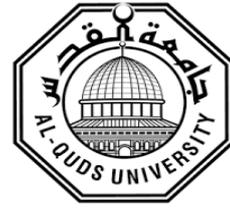


**Deanship of Graduate Studies
Al-Quds University**



**Factors Affecting The Formation Of Trans Fatty Acid
During Heat Treatment Processes Of Food Containing
Unsaturated Fatty Acids**

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M.Sc. Thesis

Jerusalem-Palestine

1438/2017

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heat treatment processes of food containing unsaturated
fatty acids**

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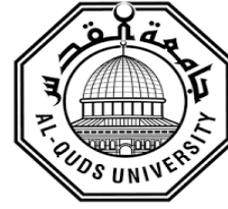
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**A thesis submitted in partial fulfillment of requirements
for the degree of Master of Applied & Industrial
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1438/2017



Thesis Approval

Factors affecting the formation of trans fatty acid during heat treatment processes of food containing unsaturated fatty acids

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Jerusalem-Palestine
1438 /2017

Dedication

To
my beloved
mother

&

the spirit of my father

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed 

Ghassan Mohammad Ahmad AlDabbas

Date : 20/05/2017

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Abstract

Trans isomers occurred in our food either naturally conjugated (conjugated) or industrial which have non-conjugated (interrupted by at least one methylene group ($-\text{CH}_2-$)) carbon-carbon double bonds. Naturally occurring trans fatty acids which have different physiological and biological functions compared to industrial (man-made) trans fatty acids that are formed by many factors in processed foods and increase the risk of coronary heart disease (CHD). Trans acid includes either monoenes mainly stereoisomers of elaidic acid or isomers of polyunsaturated fatty acid (dienes, trienes).

This work is aiming to determine the factors affecting the formation of elaidic acid during heat treatment processes of food containing unsaturated fatty acids. The research was based on two categories; control samples and fat and oil samples which were collected from four different fats and oils (two solid (margarine and ghee) and two liquids (olive oil and corn oil)). Fat and oil samples were applied to heat treatment at four different degrees (120°C , 150°C , 190°C and 250°C) and different heating time intervals (10 minutes, 30 minutes, 60 minutes and 180 minutes). Two types of samples were obtained from each fat or oil sample; heat type and extract type. Samples were stored in 100 ml dark glass containers. Percentage of each elaidic and oleic acid was evaluated using HPLC with UV detector. The correlation between elaidic and oleic acid was determined in each fat and oil treatment, in addition the correlation between heat and extract types was evaluated.

The obtained results from 38 control samples analyzed by HPLC, showed that elaidic acid was commonly present (0.75%) in margarine ($n=9$) with range (0% - 1.41%), (0.38%) in ghee ($n=5$) with range (0% - 1.09%), (0.78%) in severe heating corn oil applied to 220°C for 24 hours ($n=4$) with range (0.36% - 1.32%), (2.63%) in severe heating olive oil applied to 220°C for 24 hours ($n=3$) with range (0.58% - 4.17%), (0.85%) restaurant corn oil used in broasted chicken frying from Abu-Dees area ($n=4$) with range (0% - 1.77%), and (0.32%) in restaurant corn oil used in broasted chicken frying from Ramalla area ($n=4$) with range (0% - 1.00%), while olive oil and corn oil did not show any percentage of elaidic acid.

The results reveal that five factors chosen (Type of fat and oil, percentage of oleic acid in every fat and oil type, temperature treatment, time of heating, sample type (heat or extract)) affect the percentage of elaidic acid with Coefficient of Determination $R^2 = 0.424$. that mean five factors chosen explain the variation in the percentage of elaidic acid by 42.4% and the remaining variation can be explained by other unstudied factors.

The obtained results showed that the most effective factor was the type of fat and oil, oleic acid %, temperature treatment, time of heating and the last effective factor was the extract type.

The obtained results in margarine showed that the percentage of elaidic acid is stable and was not affected by applied 120°C for all different time intervals, but at 150°C, 190°C and 250°C for all different time intervals the results showed that there is a cubic nonlinear significant relationship between percentage of elaidic acid and time of heating.

The obtained results in terms of ghee showed that the percentage of elaidic acid is stable and is not affected by applied 120°C heat treatment for all different time intervals. At 150°C for different time intervals the results showed that there is a cubic nonlinear significant relationship between percentage of elaidic acid and time of heating. At 190°C for different time intervals the results showed a negative linear relationship may exist between the percentage of elaidic acid and time of frying. At 250°C for different time intervals the results showed oscillatory results in regards to the percentage of elaidic acid with time of heating.

