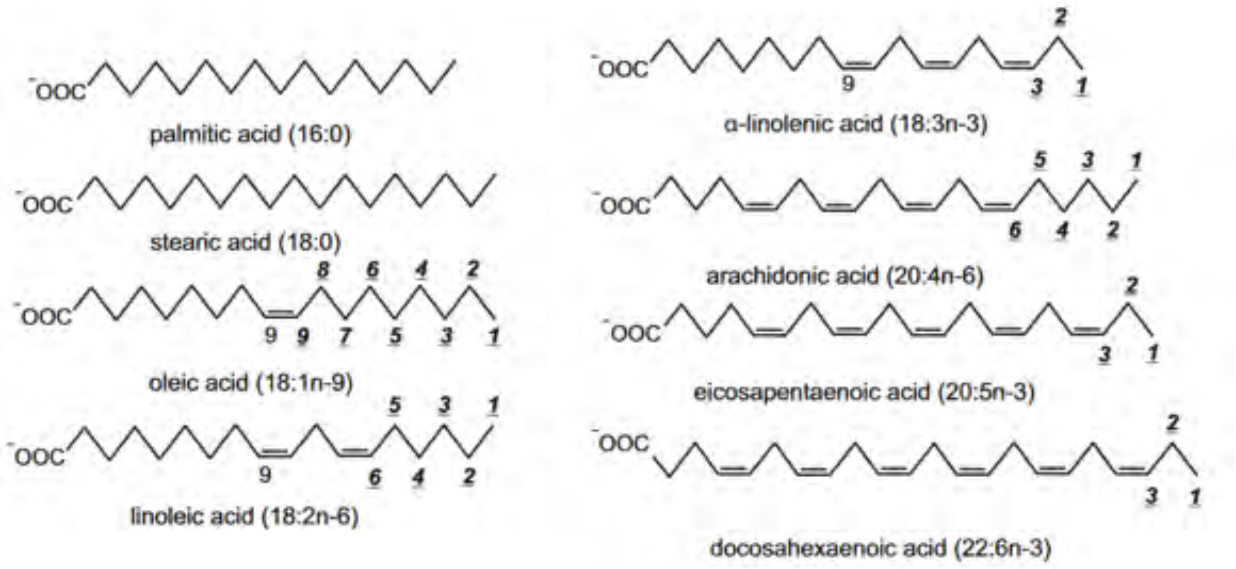


Figure 1.1. Different types of fatty acid

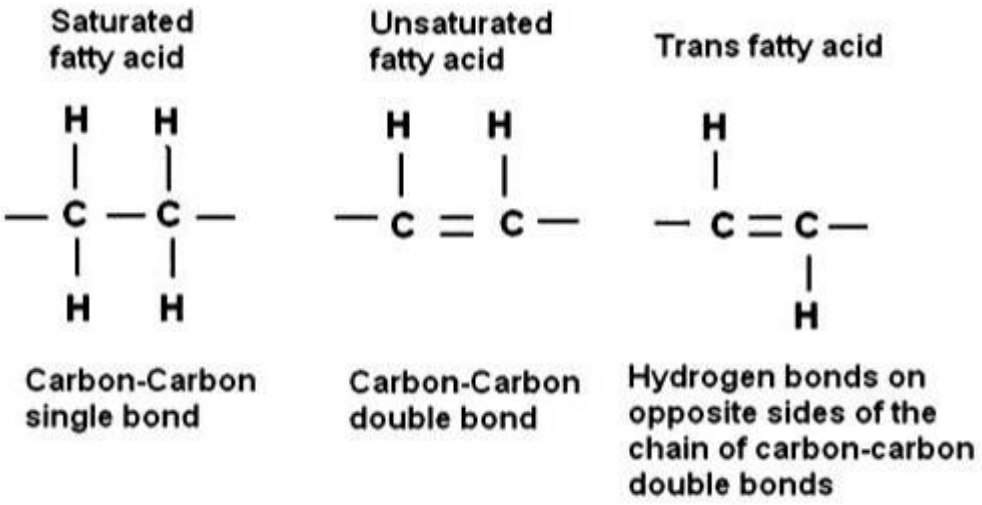
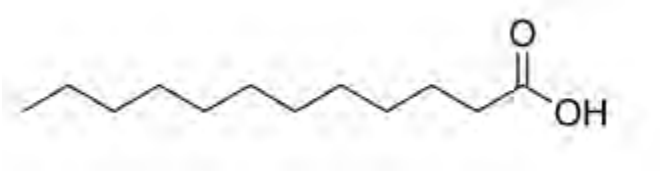


Figure 1.2. Fatty acid functional structure.

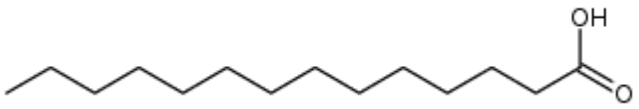
Last century the epidemic of obesity in most highly developed countries has led to an understandable public health emphasis on low fat, low energy diets and has provided a major stimulus for development of low fat products and fat substitutes. However, recent research suggests that there is a need to consider the quality as well as the quantity of fat in diets of Bangladesh populations. It is most important to know of public health nutrition which will briefly describe in research. However, there is now much evidence to suggest that specific fatty acids have bad or good effects on human health.

1.2 Saturated fatty acid (SFA)

Saturated fats are classified as a fat molecules that have no double bonds in which the fatty acid chains have all or predominantly single bonds between carbon molecules because they are saturated with hydrogen molecule (American heart association)(Figure 1.3). Saturated fatty acids have been thought to be preferred fuel for the heart [4, 9].In most developing countries have no clear conscious in dietary guidelines. But most developed country since 1980s have a dietary guidelines produced which have proposed reductions in total fat and in saturated fatty acid [10]. Many international dietary guidelines have recommended that saturated fatty acids should contribute no more than 10% dietary of total energy[11] The World Health Organization and the US Dietary Guidelines recommend consuming less than 10%E (percentage of total energy intake) from SFA [11], and the American Heart Association less than 7%E [12]. Is there strong evidence to support this dietary strategy? Second, do health effects of SFA vary depending on the chain-length, i.e. comparing 12-, 14-, 16-, and 18 carbon SFA? Current dietary recommendations generally focus on overall SFA consumption, without strong attention on specific SFA. Third, what is the relationship between SFA consumption and risk of stroke and type 2 diabetes mellitus? Historically, research on SFA has focused largely on CHD [13]. Current Centuries saturated fat of plant or animal origin such as poultry food has been an important ingredient in Bangladeshi diets for population. For the last 30 or 40 years, dietary saturated fats have attained a poor reputation especially in relation to cardiovascular health; recommendations to reduce consumption persist even in the face of equivocal or contradictory evidence [14]. In May 2009 100th AOCS annual meeting cession in, Orlando Florida a special theme issue of Lipids on saturated fat and health presents contributions [14]. High-fat diets usually mean increased intakes of saturated fat.



(a) Lauric acid



(b) Myristic acid

Figure1.3. Saturated fatty acid.

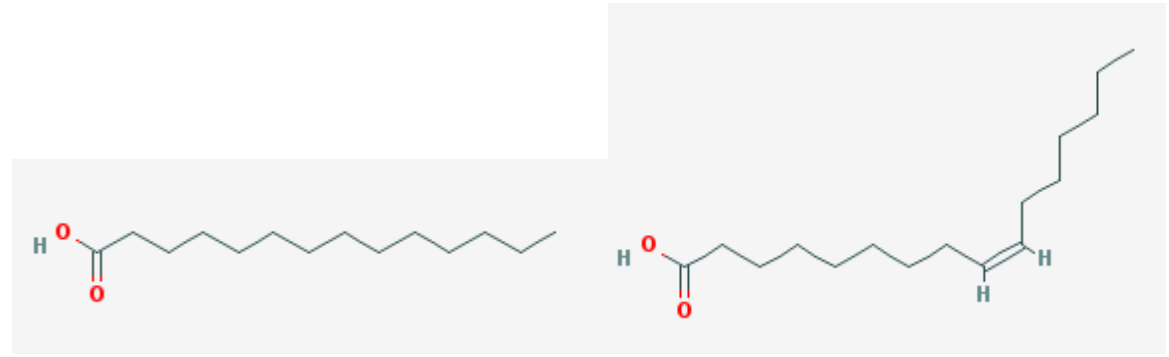
Many experimental & epidemiological studies and numerous controlled intervention studies in volunteers of varying age, sex and genotype which have demonstrated that saturated fatty acids increase blood cholesterol concentrations in a predictable and dose related fashion [15-17]. Higher intake of most dietary saturated fatty acids is associated with higher levels of blood total cholesterol (TC) and low-density lipoprotein (LDL)" [18] in the bloodstream of some persons and that elevated cholesterol concentrations heighten the risk of heart disease [5,19] .In humans saturated fat also increases high-density lipoprotein (HDL) cholesterol, the total cholesterol (TC) to HDL cholesterol ratio (a risk marker for CVD) is not altered[20].Although these fatty acid chain main reason on plasma cholesterol rising [21]. Individual saturated fatty acids have specific functions depending on chain length [14]. Now it is clear that major saturated fatty acid (SFA) mainly lauric, myristic and palmitic fatty acids which are responsible for increasing plasma total and LDL cholesterol concentration, [22],longer chain stearic acid (18:0) has been no shown to have effect on LDL or HDL cholesterol or the TC:HDL cholesterol ratio[22,23]. Major saturated fatty acids of shorter length chain have been shown to have a greater LDL cholesterol raising effect, such that lauric acid (12:0) raised LDL cholesterol the most, followed by myristic (14:0) and palmitic (16:0) acids[24]. Lauric acid also increased HDL cholesterol plasma most significantly, and it did this disproportionately to TC, so that its replacement of carbohydrate actually led to a significant decrease in the TC: HDL cholesterol ratio [25, 26]. In our health effect SFA has been suspect a risk factor insulin resistance and diabetes [27]. Aside there are other

properties and functions of the some individual saturated fatty acids that support beneficial roles in the body [5]. Butyric short-chain fatty acids are hydrolyzed preferentially from triacylglycerols and absorbed from the intestine to the portal circulation without resynthesis of triacylglycerols. These fatty acids serve as a ready source of energy, and there is only a low tendency for them to form adipose [28] Butyrate is a well-known modulator of genetic regulation [29, 30], and it also may play a role in cancer prevention [31] Dietary stearic acid decreases plasma and liver cholesterol concentrations by reducing intestinal cholesterol absorption[5]

1.3 Monounsaturated fatty acid (MUFA)

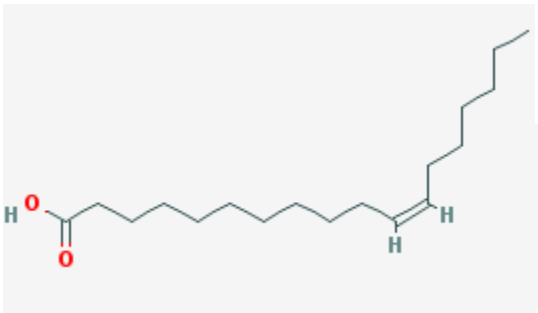
MUFAs are distinguished from the other fatty acid & chemically classified as fatty acids that have one unsaturated carbon bond in the molecule, this is also called a double bond in contrast,(Figure 1.4) polyunsaturated fatty acids (PUFAs) containing two or more double bonds, and SFAs have none [32].Some MUFA are palmitoleic acid (16:1 n-7), and vaccenic acid (18:1 n-7), elaidic acid (trans 18:1 n-9) mystrioleic (14:1 n-5), gondoic (20:1 n-9), erucic (22:1 n-9) and nervonic (24:1 n-9) acid .In a cis MUFA, the hydrogen atoms are present on the same side of the double bond, whereas in the trans configuration hydrogen atom and double bond are present on opposite sides[33]. The National Institute of Medicine, the US Department of Agriculture, European Food and Safety Authority and the American Diabetes Association suggested that have no dietary recommendations for MUFA [32, 34.].Academy of Nutrition and Dietetics, and the Canadian Dietetic Association both promote <25% MUFA of daily total energy consumption while the American Heart Association Nutrition Committee recently published a scientific statement regarding the relationship of trans MUFA to CVD risk [32, 35], and the present statement, therefore, will be limited to a discussion of dietary cis MUFAs, of which oleic acid (cis C18:1) comprises 92% of cis MUFAs [36]. In the United States, adults recommend a diet that provides,10% of calories from SFA, up to 10% from PUFA and average total MUFA intake is 13% to 14%, more of energy results in a total fat intake 30% of energy, an amount that is comparable to (or slightly greater than) SFA & PUFAs [36]. The cis-isomers are the predominant form of MUFA in food sources. The most common cis-configured MUFA in daily nutrition is oleic acid (18:1 n-9), followed by palmitoleic acid (16:1 n-7), and vaccenic acid (18:1 n-7) [33]. American heart association recommend that oleic acid represents the topmost MUFA provided in

the diet (~90% of all MUFAs). The major trans-configured MUFA is elaidic acid (trans 18:1 n-9). Some MUFA—such as (14: n-5), gondoic (20:1 n-9), erucic (22:1 n-9) and nervonic (24:1 n-9) acid—are synthesized in minor concentrations endogenously using other MUFAs as precursors. Various sources for MUFA in food are given in meat food [33].Furthermore, over the last decade commercial production of high oleic acid modified dietary oils with improved stability for the use in food (meat) processing has been markedly increased in order to replace dietary oils rich in SFA and trans fatty acids [37]. Various types of MUFA are almost completely absorbed by the intestine and are oxidized for energy production, converted into other fatty acids, or incorporated into tissue lipids. Summarizes MUFA recommendations of international authorities and organizations (Table 1.1).

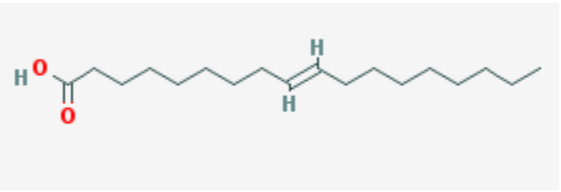


Mystrioleic acid

Palmitoleic acid



Vaccenic acid



Elaidic acid

Figure 1.4. Monounsaturated fatty acid.

Table 1.1. Summarizes MUFA recommendations of international authorities and organization.

Authority/Society	MUFA (% of TEC)	Target Group/Remarks	References
American Heart Association	<20	Healthy adults	38
Academy of Nutrition and Dietetics/Canadian Dietetic Association	<25	Healthy adults	39
Dutch Dietary Guideline	8–38	Healthy adults Upper limit for obese: 25% of TEC	40
European Food Safety Authority	No specific recommendations	Healthy adults	41

Many international dietary guidelines have recommended that in 1999, the International Society for the Study of Fatty Acids and Lipids discussion about daily intake of MUFA & they are suggest that daily provide a total fat range from 15% to 40% of TEC, the majority of fatty acids in the form of MUFAs. However, no precise value (i.e., % of TEC in the form of MUFA) was given by the panel [42]. Joint FAO/WHO Expert Consultation Committee suggest that 13 peer-reviewed background papers dealing with fats and fatty acids in human nutrition concluded that replacement of carbohydrates by MUFA beneficially increases HDL-cholesterol, while the substitution of SFA with MUFA exerts favorable effects on LDL-cholesterol and the ratio of total cholesterol to HDL-cholesterol [43]. A high MUFA diet on a range of outcome markers including postprandial lipoproteins, blood coagulation factors and immune function. Some of the data from these studies have been reported in detail elsewhere [44]. Although there is a substantive body of evidence that has shown cardio protective effects of diets high in MUFA. Collectively, these findings suggest

that high-MUFA diets may confer benefits on CVD risk factors beyond those associated with plasma lipids and lipoproteins. MUFA largely affected on fasting blood lipids in young and middle-aged men who represent subjects with a range of fasting blood lipid values [45]

1.4 Polyunsaturated fatty acid (PUFA)

From a chemical standpoint, polyunsaturated fats are simply fat molecules that have more than one unsaturated carbon bond in the molecule, this is also called a double bond [American heart association] (Figure 1.5). PUFAs can be further subdivided on the basis of the location of the first double bond relative to the methyl terminus of the chain. For example, n-3 and n-6 FAs are two of the most biologically significant PUFA classes, and have their first double bond on either the third or sixth carbon from the chain terminus, respectively. The final carbon in the FA chain is also known as the omega carbon, hence the common reference to these FAs as omega-3 or omega-6 PUFAs.[46] Long-chain n-3 and n-6 PUFAs are synthesized from the essential FAs (EFAs) alpha-linolenic acid (ALA) and linoleic acid, respectively. Basic structures of these two parent PUFAs are shown in Figure

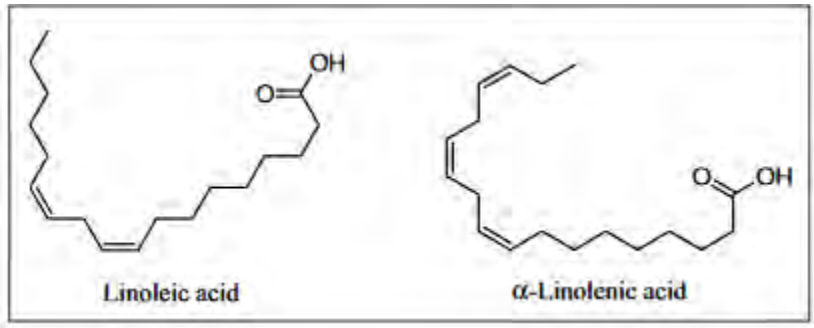


Figure 1.5 The structure of two essential fatty acids – linoleic acid and alpha-linolenic acid

Dietary polyunsaturated fatty acids (PUFA) affect to have effects on biochemical and physiological mechanisms which widely relate to numerous disease occurrences [47].Perhaps of greatest concern in PUFA bio-chemistry is the regulation effect, which act in diverse ways to influence biological processes, both positively and adversely. The PUFA quality, quantity, and

balance of these fatty acids appear to play a vital role in determining their process [48].Unfortunately, experimental evidence currently available is not sufficient to even suggest optimal dietary PUFA intake levels Polyunsaturated fatty acids (PUFAs) contribute <7% of total energy intake and 19–22% of energy intake from fat in the diets of adults, a level that is within recommended intakes for both men and women [49]. Polyunsaturated fatty acids (PUFAs) play important roles in maintaining normal physiological conditions and, consequently, in human health. Two PUFAs families, n-6 and n-3 fatty acids (FA), are physiologically and metabolically distinct. Moreover, it is believed that human beings evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids (EFA) of 1:1[50-52]. A high amount of omega-6 polyunsaturated fatty acids (PUFA) in food and a high omega-6/omega-3 ratio promote cardiovascular disease, cancer and inflammatory diseases [51]. Recent studies show that omega-6 to omega-3 fatty acid ratio play important role in obesity [52]. Omega-6 to omega-3 fatty acid ratio of 5:1 or less is considered as less harmful for health [53]. Now due to change in food habit and preference to fast food, people are having excessive amounts of omega-6 fatty acids and less amount of omega-3 in their food. This leads to increase omega-6 to omega-3 ratio to 20:1 or even more [52].