The correlation between heat type and extract type in all fat and oil samples treated showed that no significant difference in the percentage of elaidic acid except slightly difference in some points that showed more influence of extract type, its due to effects of condensation and extract heat during extraction process.

The correlation between oleic acid and elaidic acid in all fat and oil samples treated under the same condition showed the opposite relation between oleic acid and elaidic acid except margarine samples treated for 180 mins at (150°C, 190°C and 250°C).

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Chapter One:

Introduction:

1.1 Background:

In 20th century, People discovered the adverse effects of saturated fat on health and blood lipids, they turned to replacement the saturated animal fats and butters used in cooking and table spreads by plant oils, hydrogenation of plant oils and Margarine that is an emulsion composed mainly of vegetable fats and water.

last years, the trans configuration has been a matter for debate, especially after the studies showed that Trans fatty acids occurs in hydrogenation of plant oils, Margarine, fat spread, shortening vegetable oils and food containing hydrogenation of plant oils and Margarine, such as bakery, sweets, cakes, donuts, and frying foods, made with partially-hydrogenated fats.

Naturally-derived trans fatty acids is another type of trans occurs in Milk, meat, and dairy products of ruminant animals such as cows and sheep. They produce trans fatty acids by bio hydrogenation of unsaturated fatty acids in the rumen.

Fatty acids [both configurationally (*cis and trans*) and positional (double bond location vary from the $\Delta 8$ to the $\Delta 15$ position on the fatty acid carbon chain)] isomers continue to be an integral, if somewhat controversial "bad", "not bad" and/or "inconclusive" (Richard and Gary, 2004).

Consumption of diets high in hydrogenated fat and or trans fatty acids has been shown to have an adverse effect on lipoprotein profiles with respect to cardiovascular disease risk.

Dietary fat and cholesterol play an important role in the regulation of immune and inflammatory responses shown to be involved in atherogenesis (Sung et al, 2002). Trans fatty acid intake has been associated with a higher risk of cardiovascular disease,(Esther et al, 2004). Trans Fatty acid intake predicts risks of coronary artery disease and diabetes. Systemic inflammation may be involved in the pathogenesis of such conditions; however, relations between TFA intake and systemic inflammation are not well established (Dariush et al, 2006).

Within the general context of ongoing discussions on nutrition labeling regulations at both International (Codex Alimentarius) and European (European commission levels), the Direction Générale de la Consommation, de la Concurrence, et de la Répression des Fraudes (Directorate General for Fair Trading, Consumer Affairs and Fraud Control/DGCCRF), a French authority, requested the Agence Française de Sécurité Sanitaire des Aliments French Food Safety Agency/AFSSA) to provide technical and scientific support and advice regarding the relevance of the “indication of the content of trans fatty acids in food labels (Pierre et al, 2007).

In 2004, Denmark was the first country that introduced a limitation on the content industrially produced trans FAs in foods. USA and Canada were the first two countries to introduce the mandatory declaration of trans FAs (Andrzej and Jarosława, 2012).

The Food and Drug Administration (FDA) now requires that the Nutrition Facts panel list the amount of trans fat in a serving of food if a serving contains 0.5 gram or more of trans fatty acids. This is listed on the line below the listing of saturated fat.

There is no Daily Value for trans fat. Instead, the Institutes of Medicine, literature states and Researchers recommends that must be keep our intake of trans fats to as near zero as possible.

The World Health Organization (WHO) recommends that we eat no more than 1% of our daily kilojoules from TFAs.

1.2 Fatty acids

1.2.1 Introduction:

Fatty acids are one of a large and diverse group of naturally occurring organic compounds that are soluble in non-polar organic solvents and generally insoluble in water. Fatty acids with up to six carbon atoms are considered short-chain fatty acids and Fatty acids with eight to ten carbon atoms are considered medium chain, and Fatty acids with 14 and more carbon atoms are considered as long-chain fatty acids. The Fatty acids are composed of a chain of methylene groups with a carboxyl functional group at one end. The methyl chain is the fatty part, while the carboxyl group is the acid (Marais,2007).

Fatty acids can be saturated, as show in figure1.1, all the carbon atoms have the maximum number of hydrogen atoms attached to them and have a straight chain structure, it is solid at room temperature. They can also be unsaturated, with one or more double bonds connecting some of the carbons. In unsaturated fatty acids, some of the carbon atoms miss some of their hydrogen atoms and thus form a double bond between those carbons missing their hydrogen atoms. It is stay fluid at room temperature and These are mostly oils (Food and Agriculture Organization of the United Nations ,2008).