Their precursors, linoleic acid (18:2n-6; LA) and α-linolenic acid (18:3n-3; ALA) are essential fatty acids (EFA), meaning that they cannot be synthesized in the human body and must be obtained from the diet [54].Linoleic acid (18:2n -6) is the major PUFA, comprising 84–89% of the total PUFA energy, whereas a-linolenic acid (ALA;18:3n -3) contributes 9–11% of the total PUFA energy (equivalent to 1.1–1.6 g/d) in the diets of the adult population [55].Unfortunately, experimental evidence currently available is not sufficient to even suggest optimal dietary PUFA intake levels. In the light of new evidence for associations between low intakes of some PUFAs and increased risk of chronic disease that was mentioned above, optimal criteria for dietary recommendation aim to achieve optimal health and to reduce risk of developing chronic disease.[56].Perhaps ALA consumption is suggested to, reduce CHD risk but not accuretly [57]. A World Health Organization report from 1994 suggest nutrient intake values for total PUFAs, but focused on the ratio of LA/ALA in the diet .PUFAs are important constituents of the phospholipids of all cell membranes. LA, ALA and their metabolic products, AA[arachidonic], EPA (eicosapentaenoic) and DHA[all cis-4,7,10,13,16,19-docosahexaenoic] are crucial structural and

functional components of cellular and intracellular membranes in the human body, but especially in brain, heart, retina, and testes.. PUFAs of both the n-6 and n-3 series are incorporated into membrane phospholipids, and the AA/EPA ratio ranges between 1:1 and 5–10:1 and the ratio of n-6 to n-3 PUFAs is very important to human health. [54]. In the Multiple Risk Factor Intervention Trial (MRFIT [5].The diets of individuals at high risk for coronary artery disease (CAD) were monitored for 10.5 years [57].

1.5 Trans-fat

Trans fatty acid is the main interest of the research paper. Trans fatty acid (TFA) is defined as unsaturated fatty acids containing one or more isolated double bonds in a trans configuration (Figure 1.6) [58].Trans-unsaturated double bonds (transfats) from fats process occur normally in nature. [59] Most of the trans fats in the food supply have been came by food manufacturers but trans fats naturally exist in low amounts in the fat in meat and milk, etc. [60]

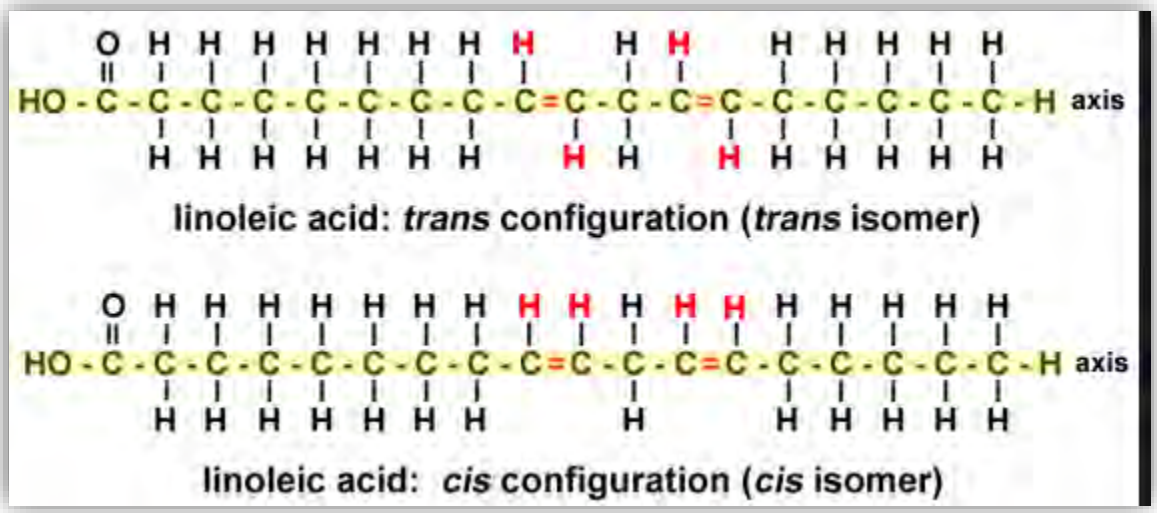


Figure 1.6.Comparison of a unsaturated fatty acid linoleic acid (cis and trans)

There are two main source of trans fat in food supply, those originating from partial hydrogenation of polyunsaturated oils and those found in ruminant-derived foods occurring as a result of bio-

hydrogenation of polyunsaturated fatty acids (PUFA) by rumen bacteria [61] or by industrial hydrogenation the double bonds tend to migrate as well as isomerize so that a hydrogenated fat may contain double bonds, which occur naturally both cis and trans, anywhere between carbon atoms 4 and 16 [6]. The process of hydrogenation was first discovered around the turn of the 20th century by French chemist Paul Sabatier using a nickel catalyst.[6] Shortly after, German chemist Wilhelm Normann developed a hydrogenation process using hydrogen gas. Modifications in the processing and formulation of hydrogenated fats continued through the mid-20th century. It was the partial hydrogenation of fats that introduced trans fatty acids (or trans fats) into fats of vegetable origin [62]. The use of partially hydrogenated fats accelerated in the 1960s, 1970s, and 1980s as food producers responded to public health recommendations to move away from animal fats and tropical oils [59]. Naturally-occurring trans fats are produced in the gut of some animals and foods made from these animals (e.g., milk and meat products) may contain small quantities of these fats. Artificial *trans* fats (or *trans* fatty acids) are creatd in an industrial process that adds hydrogen to liquid vegetable oils to make them more solid (American Heart Foundation Association)As a result these fats have therefore been incorporated into a great many foods, including snack and deep-fried foods, baked goods, margarines, and crackers (see figure) [63].Based on the restriction of US trans-fat policy on industrially manufacture kinds of food, an update on some of the food items can turn out without containing trans-fats and the fat contents are vehemently labelled on each of the items[64].

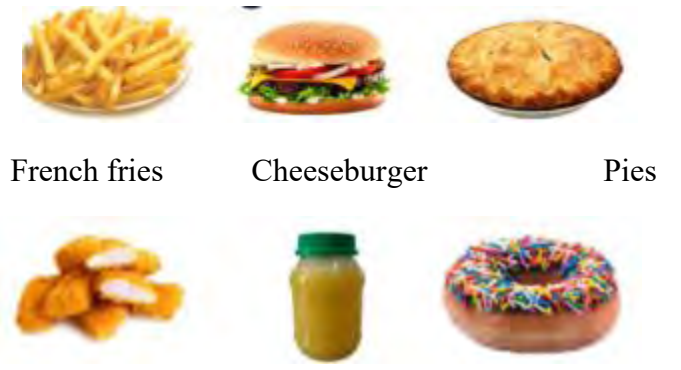


Figure 1.7. Some example of (industrial) Trans- fats.

Trans fats are easy to use, inexpensive to produce and last a long time. *Trans* fats give foods a desirable taste and texture. Many restaurants and fast-food outlets use *trans* fats to deep-fry foods because oils with *trans* fats can be used many times in commercial fryers. Several countries (e.g., Denmark, Switzerland, and Canada) have reduced or restricted the use of *trans* fats in food service establishments**.** The primary dietary TFA are vaccenic acid and elaidic acid. Vaccenic acid (18:1, trans-11) is the major ruminant TFA, whereas elaidic acid (18:1, trans-9) is the main TFA isomer in industrial hydrogenation [65]. The trans fatty acid content of industrially hydrogenated fats varies widely and may account for up to 60% of the fatty acid content, whereas the trans fatty acid content of meat and dairy products is considerably lower and accounts for 2%–8% of the fatty acid content [66].In the case of special dietary choices, this allows for a daily intake of up to 10 times more industrially produced trans fatty acids than trans fatty acids from ruminants. Processed foods and oils provide approximately 80% of trans fats in the diet, compared to 20% that occur naturally in food from animal sources. TFAs have received intense interest as risk factors to public health in recent years [67]. Epidemiological studies linking TFAs to certain diseases like cancer and coronary heart diseases and blood lipoprotein have been undertaken in different geographical locations [67].However, recent research has shown that TFAs also contribute to chronic diseases associated with high-fat intake [68]. Study showed TFAs correlated to chronic heart disease (CHD) [69], metabolic disease and inflammation diseases, obesity and cancer [70]. The intake of TFAs increases LDL to a degree similar to saturated fatty acids but also reduces circulating HDL [67]. With respect to human health, WHO endorsed the recommendation of TFA intake should not exceed <1% of the total energy intake [70].

Although majority of those studies have indicated a positive correlation between TFA intake and coronary heart disease (CHD), the reports about TFA intake in association with cancer are still inconsistent [71]. Among the two subgroups of TFAs are, industrially obtained TFA (iTFA) and TFA obtained from ruminant animals (rTFA), coronary heart diseases and cancer are strongly linked with iTFAs as reported by many studies. iTFAs have also received further implications like increasing inflammatory markers, lipid and lipoproteins content of plasma.[50].As compared with the consumption of an equal number of calories from saturated or cis unsaturated fats, the consumption of trans fatty acids raises levels of low density lipoprotein (LDL) cholesterol, reduces levels of high-density lipoprotein (HDL) cholesterol, and increases the ratio of total cholesterol to

HDL cholesterol, a powerful predictor of the risk of CHD[72]. Trans fats also increase the blood levels of triglycerides as compared with the intake of other fat[72] increase levels of Lp(a) lipoprotein[6] and reduce the particle size of LDL cholesterol, each of which may further raise the risk of CHD(Figure 1.8)[73]. Thus, trans fatty acids have markedly adverse effects on serum lipids. Although these effects would be expected to increase the risk of CHD, the relation between the intake of trans fats and the incidence of CHD reported in prospective studies has been greater than that predicted by changes in serum lipid levels alone, suggesting that trans fatty acids may also influence other risk factors for CHD[72].

In 1993 Willett and colleagues published the results from the Nurses' Health Study of 85,095 women without diagnosed CHD, stroke, diabetes,or hypercholesterolemia at the start of the study, and reported that the intake of TFA isomers was related to the risk of CHD after 8 years of follow-up.[74(a)]

1.6 Aim of the project

a) Objectives with specific aims:

The aim of this research is to create public awareness about the types of fat consumed from popular fast food products in Bangladesh and to help make new policies to regulate harmful fat content in processed foods.

The main objectives of the present work are to:

1. extraction of total fat by soxhlet extraction method from raw, procesed/fried poultry products .
2. preparation of fatty acid methyl esters (FAME) by transmethylation of the extracted samples for analytical purpose.
3. application of Gas Chromatography-Mass Spectrometry (GC-MS) and Attenuated Total Reflection (ATR) Infrared Spectroscopy (ATR-FTIR) to identify as many saturated, cis and trans unsaturated fatty acids as possible
4. determination of total fat and the amount of trans fats present in the chicken meat based

fast foods.

(b) Possible outcome:

By completing the studies mentioned above, it is expected that,

1. the level of total fat content including the harmful saturated and trans fatty acids in poultry based popular fast foods in Dhaka city will be known.
2. Because the contribution in TFA content from the natural/ruminant source for poultry products is very low, the contribution from cooking oils and cooking practice could be identified from the TFA content of these products.
3. We investigated some fast foods of Dhaka city to know what kinds of fatty acids are present in them, what is the ratio of omega-6 to omega-3 in fast foods and to find presence of any harmful trans fatty acids.

Chapter-2

Experimental

2.1 Method

A brief description in extraction, fractionation & purification of following the methods in experimental works.

2.2 Purification of solvents and chemicals

All chemical such as (chloroform, methanol, ethanol, n-hexane, acetone, pet-ethare) analytical or laboratory grade solvents and chemicals had used in the experiments. All solvents and reagents used in the experiments were produced from Merck (Germany), Sigma aldrich. The commercial grade solvents (chloroform, methanol, ethanol, n-hexane, acetone, pet-ethere) were distilled before use. The solid samples were purified by recrystallizing in different solvents

2.3 Soxhlet extraction

Samples were grinded as fine as possible without any pretreatment. Fatty acids in various food samples were extracted by the Soxhlet extraction method [74(b)].Lipid in food present in various forms like monoglycerides, diglycerides, triglycerides and sterol and free fatty acid and phospholipid and carotenoids and fat-soluble vitamins. Lipid is soluble in organic solvent and insoluble in water, because of this, organic solvents like hexane, petroleum ether, chloroform have the ability to solubilize fat and fat is extracted from food in combination with the solvent. Later the fat is collected by evaporating the solvent. Almost all the solvent is distilled off and can be reused. There are two ways to find out the fat present in food, either by acid hydrolysis or by solvent extraction. The solvent extraction method is more pronouncedly known as Soxhlet method. Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. This method is widely used in almost all food industries and primarily used in oil extraction industries.

2.3.1 Preparing the sample

First of all, we have to dry the meat product and remove moisture in order to facilitate entry of the organics solvent, because moisture restricts the entry of organic solvent. Then size reduction is there to increase the surface area and due to it, there is larger exposed surface. After this, we go

for acidic hydrolysis which helps in breaking of protein fat emulsion and increases the availability of fat for the solvent. Furthermore, we can collect the solvent by distillation.

***REQUIREMENTS*:**

- Weighing balance
- Soxhlet apparatus
- Drying oven
- Thimble
- Heating mantle
- Glass rod
- Desiccator with silica gel
- Petroleum ether/ Chloroform (Boiling temperature 60°-80°c)
- Cotton plugs

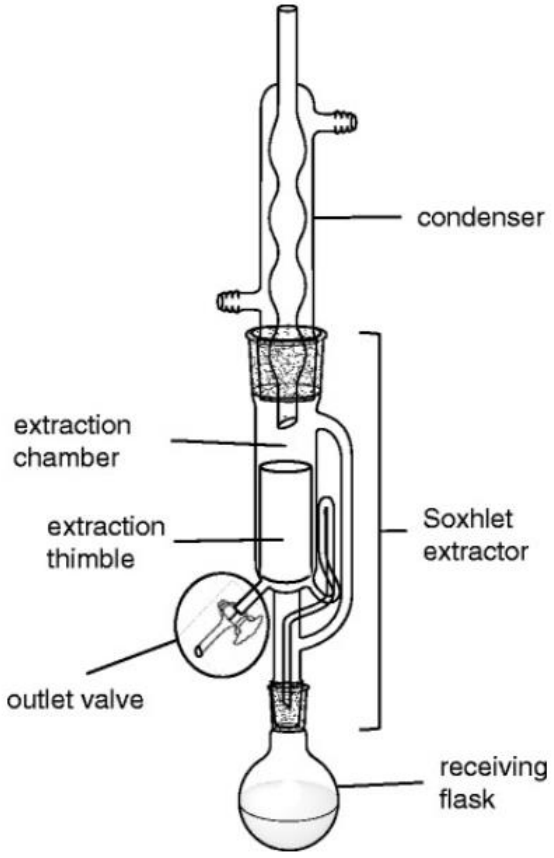


Figure 2.1 Structure of a Soxhlet extractor

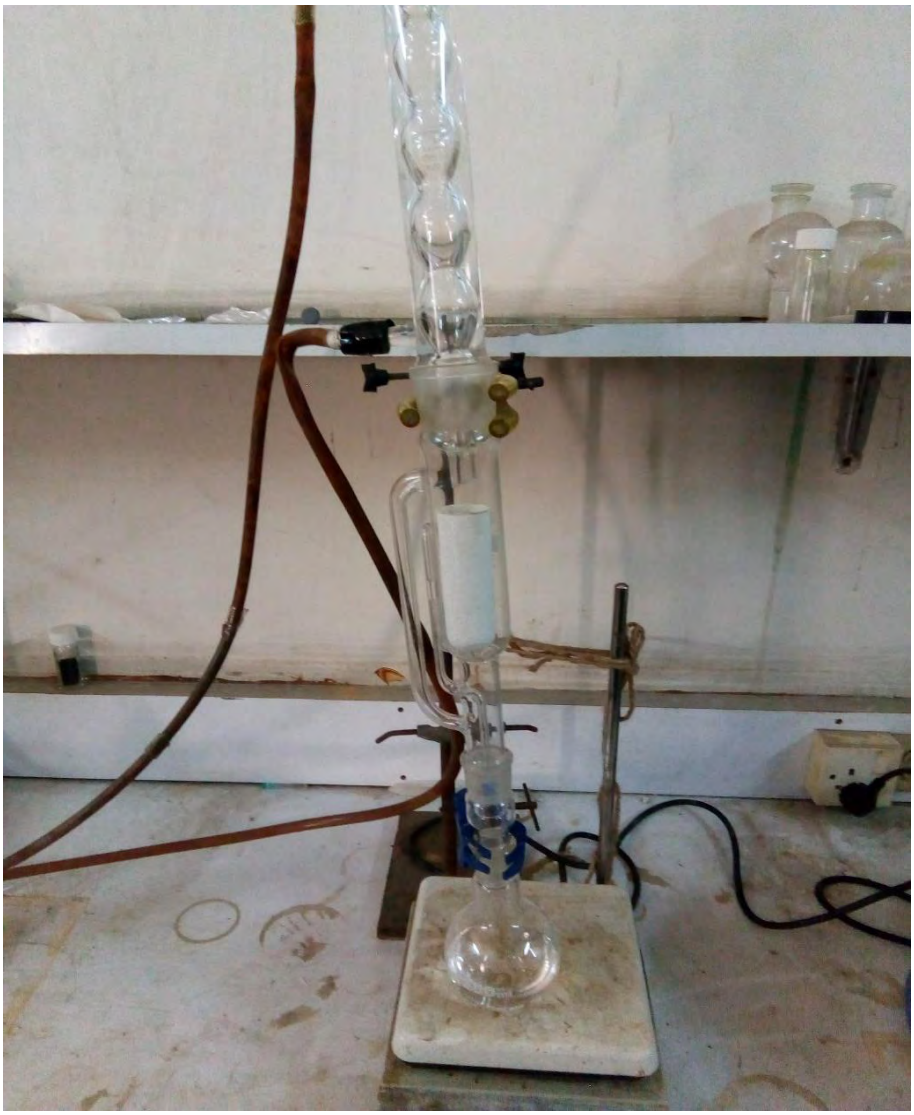


Figure 2.2 Soxhlet extractor

2.3.2 Procedure

1. First of all, rinse all the glass apparatus by Chloroform and dry it in the oven at 110°c and after removing it keep in the desiccator.

2. Weigh 10 gram of grounded and dried sample and place it in the thimble.

3. Place the thimble in the soxhlet extractor.

4. Take a 250ml round bottom flask and clean it and fill the flask with 150 ml Chloroform.

5. Place the whole setting on a heating mantle and allow the Chloroform to boil.

6. Continue the extraction process for several hours, almost 8 hours.

7. Remove the condensing unit from extraction unit and allow the sample to cool down & evaporation. Finally, it removes all the lipid.

8. Collect almost all the solvent after distillation.

9. Place the sample in the oven and after removing it place in the desiccator

10. Take the weight of the sample.

11. As a result, we get a defat sample.

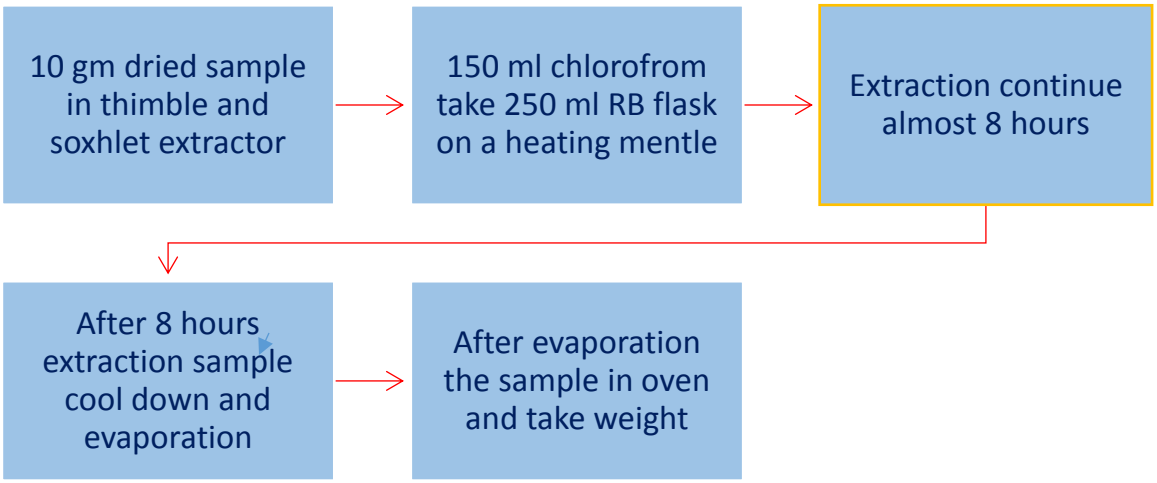


Figure: 2.3 Fat extraction

Calculation-

Empty thimble= w1

Thimble with sample= w2

Weight of sample= p

Then crude fat percentage ig= (w2-w1)/p × 100

This method is an efficient method to extract all the fat present in the food

2.3.3 Evaporation

The solvents were evaporated under reduced pressure in rotatory evaporator (West Germany) with a bath temperature not more than 60°C. The residual solvent in the extract and compounds were removed under high vacuum.

Figure 2.4 Rotary vacuum evaporator

2.4 Fatty acid methyl esters (FAMEs)

FAMEs were prepared according to method AOAC 969.33. To prepare FAME, lipids/ fats were hydrolyzed with methanolic KOH solution and the hydrolyzed lipids/ fats were transesterified by using BF_3-methanol complex solution. Finally FAMEs were extracted with petroleum ether.

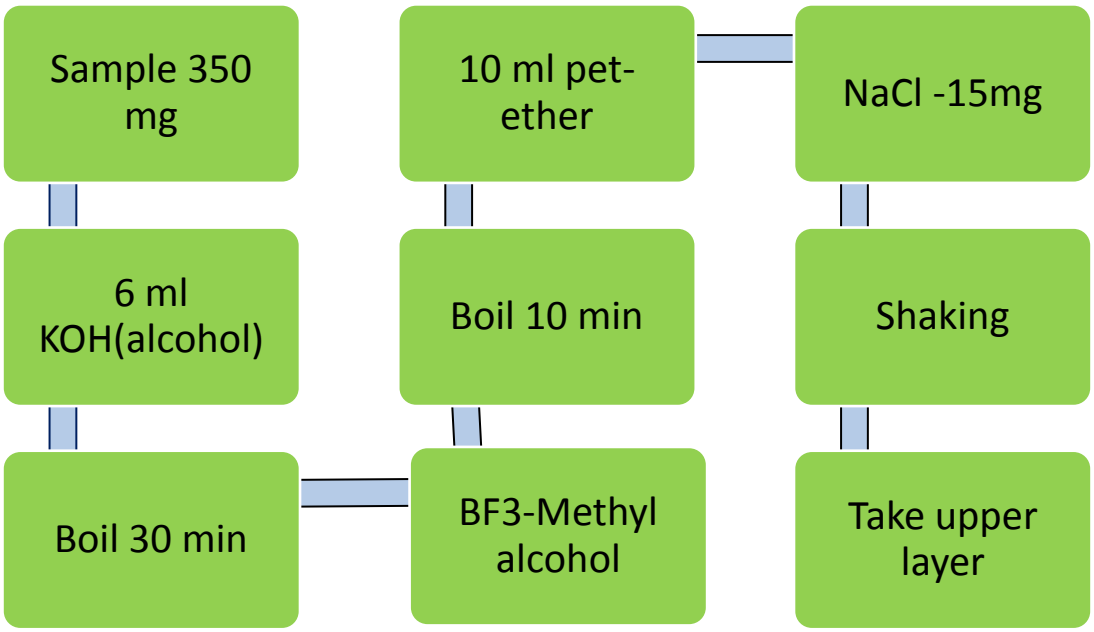


Figure 2.5 FAMEs preparation for GC analysis.

2.5 Chromatographic techniques

Two types of chromatographic techniques such as Ag- Thin Layer Chromatography (Ag-TLC) and Gas Chromatography (GC) were used.

2.6 Silver ion Thin-layer chromatography

Silver ion thin-layer chromatography (Ag-TLC) as used for the separation of fatty acids The efficiency of Ag-TLC/gas chromatography for determination of *trans*-fatty acids in food is emphasized.0.2 mm pre-coated uniform layer of silica gel or aluminum sheets were used throughout the experiment.

2.6.1 Sample preparation

The working sample is prepared by dissolving the isolated material in hexane to give a 1.5-2.0% solution.

Plate preparation:

A pencil line is drawn near the bottom of the plate and a small drop of a solution of the dye mixture is placed on it. Any labelling on the plate to show the original position of the drop must also be in pencil. If any of this was done in ink, dyes from the ink would also move as the chromatogram developed. The binding material in aluminum pre-coated silica gel 60 plates layer is uniform and smooth with a thickness 200-250 µm and particle size of ~10 µm. We use 20 × 20 cm silica gel 60 aluminium-backed plates cut into 4 × 20 and 4 × 10 cm sheets for purity check or identification. The plates are subjected to a single development with 2-4 mL freshly prepared mobile phase

Figure 2.6 Process of spotting

Solvent system:

Mobile phases will be discussed below, but generally they consist of two,rarely three component mixtures. Hexane or petroleum ether (b.p. 40-60°C),chloroform, benzene, and toluene are most often the major components, while smaller proportions of diethyl ether, acetone, methanol, ethanol, or acetic acid may be added to these. The solvents of different polarity used in different ratio. It is important that the solvent level is below the line with the spot on it.

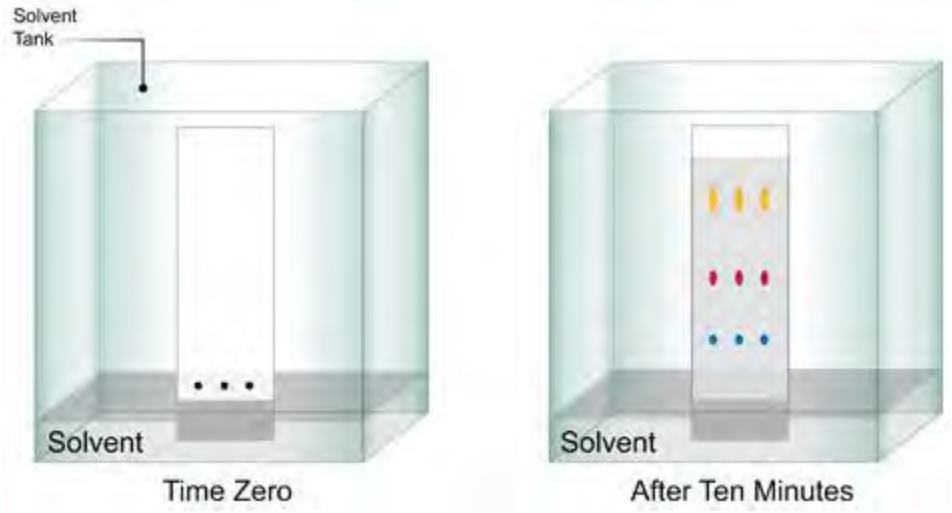


Figure 2.7 Processing of TLC plate.

2.6.2 Sample application

The working sample solution for analytical Ag-TLC depends on the purpose, and for purity checks or identification it is usually 1.5-2% in hexane. An aliquot of 5-10 μL of the solution is applied (automatic or Pasteur pipette), as a spot, at 1.5-2.0 cm above the bottom edge of the plate. The sample amount should not exceed 200 μg (limited by the capacity of the layer). FA are subjected to Ag-TLC usually in the form of methyl esters. Methyl esters are particularly suitable when Ag-TLC is used as a complementary method with GC. We soak pre-coated plates for 5 min in 0.5% methanolic silver nitrate in order to obtain reliable analytical separation. After impregnation, the plates are air-dried, preserved in a dark place, and activated (between 5 min and 30 min,at 110-120°C in an oven) prior to sample application.

2.6.3 Spot detection

When the spot of mixture is dry, the plate is stood in a shallow layer of solvent in a covered beaker. Detection is the critical moment when working with pre-coated plates and especially those with an aluminium back. Detection of lipids, FA, is troublesome in general because of the lack of chromophores in the molecule. The non-destructive fluorescent reagents, widely used in preparative Ag-TLC, are not suitable in analytical mode because of the low sensitivity (note the low sample load mandatory in analytical Ag-TLC). Sensitive but destructive reagents like sulfuric or phosphomolybdic acids (50% and 10% solutions in ethanol, respectively) are the most common (**Note:** Both are hazardous!). The plate should be sprayed as thoroughly as possible (fume cupboard!) and heated (any kind of thermostated hot plate will do) at 160-180°C. The result is clearly visible spots/bands of the components with good contrast to the background

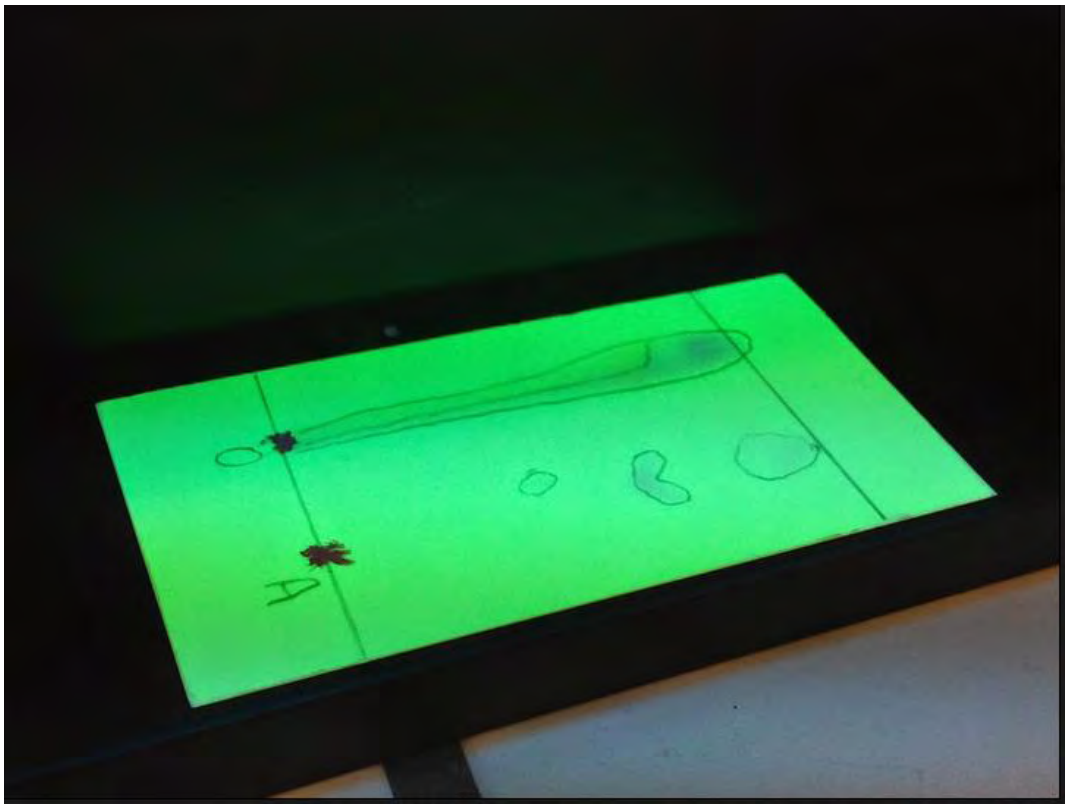


Figure 2.8 Spotted TLC paper**.**

2.7 Attenuated total reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR)

In recent years Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy is most important technique for monitoring to liquid phase concentration during crystallization process [75(a)]. It is used to determine energy difference between vibrational state of molecule solid, liquid or gaseous phase. ATR-FTIR spectroscopy is based on absorption in the mid-infrared region, that is a photon of infrared radiation of frequency vs is absorbed and the molecule presented higher vibrational state [75(b)]

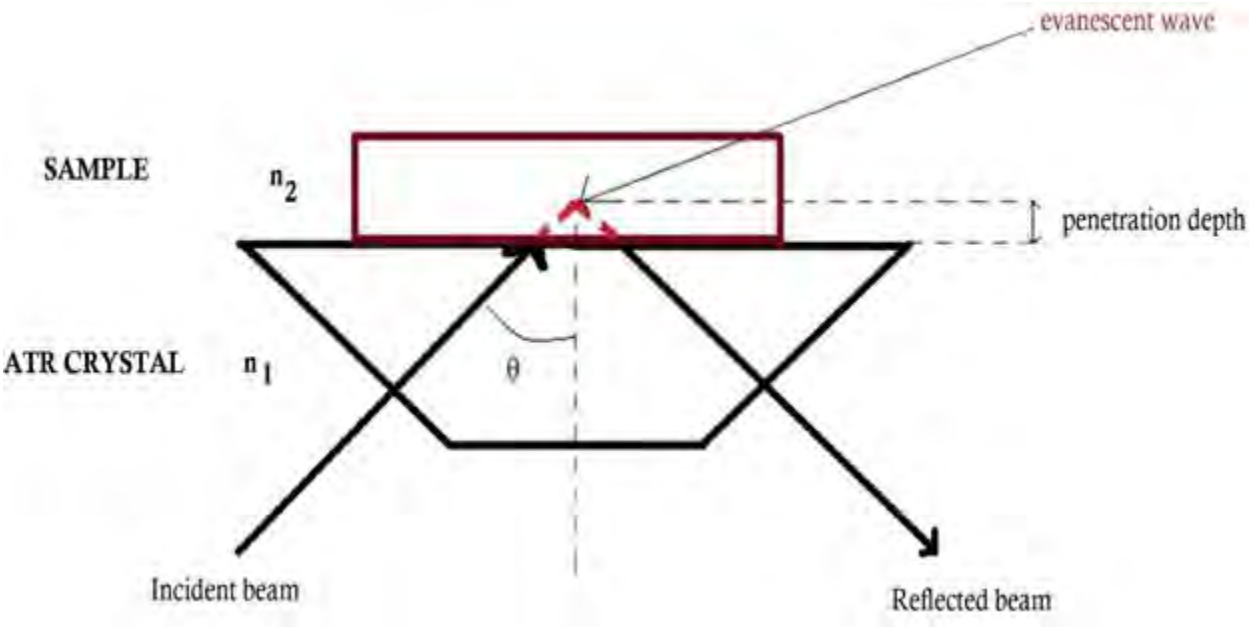


Figure 2.9. Schematic diagram of horizontal ATR sampling accessory illustrating the important parameters [75(b)]

ATR spectroscopy measuring beam, when the sample reflected internally interface between an auxiliary medium. This medium must be infrared transparent and of high refractive index [76].FTIR has been the dominant technique used for measuring the infrared (IR) absorption and emission spectra. The major advantage of FTIR technique is that practically all compounds show characteristic absorption emission in the IR spectral region. This reason FTIR is highly sensitive detection methods for trace gas, solid or liquid samples and some components of an unknown mixture of functional.In many ways, Fourier Transform Infrared spectroscopy would appear to be

the ideal technology for on-line chemicals analysis, and that is reduce measuring time and increased light though put. This technology is a major advantage over conventional dispersive-based IR spectroscopy and, being based on interferometry, provides enhanced energy throughput and a better signal-to-noise ratio, high resolution, and wide range spectrum.[63]. The IR samples are either dried on the window material, or if they had been dried in advance, an aqueous suspension is prepared where an aliquot is transferred to the window and dried. Solid samples in powder form can also be mixed with an IR transparent material such as KBr and moulded compound into pellets under high pressure.