The common fatty acids in plant tissue are C16 and C18 with zero to three double bonds in the cis configuration. These fatty acids are also abundant in animal tissues, together with other fatty acids with a wider range of chain lengths and up to six cis double bonds separated by methylene groups. These methylene-interrupted double bonds are also referred to as nonconjugated double bonds as show in Figure 1.2 that gives the chemical structure of a non-conjugated unsaturated fatty acid (Marais, 2007), (Eckel et all, 2006) and (Food and Agriculture Organization of the United Nations, 2008).

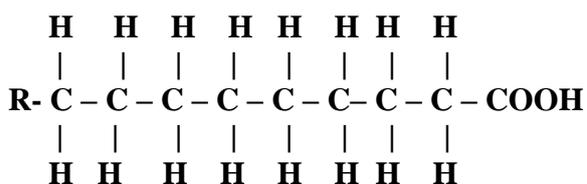


Figure 1.1 Saturated Fatty Acid Structure

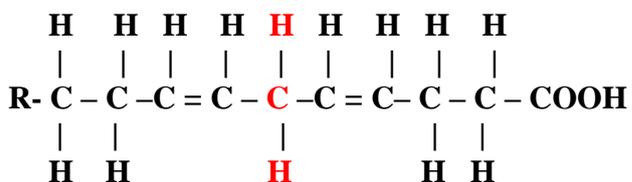


Figure 1.2 Non-conjugated Polyunsaturated Fatty Acid Structure

Polyunsaturated fatty acids can also be conjugated. Conjugated fatty acids do not have a methylene group between the two double-bonded carbons as can be seen in Figure 1.3 (Marais, 2007).

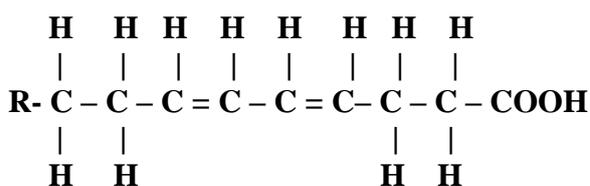


Figure 1.3 Conjugated polyunsaturated fatty acid structure

Another group of natural occurring fatty acids are the omega-3 and omega-6 long-chain unsaturated fatty acids. The human body needs, but cannot synthesise these fatty acids and therefore they are called essential fatty acids (Food and Agriculture Organization of the United Nations, 2008).

1.2.2 Name of Fatty Acid:

The three common named of fatty acid as show in Table 1, scientific or (systematic) names, according to official International Union of Pure and Applied Chemistry (IUPAC), shorthand designation or (abbreviation) and common names, depend on sources of fatty acid (Marais,2007).

Table 1.1: The scientific names, shorthand designation and Common names of some of the fatty acids

Saturated fatty acids		
Scientific name	Short and designation	Common name
Dodecanoic acid	12:0	Lauric acid
Tetradecanoic acid	14:0	Myristic acid
Hexadecanoic acid	16:0	Palmitic acid
Heptadecanoic acid	17:0	
Octadecanoic acid	18:0	Stearic acid

monounsaturated fatty acids		
Scientific name	Short and designation	Trivial name
Cis-9, Tetradecenoic acid	9-14:1	Myristoleic acid
Cis-9, Hexadecenoic acid	9-16:1	Palmitoleic acid
Trans-9, Hexadecenoic acid	9-16:1	Palmitelaidic acid
Cis-6, Octadecenoic acid	6-18:1	Petroselinic acid
Cis-9, Octadecenoic acid	9-18:1	Oleic acid
Cis-11, Octadecenoic acid	11-18:1	Vaccenic acid
Trans-6,Octadecenoic acid	6-18:1	Petroselaidic acid
Trans-9,Octadecenoic acid	9-18:1	Elaidic acid
Trans-11,Octadecenoic acid	11-18:1	Trans-vaccenic acid

polyunsaturated fatty acids		
Scientific name	Short and designation	Trivial name
Cis-9,Cis-12,Octadecadienoic acid	9c,12c-18:2	Linoleic acid
Cis-9,Trans-11,Octadecadienoic acid	9c,11t-18:2	Conjugated linoleic acid

1.3 Trans Fatty Acids

1.3.1 Definition:

Trans Fatty acid is defined as the geometrical isomers of monounsaturated and polyunsaturated fatty acids having non-conjugated [interrupted by at least one methylene group ($-\text{CH}_2-$)] carbon-carbon double bonds in the trans configuration (Codex Alimentarius, 2004). This includes the trans monoenes mainly stereoisomers of elaidic acid, and the trans isomers of polyunsaturated FAs (trans dienes, trans trienes).

with non-conjugated carbon-carbon double bonds, produced through hydrogenation of oils and fats (both vegetable and animal/marine origin) in the presence of a suitable chemical catalyst. The definition, however, excludes conjugated trans FAs present naturally in animal fats and their products that include conjugated linoleic acid. The US FDA defined trans FA as “unsaturated FAs that contain one or more isolated (i.e. nonconjugated) double bonds in a trans configuration (Stolyhwo and Rutkowska, 2012).