The term "infrared" generally refers to any electro-magnetic radiation, functional groups of molecules absorb in a range between (14000 ~ 4)cm-1. However, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy is a more convenient method as liquid fatty acid can be analyzed directly without making KBr plate [78,79]. In FTIR, trans-ethylenic double bonds absorb in between 976 and 956 cm^{-1} with a maximum at 966 cm^{-1}. This is due to the deformation of the C-H bond adjacent to the trans double bond and bending vibrations of trans =CH groups [79]. According to Sherazi [78], specific region of trans peak in FTIR is in between 990-945 cm^{-1} where trans peak at 967 cm^{-1} is considered to be the most prominent in the partially hydrogenated oil.

Calculation: The law of Lambert- Bear is commonly used to determine IR spectroscopy to relate the measures of absorbance,

$$A= \varepsilon lc$$

Where A is absorbance, C is concentration, ε is extinction coefficient and l is the path that the light travel through material. If the sample is shown low concentration and deviation at high concentration the law is only valid. To apply this equation, path length also has to be known which is very difficult for keeping consistent in KBr pellets. Although ATR has higher prospective in quantitative analysis by utilizing a defined evanescent field, data quality may differ because of different sensitivities according to the physical state of the sample. Due to low signal density and low signal -to –noise ratio the measurement shows error at low concentration of mixed samples.

2.8 GC-FID [Gas Chromatography – Flame Ionization Detector]

Gas Chromatography – Flame Ionization Detector or GC-FID is a most commonly used analytical technology for the analysis of fatty acids separating and analyzing compounds that can be vaporized without decomposition. that is widely used in the petrochemical, pharmaceutical and natural gas markets. An FID typically uses a Helium/Hydrogen/Air flame into which the sample is passed to oxidise organic molecules and produces electrically charged particles (ions). The ions are collected and produce an electrical signal which is then measured. As common with other GC techniques, a carrier gas is mobile phase usually such as helium or an unreactive gas such as nitrogen required with perform gas chromatography. The FID is also extremely sensitive to Hydrocarbon impurities in the Hydrogen and Air supply for the flame. Hydrocarbon impurities can cause increased baseline noise and reduce the detector sensitivity.The determination of FAME by GC is among the most commonplace analyses in lipid research. Quantification of FAME by GC with FID has been effectively performed for some time, whereas detection with MS has been used chiefly for qualitative analysis of FAME.

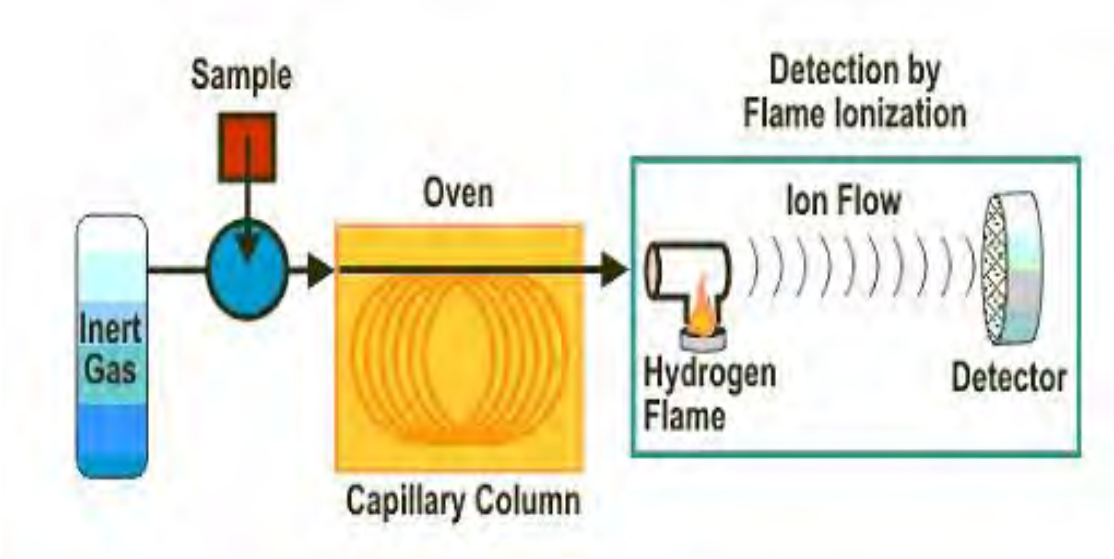


Figure 2.10. Flame Ionization Detector

In an FID the sample undergoes a combustion in a hydrogen/synthetic air flame. Ions and free electrons are formed in the flame. The charged particles produce a measurable current flow in the gap between two electrodes in the detector. The resulting current flow is of greater strength than the signal produced by the pure carrier gas and the fuel gas flame alone. This signal differential provides information about the sample. The current is proportional to the information which depends on the composition of the separated sample. The FID is a general detector which, with extra configurations, can be used for more specific components. For example, with placing a methanizer ahead of the FID, components containing carbon can undergo a formation to methane and thereby be suited for further FID analysis. Flame ionization detectors are extremely sensitive and have a wide range of linearity. An important facet of the GC-FID is the use of a carrier gas to transfer the sample from the injector, through the column and into the FID-detector. He carrier gas must be inert and may not be adsorbed by the column material. Helium or nitrogen are normally used as carrier gas with GC-FID, and sometimes hydrogen. The detector gases, hydrogen and synthetic air, serve respectively as fuel gas and oxidizing gas during the combustion process. Since hydrocarbon impurities, moisture and oxygen produce a greater baseline noise which has an adverse effect on the detection limit, these impurities in the detector gases should be kept as low as possible.

GC-FID was performed in a Shimadzu, GC-2010 Plus (Tokyo, Japan) gas chromatograph fitted with a fused silica polar capillary column (75 m x 0.18 mm i.d x 0.14 µm film thickness) coated with poly(biscyanopropyl siloxane) and flame ionization detector. GC-FID analysis was performed under following conditions: Initial column temperature was maintained at 180 ºC and held for 45 min. Then the temperature was increased to a final temperature of 240 ºC at a rate of 4 ºC/min and hold for 15 min. Injector temperature: 250 ºC, Injected volume: 1 µL, Injection mode: split, split ratio- 50:1. Detector temperature: 250 ºC, column flow rate: 0.34 ml/min and carrier gas: nitrogen.

2.9 Statistical Analysis

The sample fatty acid quantification were analyzed one by one. Result for fatty acids composition were expressed as g/10g of total fatty acids while the data for fatty acids in sample were expressed as percent of total fatty acids.

2.10 Gas chromatography-flame ionization detector (GC-FID) analysis

Trans fatty acids isomers including elaidic acid (C18:1 trans-9), vaccenic acid (C18:1 trans-11) and palmitelaidic acid (C16: 1 trans-9),saturated fatty acid isomers, monounsaturated fatty acid isomers, polyunsaturated fatty acid isomers have been used as standard to were identified by comparing retention times with the authentic standards (>98% purity, Sigma-Aldrich, Germany). Each FA isomer was analyzed according to the following formula

FA isomers (% fat)

$$= \frac{\text{Area FA isomers} \times \text{Conc. IS}}{\text{Area}_{IS}}$$

$$= X\ ppm$$

$$= \frac{X \times 10}{1000}\ {}^{mg}/_{10ml}$$

$$= Y\ {}^{mg}/_{10ml}$$

$$= \frac{Y \times 100}{Sample\ weight}\ {}^{mg}/_{100g}$$

$$= Z\ {}^{mg}/_{100g}$$

$$= \frac{Z}{100}\ {}^{g}/_{100g}$$

$$= Sample\ \%$$

Chapter -3

Result and Discussion

3.1 Sample Information

On the basis of evidences from many experimental studies, dietary trials, and prospective observational studies, the consumption of fatty acids from partially poultry foods provides no apparent nutritional benefit and has considerable potential for harm. Fatty acids have been extracted from fast foods using Soxhlet extraction method in chloroform solvent according to standard method AOAC [74(b)]. Infrared (IR) spectroscopy [80] and Gas Chromatography – Flame Ionization Detector or GC-FID [81] have been used to determine the trans fatty acids in the collected chicken based fast foods from around Dhaka city. Fast food items have been collected from Dhanmondi, Mohammadpur, Shamoli and Mirpur of Dhaka. Fast food sample information is given in table 3.1

Table 3.1 Fast Food Sample Information

Sample	Sample Type	Sample Address
A	Chicken Winglet	KFC, House-84,Rd-7/A, Dhanmondi-15, Dhaka-1209
B	Chicken Hot Wings	KFC, House-84,Rd-7/A, Dhanmondi-15, Dhaka-1209
C	Chicken Drumst	Agora Superstores, 24/A ,Ring Road, Block-C, Japan garden city,Mohammadpur,Dhaka-1207
D	Chicken Botik	Agora Superstores,24/A ,Ring Road, Block-C, Japan garden city,Mohammadpur,Dhaka-1207
E	Chicken Fiery Grilled	KFC, House-84,Rd-7/A, Dhanmondi-15, Dhaka-1209
F	Chicken Meet Ball	Kazi farm, Sompa Market ,Shamoli,Dhaka
G	Chicken Naget Fry	Kazi farm, Sompa Market, Shamoli,Dhaka

3.2 ATR-FTIR spectrum & Result of meat product

In this study elaidic acid (C18:1 trans-9), vaccenic acid (C18:1 trans-11) and palmitelaidic acid (C16: 1 trans-9), Linoleic acid (LA), (C18:2 *cis*-9,12) Linolenic acid have been used as standard because elaidic acid (C18:1 trans-9) and vaccenic acid (C18:1 trans-11) are considered to responsible for coronary heart disease (CHD). The ATR-FTIR of sample A (Chicken Winglet) is shown in Figure 1. ATR-FTIR spectrum of three standard trans fatty acids and all fast food samples (A-G) are shown in Figure 2. Both Figure 3.10.1 and Figure 3.10.2 clearly show the presence of typical functional groups in fatty acids of the fast food samples.

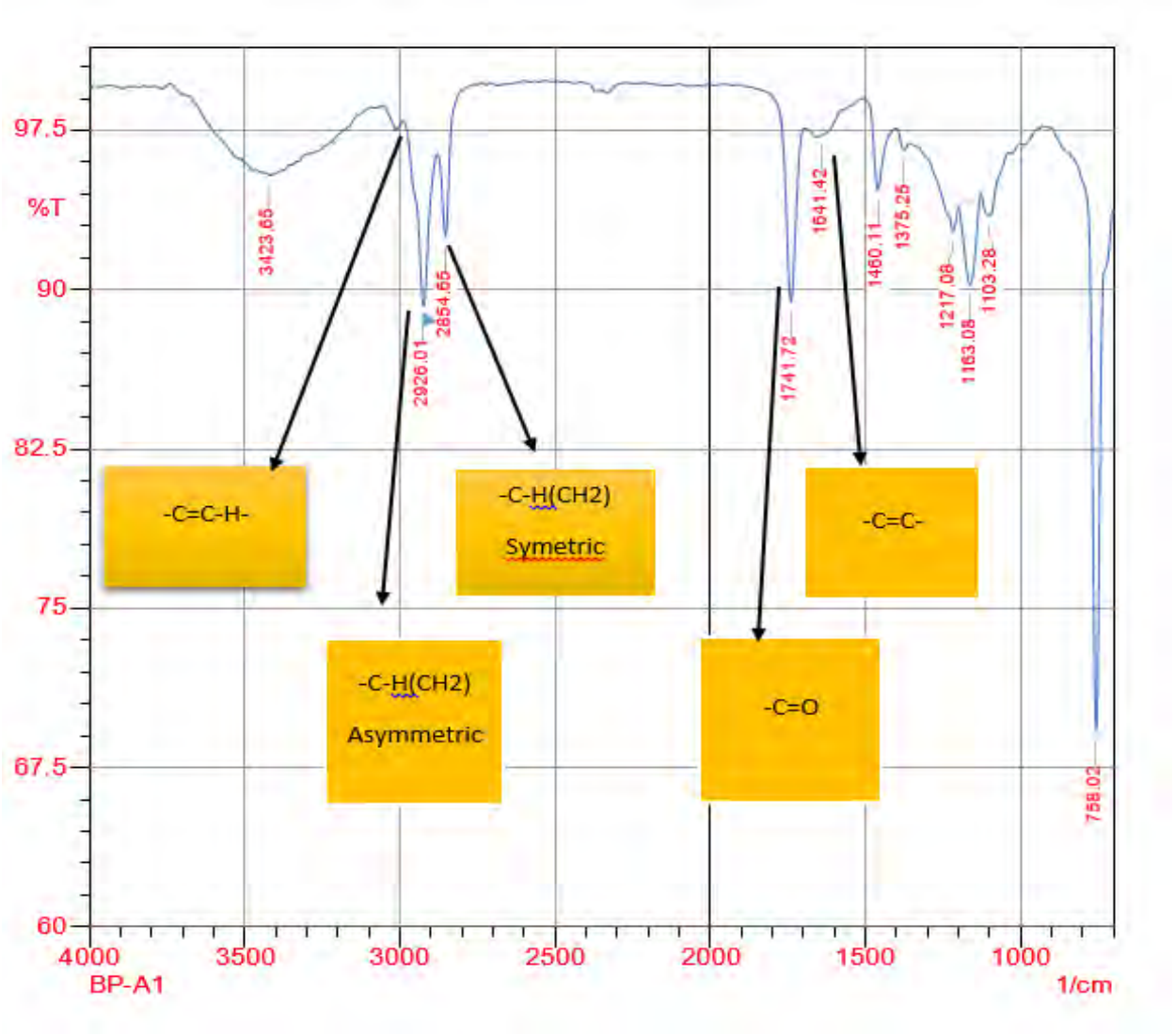


Figure 3.1 ATR-FTIR of Chicken Winglet (A)

In the Figure 3.1, C-H stretching vibration of cis- double bond appears at 3008 cm^{-1}. The band at 2924 cm^{-1} is due to asymmetric stretching of aliphatic methylene group, -C-H (CH_2) present in fatty acid of sample. The symmetric stretching of –C-H (CH_2) is at 2855 cm^{-1}. The band at 1743 cm^{-1} is due to –C=O stretching which arises from ester carbonyl group of triglyceride of fatty acid. The C=C stretching vibration of cis-olefin appear at 1639 cm^{-1} as a very weak band. The –C-H bending of the CH_2 and CH_3 of aliphatic groups appear at 1463 cm^{-1}. The band at 1218 cm^{-1}, 1157 cm^{-1}, and 1103 cm^{-1} are due to stretching vibration –C-O ester group [77]

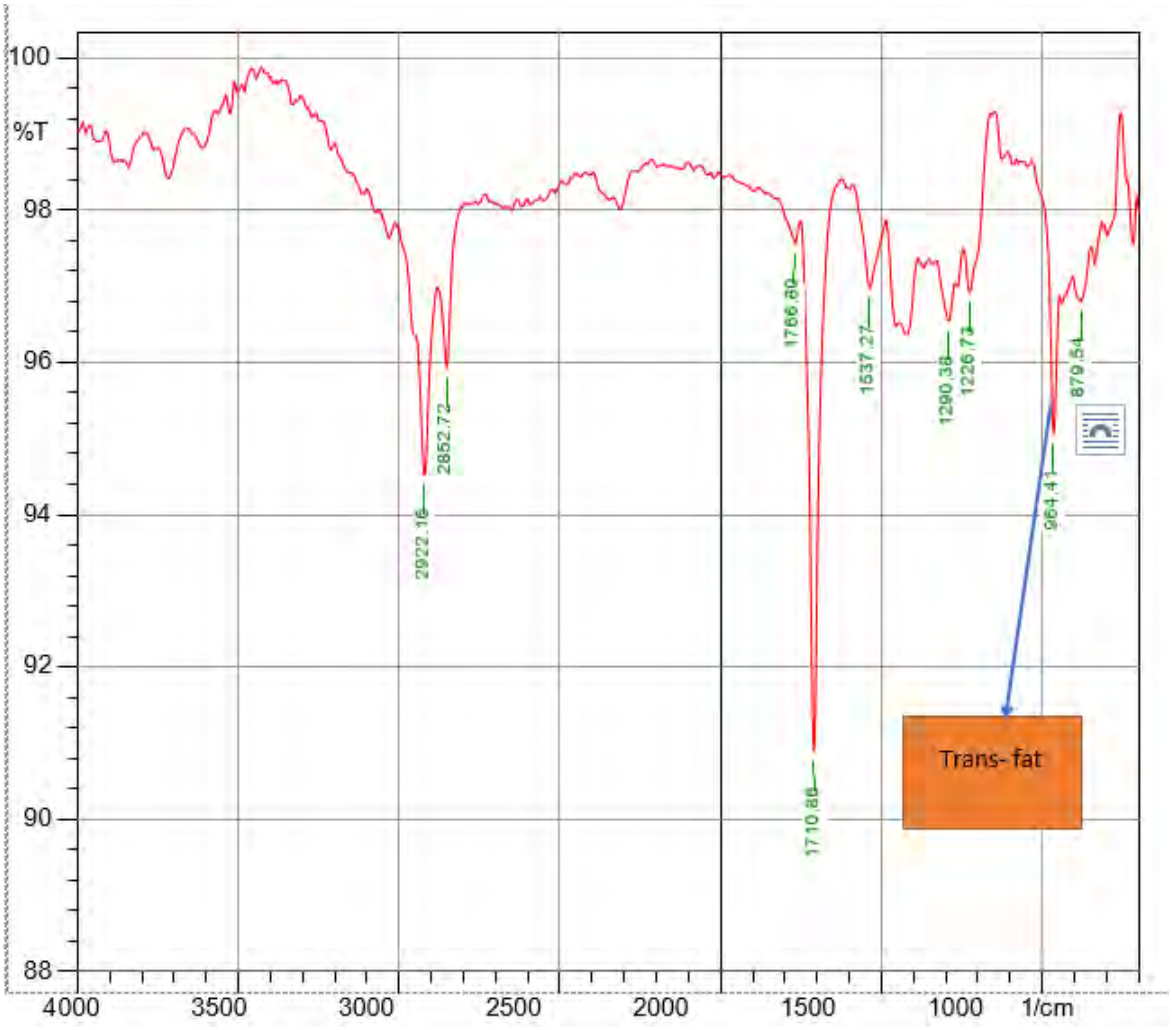


Figure 3.2. . ATR-FTIR of Palmiteladic acid

An expanded ATR-FTIR spectrum of three standard trans fatty acids are shown in Figure 3.2 to 3.4 show trans peak more clearly. Trans peak at 964 cm^{-1} is clearly found in all the three standard trans fatty acids namely palmitelaidic acid, elaidic acid & trans-vaccenic acid.

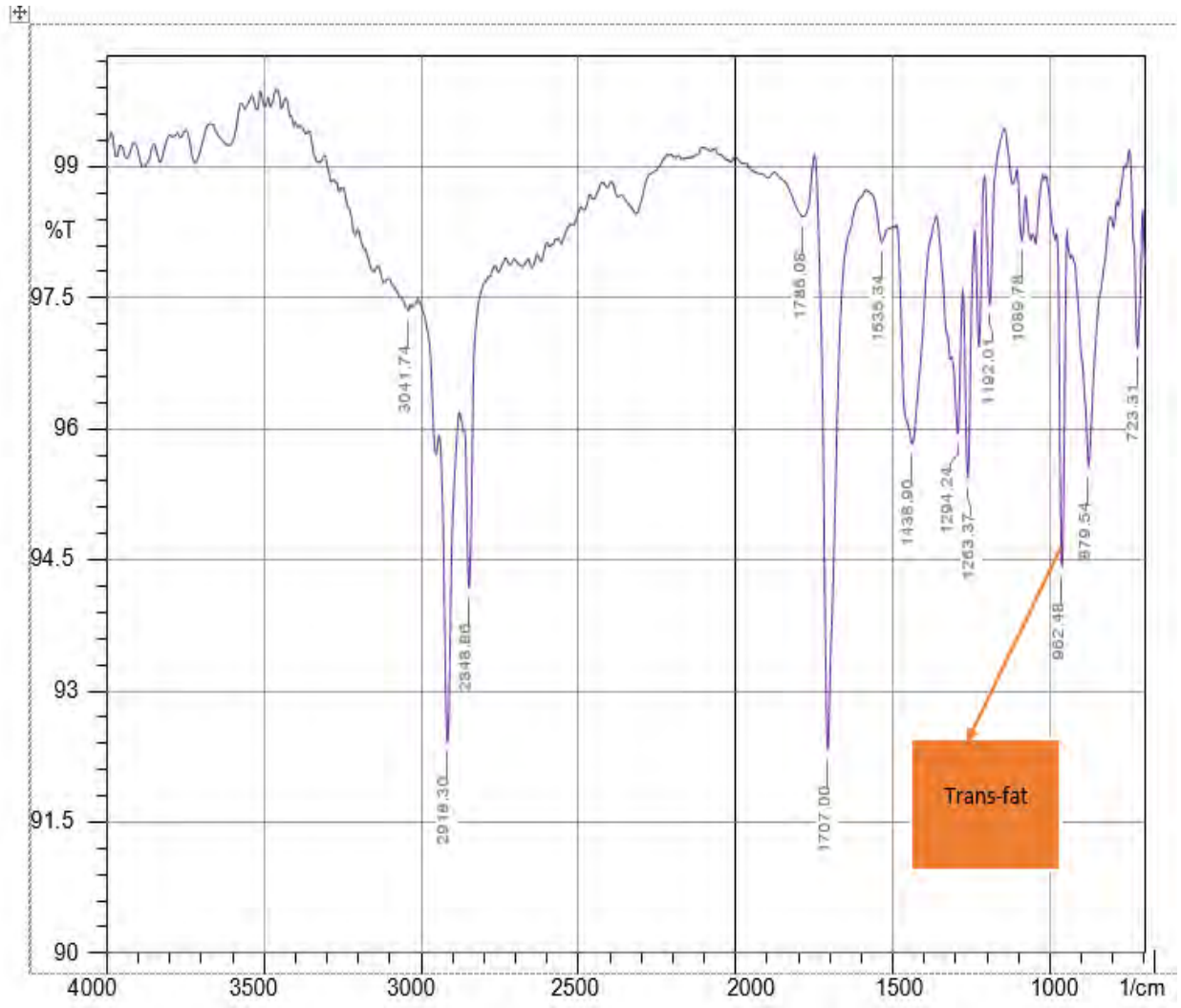


Figure 3.3. ATR-FTIR of Elaidic acid

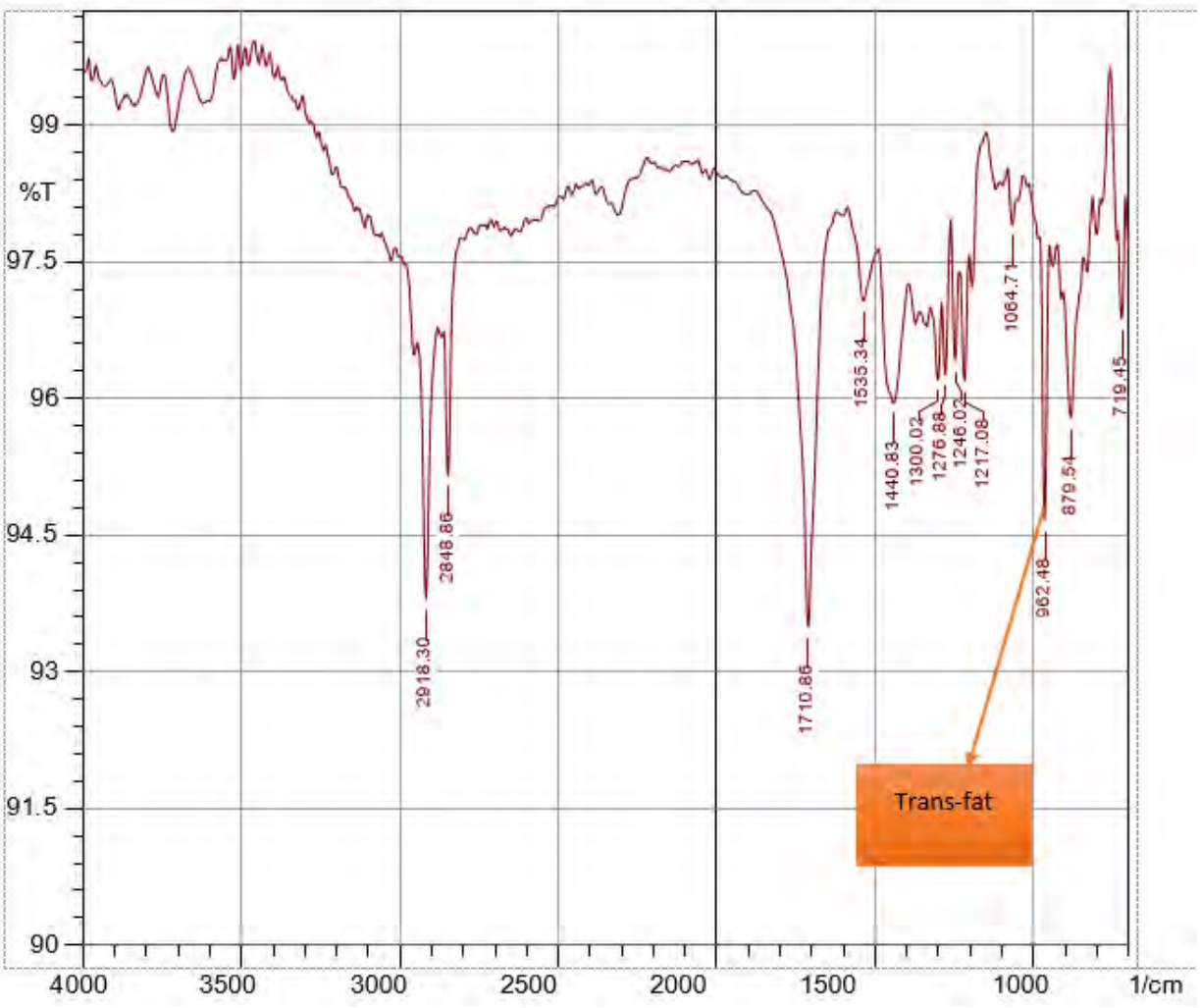


Figure 3.4. ATR-FTIR of trans-vaccenic acid

ATR-FTIR spectrum of smples (B-G) are shown in Figure 3.5-3.10.

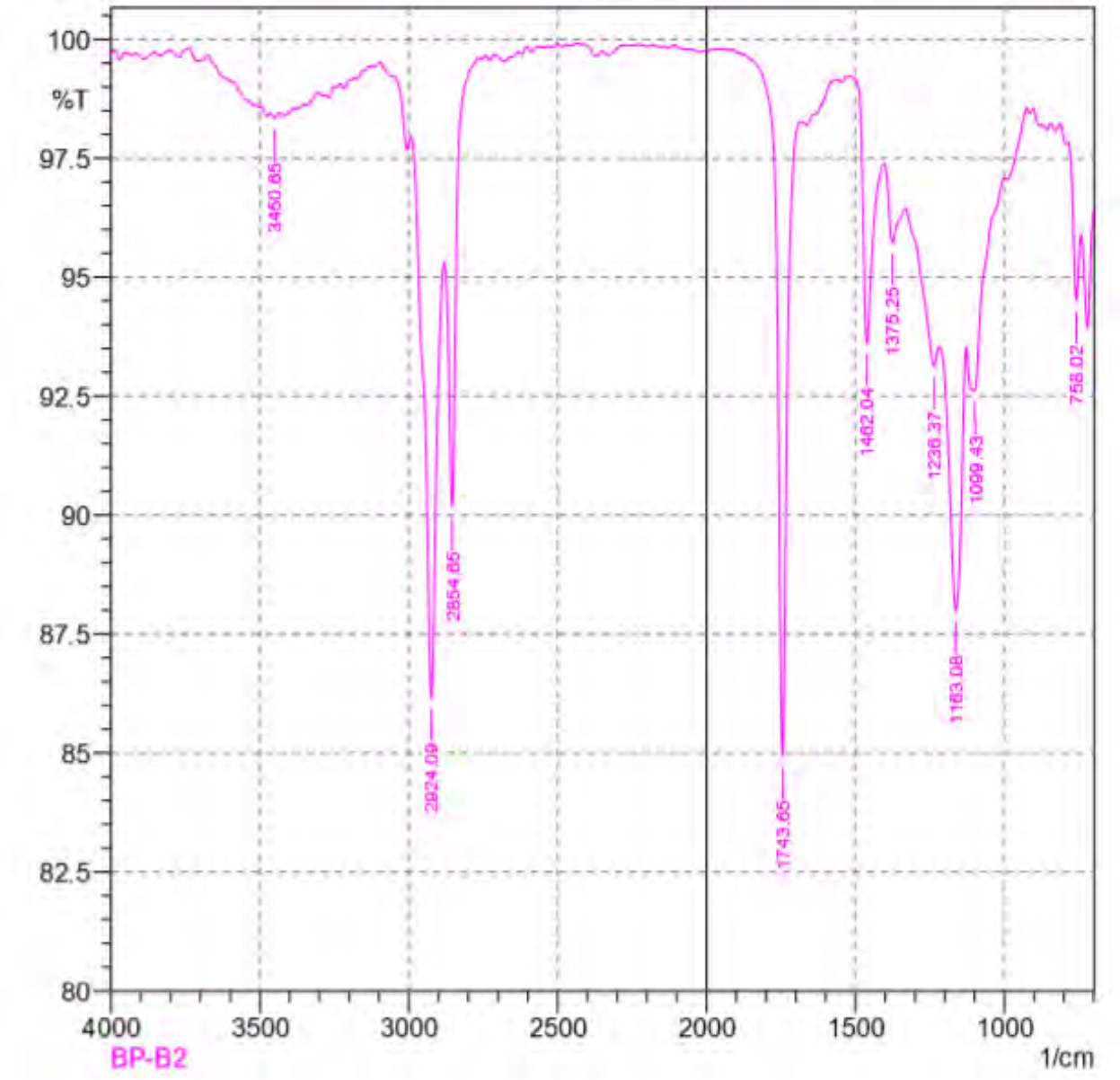


Fig 3.5. ATR-FTIR of Chicken Hot Wings (B)

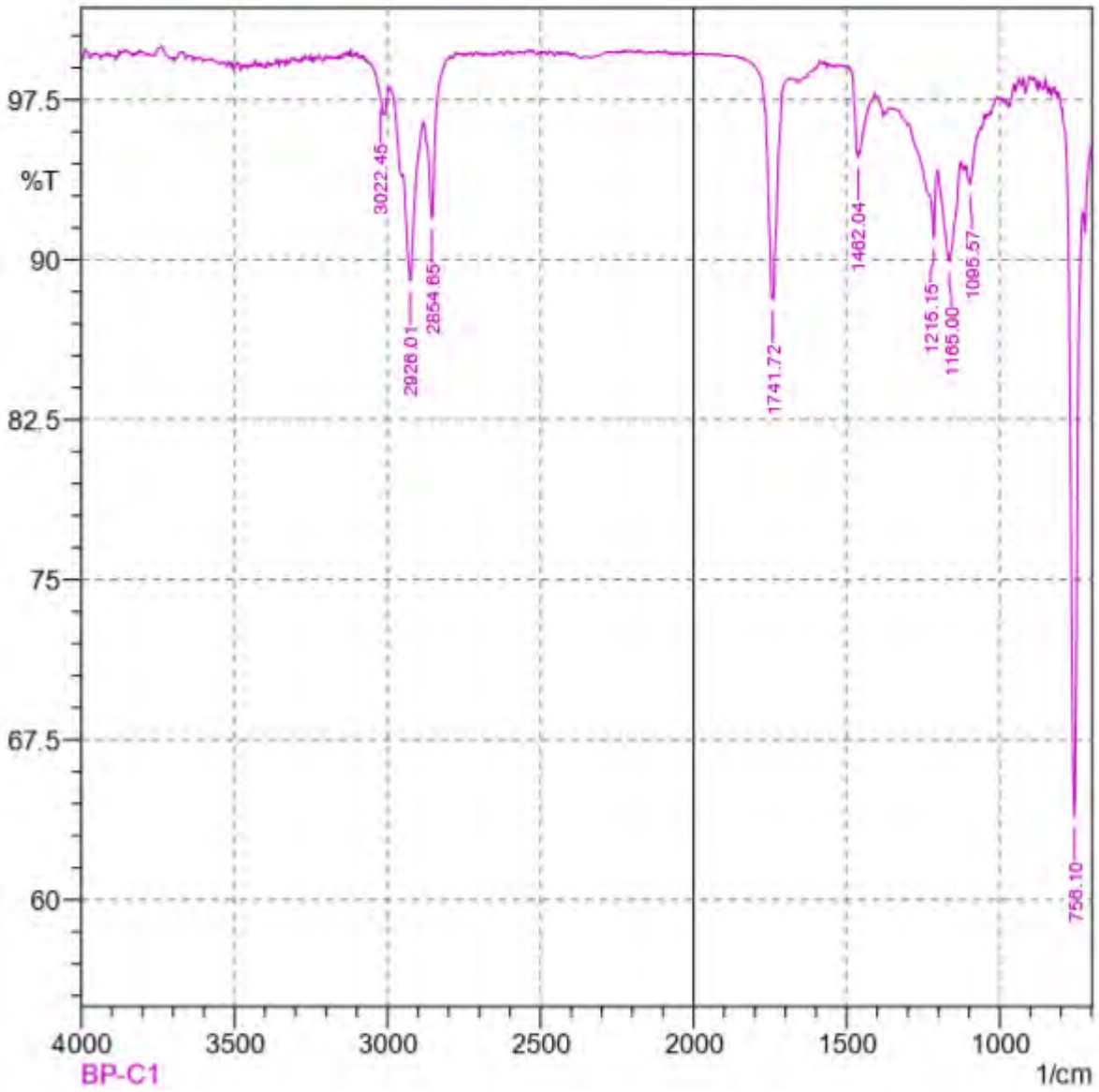


Figure 3.6. ATR-FTIR of Chicken Drumst (C)

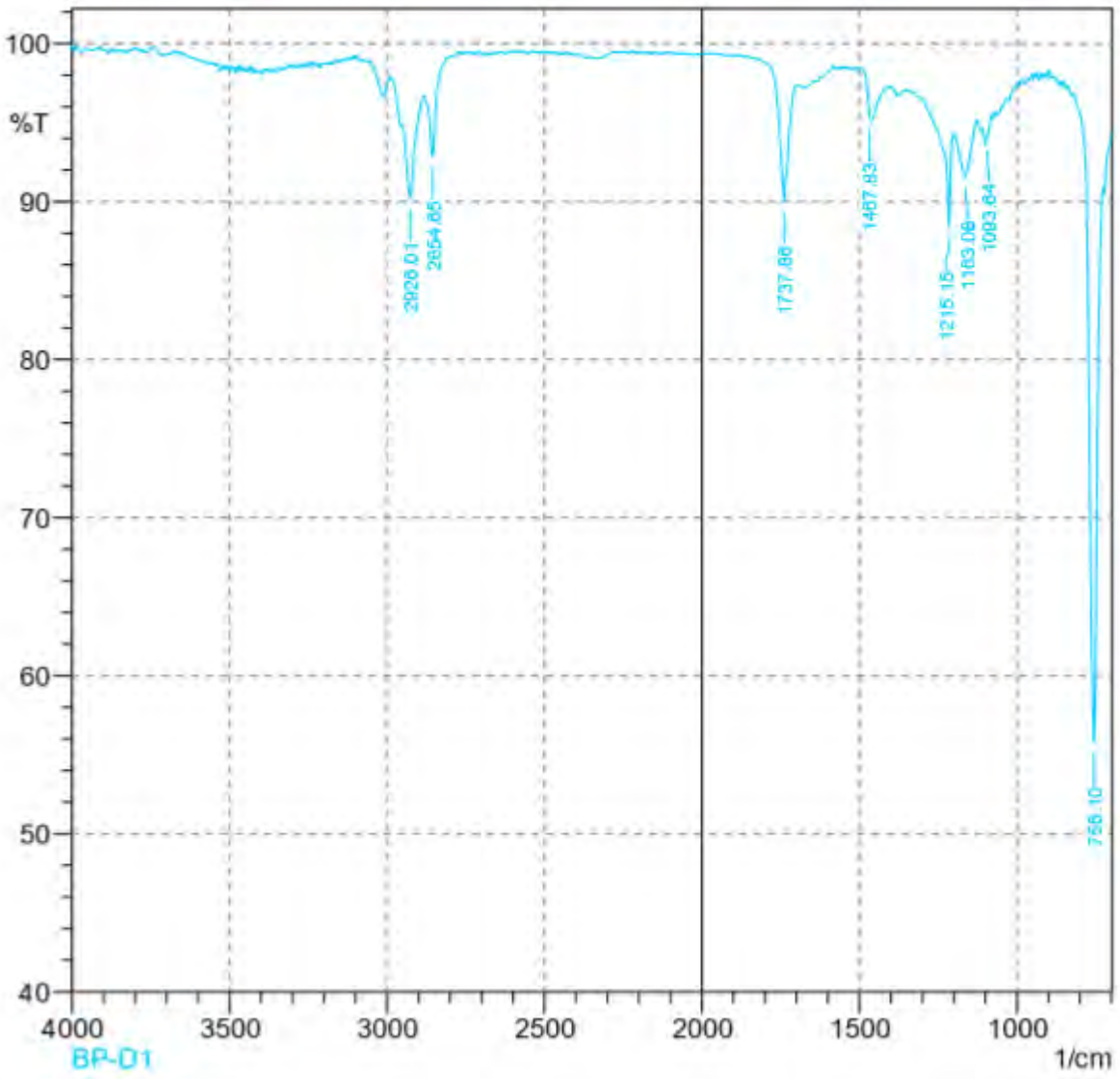


Figure 3.7. ATR-FTIR of Chicken Botik (D)

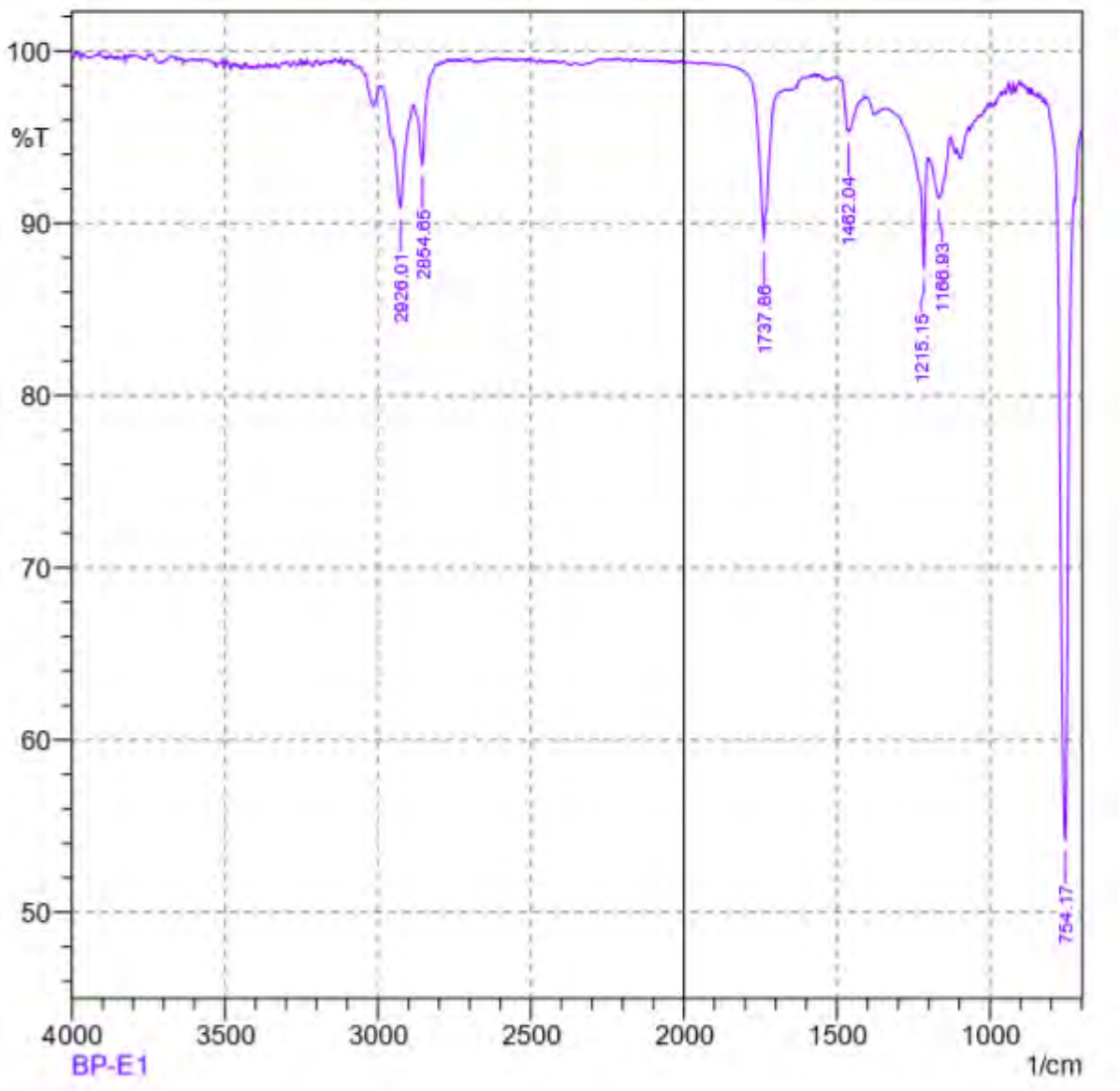


Figure 3.8. ATR-FTIR of Chicken Fiery Grilled (E)

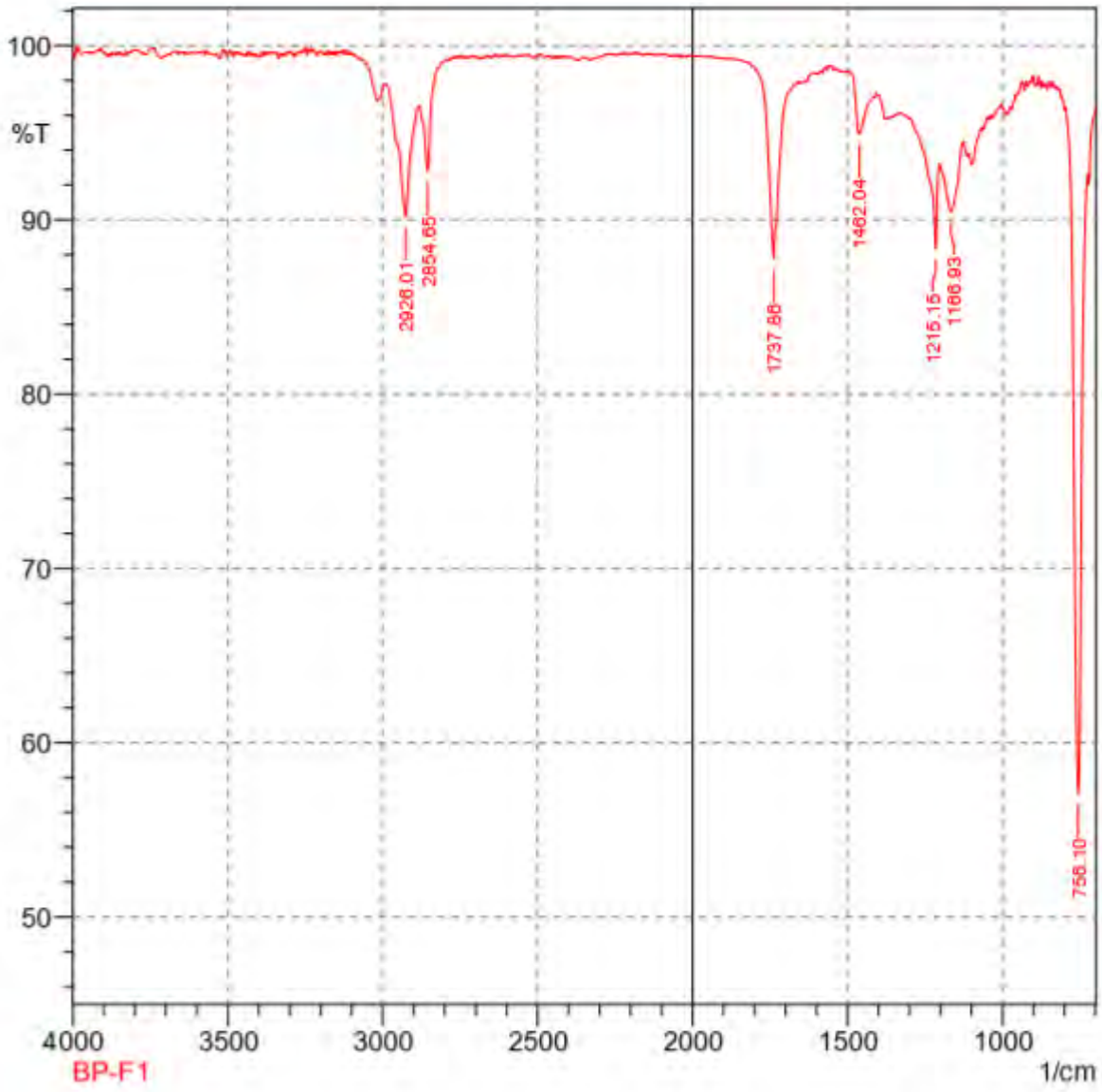


Figure 3.9. ATR-FTIR of Chicken Meet Ball (F)

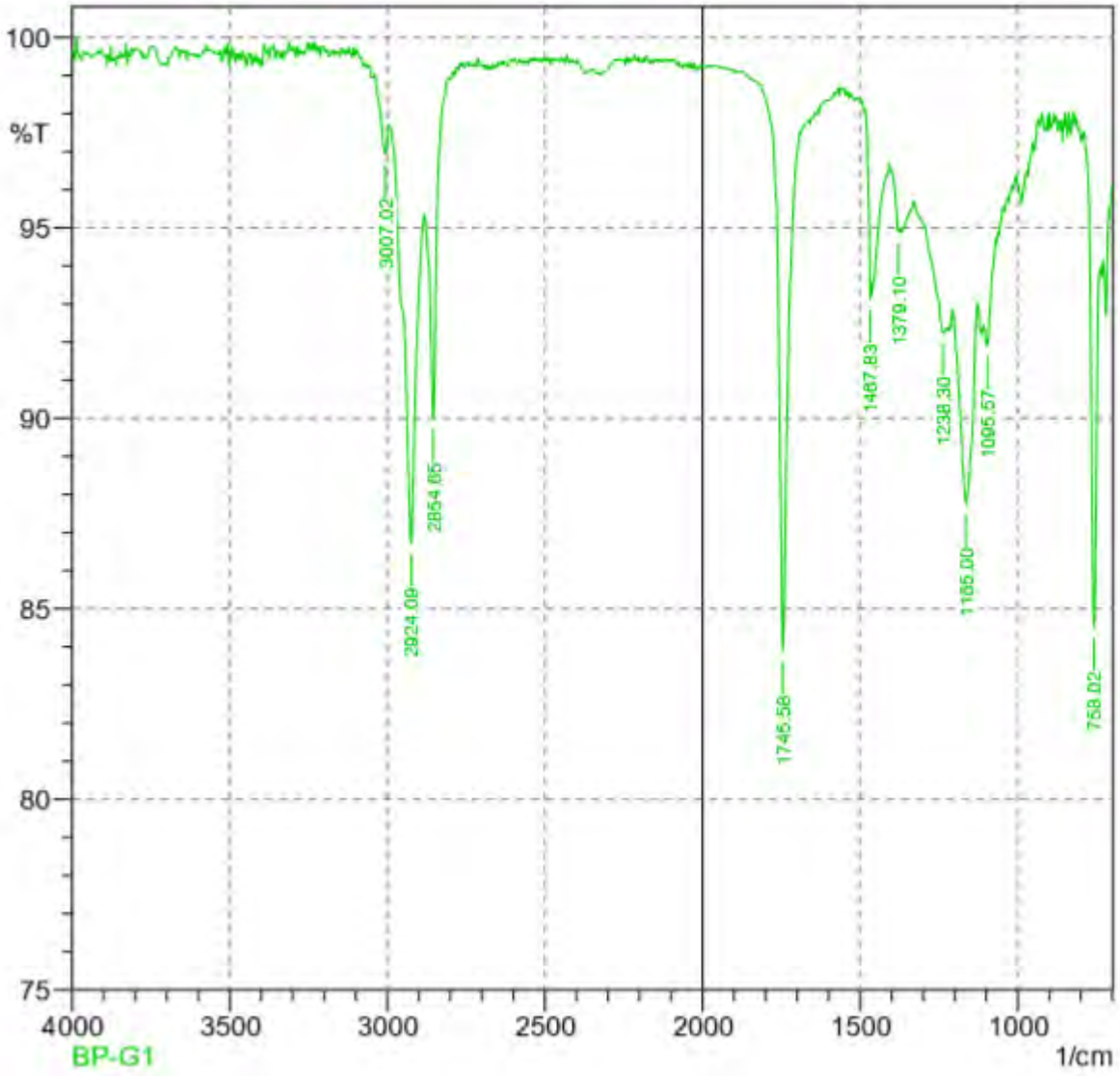


Figure 3.10. ATR-FTIR of Chicken Naget Fry (G)

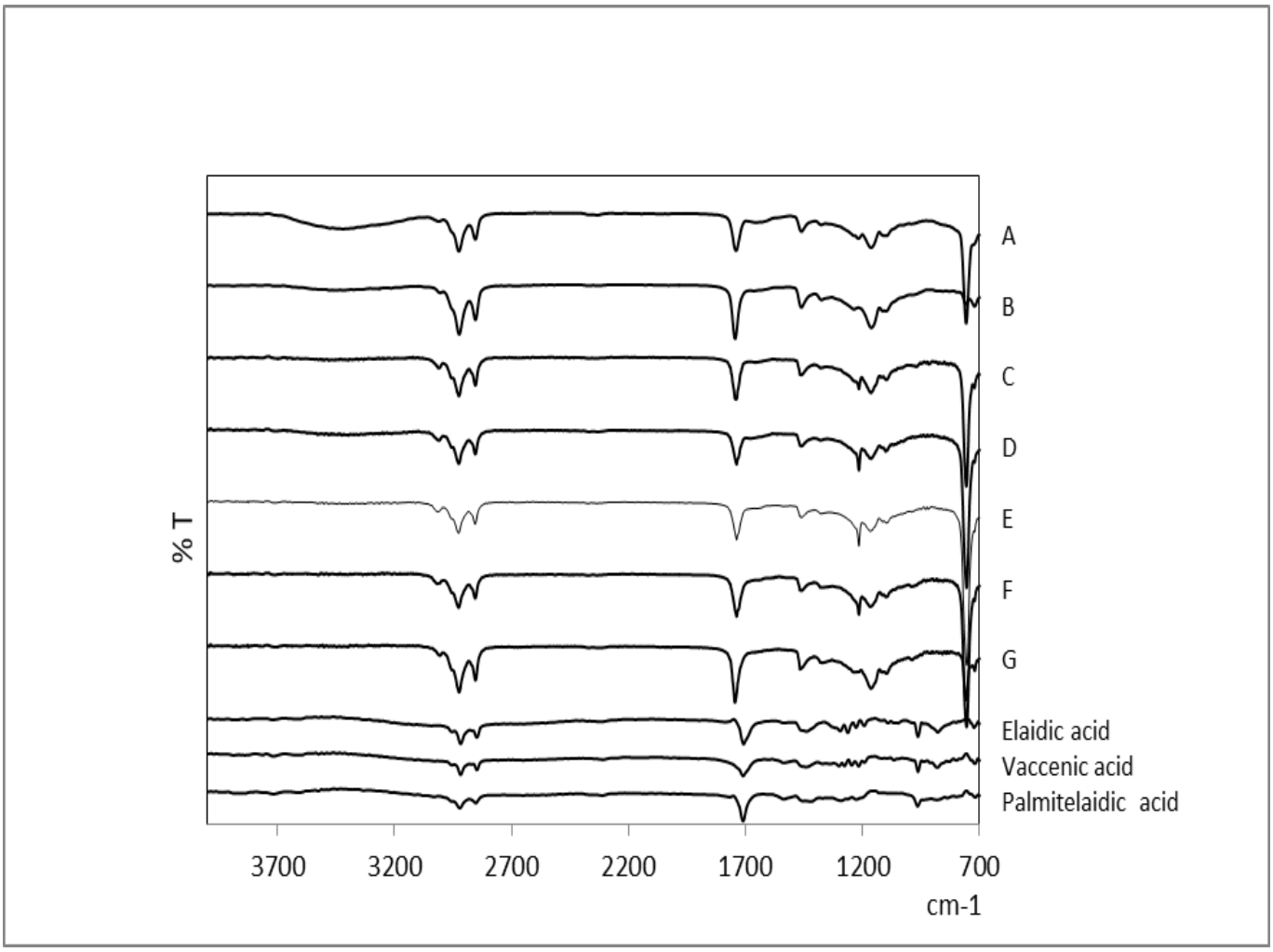


Figure 3.11. ATR-FTIR of three standard trans fatty acids and fast food samples (A-G) from 700-4000 cm^{-1}.

However, none of the fast food samples (A-G) has any peak in the characteristic trans peak region from 976 to 956 cm^{-1}. According to literature, trans peak is found in the region from 976 - 956 cm^{-1} with a maximum at 966 cm^{-1} which is due the CH out-of-plane deformation absorption.[78,79].In the table all sample (A-G) and standard wave number and functional group have submitted

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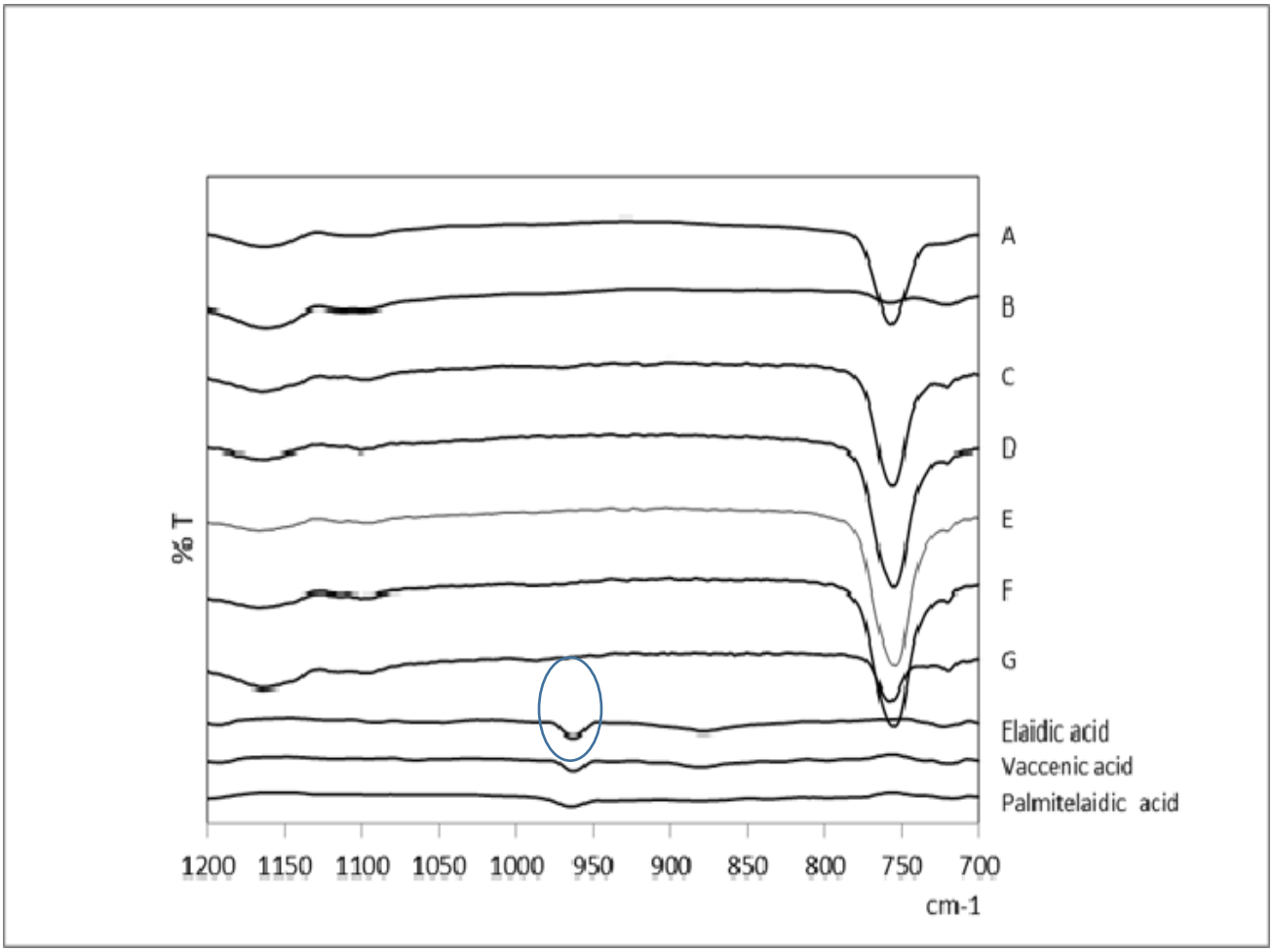

Figure 3.12. ATR-FTIR (expanded) of three standard trans fatty acids and fast food samples (A-G) to show trans peak region from 976 to 956 cm^{-1} more clearly.

Table 3.2. List of ATR-FTIR absorption data (wave number) according to functional groups

Sample identiy	Functional group	Wave number (cm^{-1})
A	–OH, –C=C–H–, asymmetric–C–H(CH_2), symmetric –C-H (CH_2), –C=O,	3424,3008,2924,2855,1743
B	–OH, –C=C–H–, asymmetric–C–H(CH_2), symmetric –C-H (CH_2), –C=O,	3424,3008,2924,2855,1743
C	asymmetric–C–H(CH_2), symmetric –C-H (CH_2),–C=O,	2924,2855,1743
D	–C=C–H–, asymmetric–C–H(CH_2), symmetric –C-H (CH_2),–C=O,	3008,2924,2855,1737
E	–C=C–H–, asymmetric–C–H(CH_2), symmetric –C-H (CH_2),–C=O,	3008,2926,2855,1737
F	C=C–H–, asymmetric–C–H(CH_2), symmetric –C-H (CH_2),	3008,2926,2855,1737
G	C=C–H–, asymmetric–C–H(CH_2), symmetric –C-H (CH_2),	3007,2924,2855,1746
Palmiteladic acid	Trans fat	964.41
Elaidic acid	Trans fat	962.48
trans-vaccenic acid	Trans fat	962.48

In ATR-FTIR recomond that functional group of –OH wigging and stretching vibration at 3024 cm^{-1},C-H stretching vibration of cis- double bond appears at 3008 cm^{-1}. The assymetric stretching of CH (-CH_2) is at 2924 cm^{-1}, symmetric stretching of CH (-CH_2) is at 2855 cm^{-1}. The ester group (-C=O) is observed at 1746 cm^{-1}, bending of –CH (-CH_2-, CH_3) is at 1463 cm^{-1} and stretching of –C-O is at 1157 cm^{-1} of fatty acids present in the sample. The band at1639 cm^{-1} is due to C=C stretching vibration of cis-olefin.

3.3 GC –FID Spectrum & result

ATR-FTIR provides information of trans fat containing more than 5%[78]. However, GC can give measurement at lower level of trans fat. The results obtained from ATR-IR were further verified by the GC-FID. It is known that very long (around 100 m) and highly polar capillary columns can provide better resolution of trans fatty acid isomers [78]. For this reason, a polar capillary column (75 m long) coated with poly(biscyanopropyl siloxane) is used in GC analysis to maximize the resolution of trans fatty acid isomers. For GC-FID analysis, methyl esters of standards and samples were performed. The Figure 3.13 shows the GC-FID chromatogram of standard fatty acids.

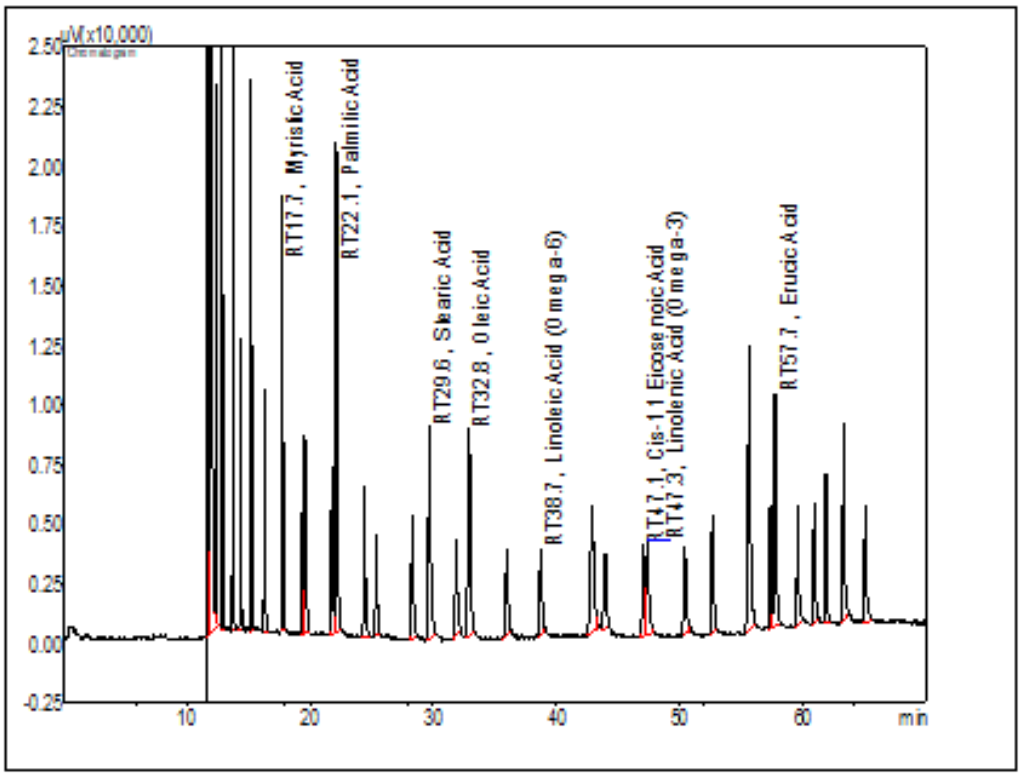

Figure 3.13. GC-FID chromatogram of standard fatty acids.

The Figure 3.14 shows the GC chromatogram of sample D (Chicken Botik). Trans fatty acids were not found in any of the samples in GC-FID analysis (Fig. 5 and Table 1). This result is consistent with the ATR-FTIR analysis. Table 1 shows the fatty acids and their contents that are present in

each of the fast food samples by GC-FID analysis. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) have been found in the fast food samples. Chicken Winglet (sample A) and Chicken Hot Wings (sample B) of KFC have higher amount of saturated fatty acids (SFA) which are 28.73% and 25.92% respectively. The amount of saturated fatty acids (SFA) in Chicken Drumst, Chicken Botik, Fiery Grilled Chicken, Chicken Meatballs, and Chicken Nuggets are in between 10.94-19.38%. Saturated fatty acids found in the fast food samples are palmitic acid, stearic acid, and myristic acid. All fast food samples contain oleic acid and linoleic acid (omega-6) in various amounts ranging from 12.47% to 28.53% and 8.30% to 18.90% respectively. Except sample D (Chicken Botik of Agora) none of the sample contains linolenic acid (omega-3) fatty acid. In sample D, 8.30 % linoleic acid (omega-6) and 3.57 % linolenic acid (omega-3) are present. This shows omega-6 to omega-3 ratio in sample D is 2.32:1.

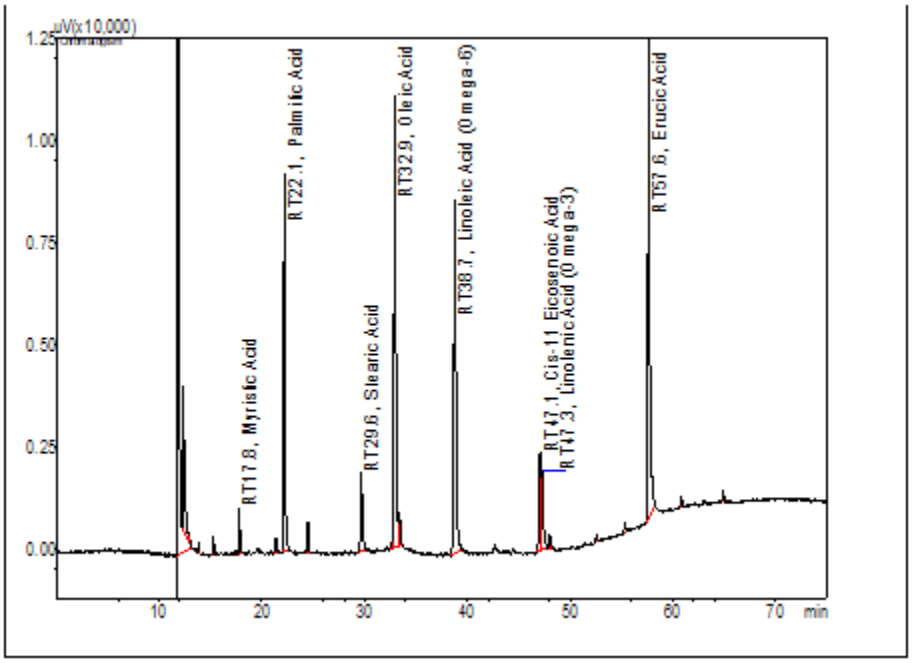

Figure 3.14. GC-FID chromatogram of sample D (Chicken Botik).

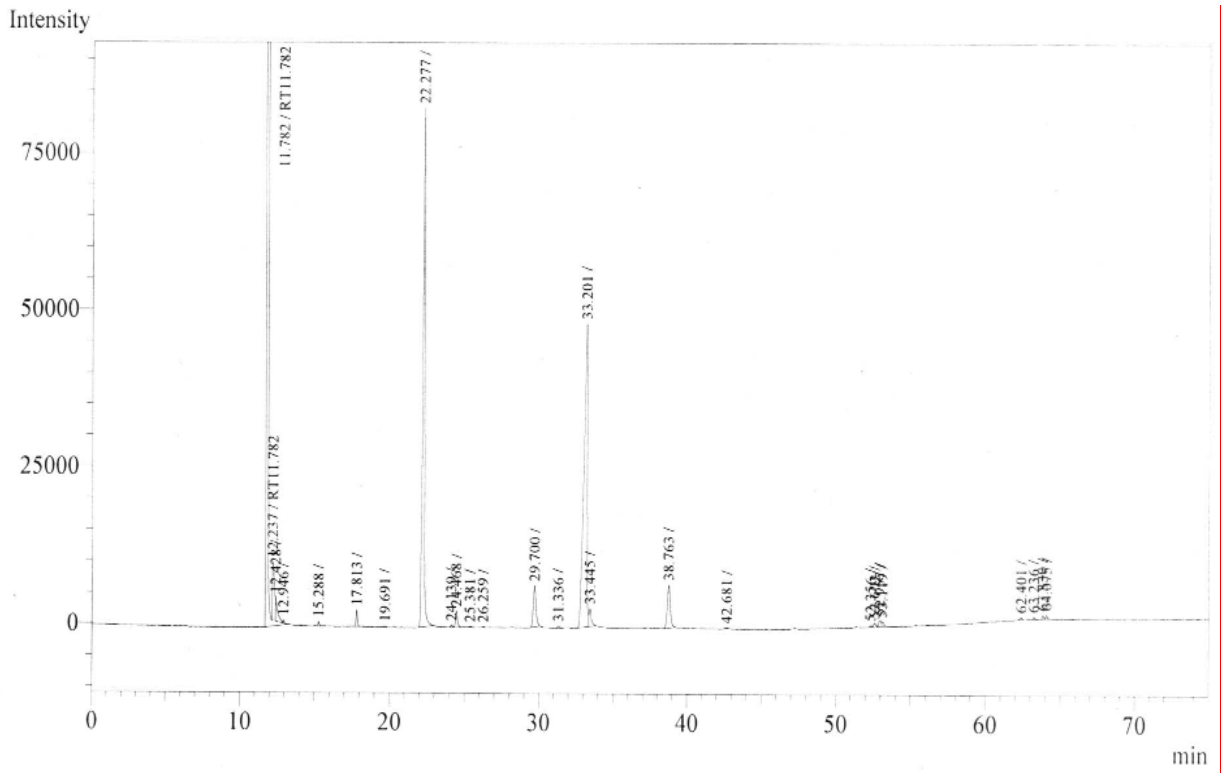

Figure 3.15. GC-FID chromatogram of Chicken Winglet(A)

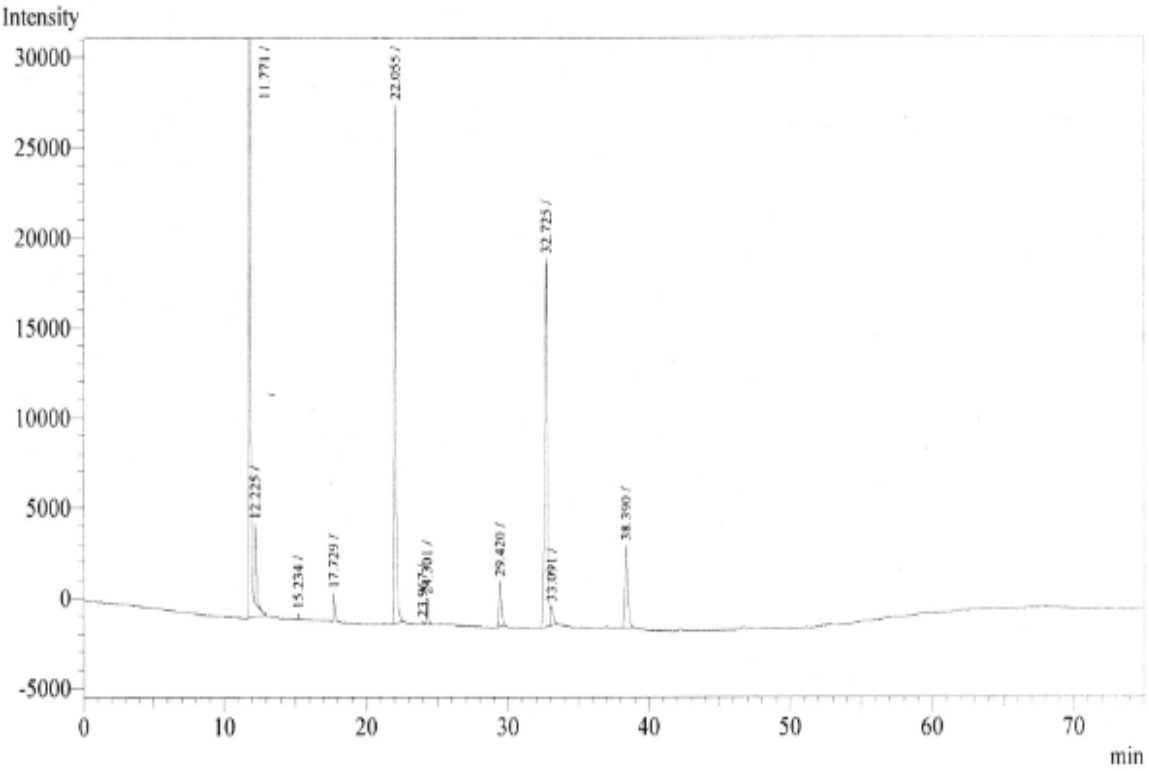

Figure 3.16. GC-FID chromatogram of Chicken Hot Wings (B)

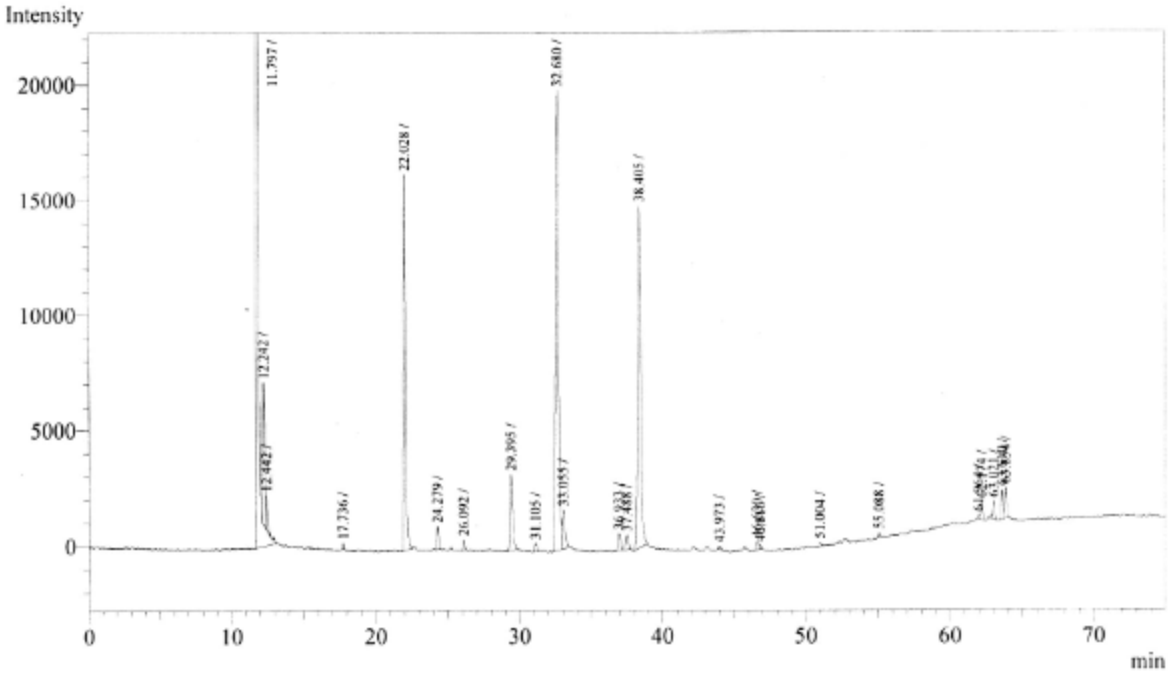

Figure 3.17. GC-FID chromatogram of Chicken Drumst (C)

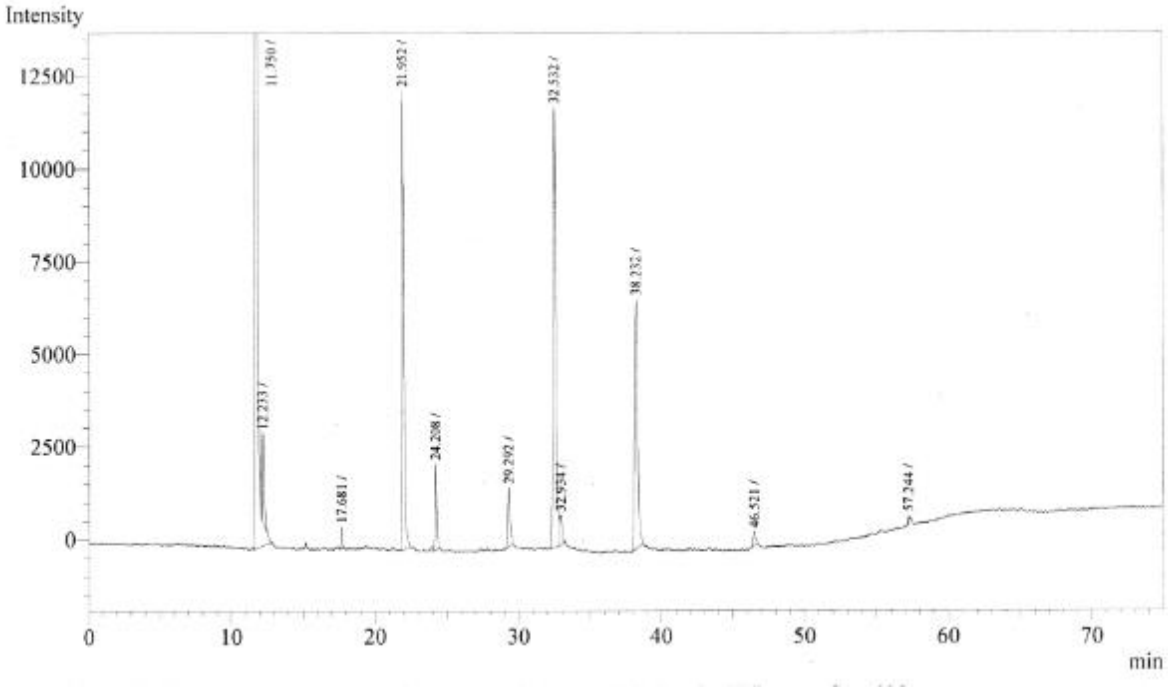

Figure 3.18. GC-FID chromatogram of Chicken Fiery Grilled (E)

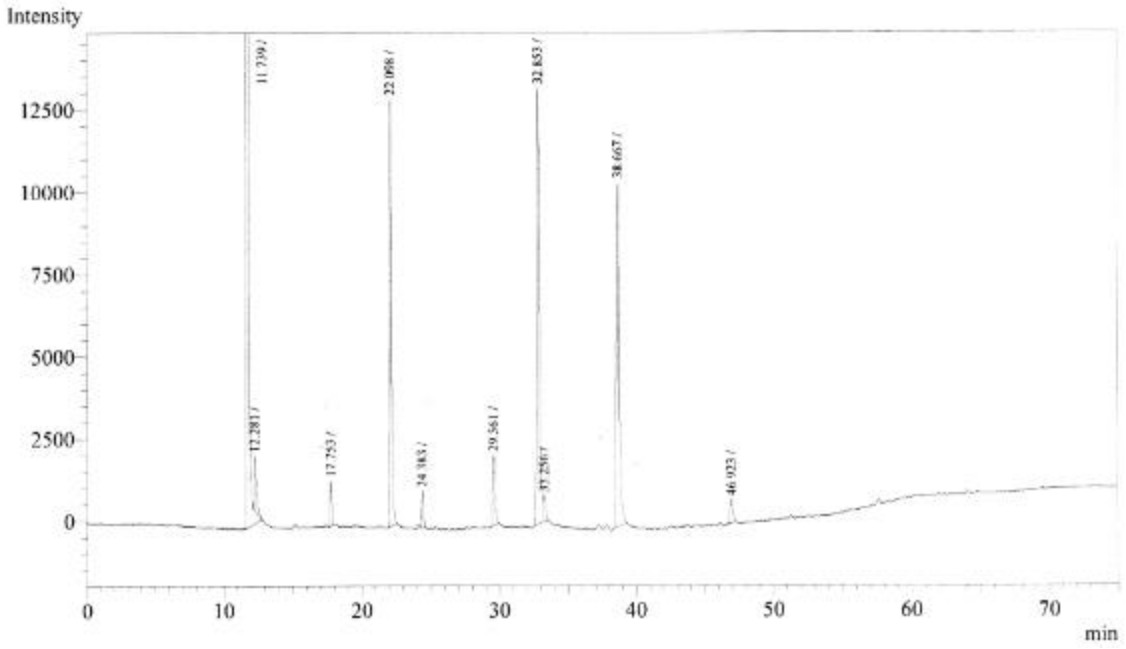

Figure 3.19. GC-FID chromatogram of Chicken Meet Ball (F)

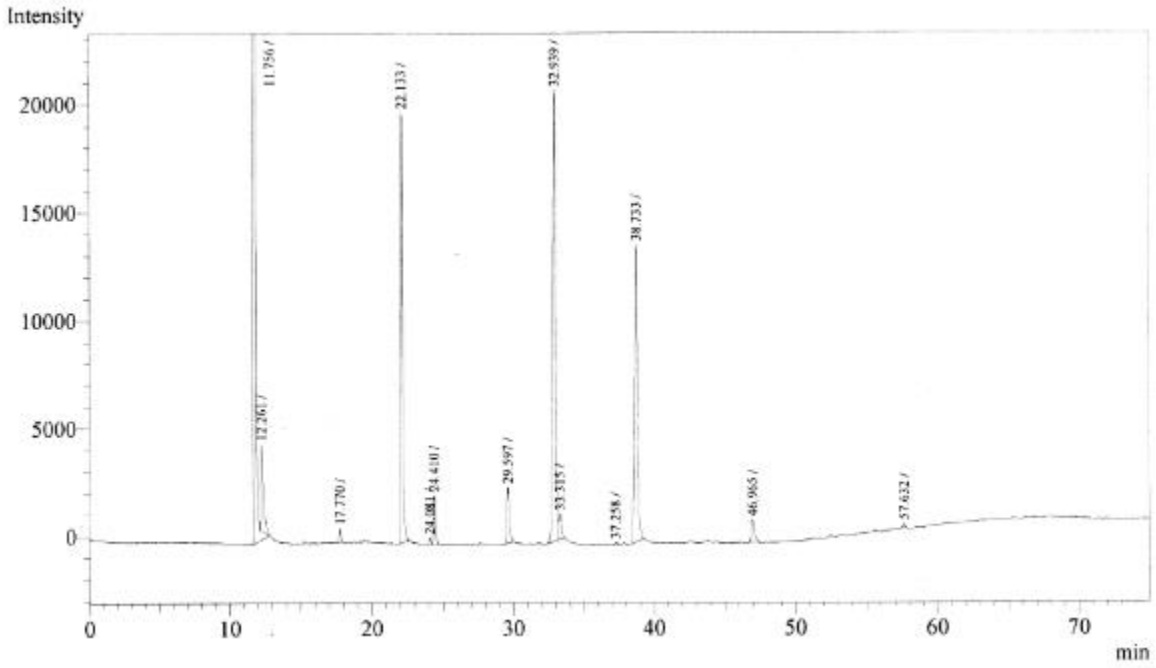

Figure 3.20. GC-FID chromatogram of Chicken Naget Fry (G)

Table 3.3. GC-FID analysis of fast food samples (A-C) by percent (%)

Sample ID	Type	Name of Acid	Unit	Amount (%)
A	SFA	Palmitic Acid	%	20.64
		Stearic Acid	%	6.07
		Myristic Acid	%	2.02
	MUFA	Oleic Acid	%	12.47
	PUFA	Linoleic Acid (Omega-6)	%	13.49
			Total=	**54.69**
B	SFA	Palmitic Acid	%	24.33
		Stearic Acid	%	1.59
	MUFA	Oleic Acid	%	28.53
		Palmitoleic Acid	%	1.19
	PUFA	Linoleic Acid(Omega-6)	%	8.53
			Total=	**64.17**
C	SFA	Palmitic Acid	%	10.93
		Stearic Acid	%	1.78
	MUFA	Oleic Acid	%	19.89
		Palmitoleic Acid	%	0.59
	PUFA	Linoleic Acid(Omega-6)	%	17.20
			Total=	**50.39**

Table 3.4. GC-FID analysis of fast food samples (D-G) by percent (%)

D	SFA	Palmitic Acid	%	8.92
		Stearic Acid	%	1.04
		Myristic Acid	%	0.98
	MUFA	Oleic Acid	%	17.85
		Erucic Acid	%	32.67
		Cis-11 Eicosenoic Acid	%	4.46
	PUFA	Linoleic Acid(Omega-6)	%	8.30
		Linolenic Acid(Omega-3)	%	3.57
			Total=	**77.79**
E	SFA	Palmitic Acid	%	8.75
		Stearic Acid	%	3.56
	MUFA	Oleic Acid	%	13.63
		Palmitoleic Acid	%	1.75
		Cis-11 Eicosenoic Acid	%	0.43
	PUFA	Linoleic Acid(Omega-6)	%	9.43
			Total=	**37.55**
F	SFA	Palmitic Acid	%	16.64
		Stearic Acid	%	1.40
		Myristic Acid	%	1.34
	MUFA	Oleic Acid	%	18.29
		Palmitoleic Acid	%	1.03
		Cis-11 Eicosenoic Acid	%	1.41
	PUFA	Linoleic Acid(Omega-6)	%	18.90
			Total=	**59.01**
G	SFA	Palmitic Acid	%	13.33
		Stearic Acid	%	1.5
	MUFA	Oleic Acid	%	21.66
		Palmitoleic Acid	%	3.00
		Cis-11 Eicosenoic Acid	%	1.41
	PUFA	Linoleic Acid(Omega-6)	%	16.66
			Total=	**57.56**

Table 3.5. GC-FID analysis of fast food samples (A-C) by g/10gm

Sample ID	Type	Name of Acid	Unit	Amount
A	SFA	Palmitic Acid	gm	0.612 g
		Stearic Acid	gm	0.18
		Myristic Acid	gm	0.06
	MUFA	Oleic Acid	gm	0.37
	PUFA	Linoleic Acid	gm	0.40
			Total=	**1.62 (2.965)**
B	SFA	Palmitic Acid	gm	1.10
		Stearic Acid	gm	0.072
	MUFA	Oleic Acid	gm	1.29
		Palmitoleic Acid	gm	0.054
	PUFA	Linoleic Acid	gm	0.386
			Total=	**2.894(4.52)**
C	SFA	Palmitic Acid	gm	0.61
		Stearic Acid	gm	0.094
	MUFA	Oleic Acid	gm	1.11
		Palmitoleic Acid	gm	0.033
	PUFA	Linoleic Acid	gm	0.96
			Total=	**2.80 (5.58)**

Table 3.6. GC-FID analysis of fast food samples (D-E) by g/10gm

D	SFA	Palmitic Acid	gm	0.10
		Stearic Acid	gm	0.017
		Myristic Acid	gm	0.011
	MUFA	Oleic Acid	gm	0.20
		Erucic Acid	gm	0.366
		Cis-11 Eicosenoic Acid	gm	0.05
	PUFA	Linoleic Acid	gm	0.093
		Linolenic Acid	gm	0.040
			Total=	**0.874 (1.12)**
E	SFA	Palmitic Acid	gm	.14
		Stearic Acid	gm	0.057
	MUFA	Oleic Acid	gm	0.218
		Palmitoleic Acid	gm	0.028
		Cis-11 Eicosenoic Acid	gm	0.007
	PUFA	Linoleic Acid	gm	0.151
			Total=	**0 .60 (1.60)**

Table 3.7. GC-FID analysis of fast food samples (F-G) by g/10gm

F	SFA	Palmitic Acid	gm	0.272
		Stearic Acid	gm	0.023
		Myristic Acid	gm	0.022
	MUFA	Oleic Acid	gm	0.30
		Palmitoleic Acid	gm	0.017
		Cis-11 Eicosenoic Acid	gm	0.023
	PUFA	Linoleic Acid	gm	0.31
			Total=	**0.967 (1.64)**
G	SFA	Palmitic Acid	gm	0.16
		Stearic Acid	gm	0.018
	MUFA	Oleic Acid	gm	0.26
		Palmitoleic Acid	gm	0.036
		Cis-11 Eicosenoic Acid	gm	0.017
	PUFA	Linoleic Acid	gm	0.20
			Total=	**0.69 (1.20)**

3.4 Conclusion

Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) have been found in the fast food samples. None of the fast food samples that have been analyzed contain any trans fatty acids. The results of ATR-FTIR spectroscopy were found in good agreement with the results of GC-FID. All fast food samples contained omega-6 (linoleic acid) fatty acids in various amounts ranging from 8.30% to 18.90%. The ratio of omega-6 and omega-3 in Chicken Botik (D) was 2.32:1. This ratio is in the acceptable range. Except sample D, none of the fast food sample contains linolenic acid (omega-3). Chicken Winglet (A) and Chicken Hot Wings (B) of KFC have higher amount of saturated fatty acids which are 28.73% and 25.92% respectively. Intake of saturated fat is known to increase low density lipoprotein (LDL) cholesterol, and therefore has been associated with increased risk of cardiovascular disease (CVD).This study gave some vital information about fatty acid contains of chicken based fast food in Dhaka city, however detailed study about the dietary practice of the chicken/poultry is required to identify the sources of excess amount of SFA and omega 6 in their next product.

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