Trans fatty acids (TFAs) are unsaturated fatty acids with at least one double bond in the trans configuration and has a straight chain that is similar to those structure of saturated fatty acid as show Elaidic acid in figure 1.4. TFAs are found in two major sources, natural and industrial. In natural source, TFAs originate from milk fat and tissue fat of ruminants such as cows, goat and sheep. Bacteria in their stomach can be producing TFAs by a biological hydrogenation process. Industrial TFAs are mainly generated from vegetable oil polyunsaturated fatty acids during many factors that are created man-made trans fatty acid (Narkwichian et al ,2009).

1.3.2 Natural occurring trans fatty acids:

A type of trans fat occurs naturally in the milk and meat of ruminants animals (such as cattle and sheep) at a level of 2–5% of total fat.(Marais,2007). Natural trans fats, which include conjugated linoleic acid (CLA) and vaccenic acid, originate in the rumen of these animals. CLA has two double bonds, one in the *cis* configuration and one in trans, which makes it simultaneously a *cis*- and a trans-fatty acid.

Trans fatty acids which occur naturally have very different physiological and biological functions compared to man-made trans fatty acids that are found in processed foods. Data from the Nurses' Health study reveal that while man-made trans fatty acids increase the risk of coronary heart disease (CHD), naturally occurring trans fatty acids of animal origin does not increase this risk (Marais, 2007 and Christie, 2007).

1.3.3 Commercially produced trans fatty acids (man-made trans fatty acid):

Another type of trans fatty acids are man-made. partial hydrogenation, shortening, refining process and Processing foods such as, fast food, snack food, fried food, and baked food. All of them can only be made by cooking with a very high heat, at high temperatures the oil with one double bond at least in a fatty acid chain, there is a possibility for the formation of trans fatty acid positional and/or geometrical isomers (Marais,2007 and Christie, 2007).

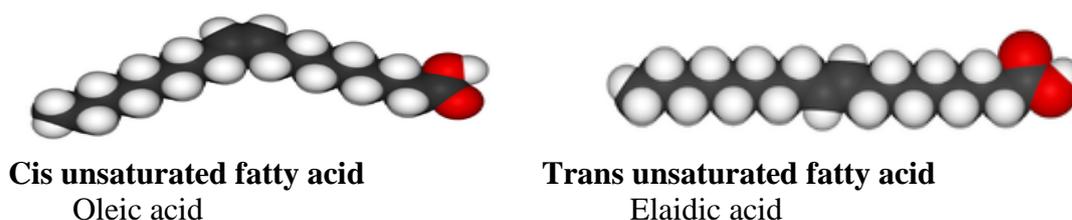


Figure 1.4. The geometrical structures of the cis and trans mono-unsaturated fatty acids

1.4 Factors affecting conversion of cis fatty acid to trans fatty acid

1.4.1 Introduction:

There are many factors that affect the formation of trans fatty acid, e.g partial hydrogenation, refining process, baking and frying (Narkwichian et al, 2009). this factors may change a double bond from a cis position to a trans position (geometric isomerization) or move to another position in the carbon chain (positional isomerisation) and both types of isomerization may occur in the same molecule. Different trans isomers can be formed depending on difference factory effect (Marais, 2007). However, according to literature

states, partial hydrogenation and frying are the most important factors that affecting to creation man-made trans fatty acid .

1.4.2 hydrogenation:

In hydrogenation , All the double bonds can be removed to form saturated fatty acids, or only some of the double bonds can be removed to change polyunsaturated fatty acids into monounsaturated fatty acids. Some of the double bonds may remain, but be moved in their positions on the carbon chain. Some of the *cis* double bonds can be changed into the *trans* position to produce several geometrical and positional isomers (Marais, 2007).

During the partial hydrogenation process, which is easily controlled, hydrogen atoms are added in no particular order. When the hydrogenation process is stopped, unsaturated fatty acids are in varying stages of hydrogenation. Some molecules are totally hydrogenated (saturated) while in others, some of the double bonds have changed from the natural *cis* configuration to the unnatural *trans* configuration. Some of the double bonds have even shifted to unnatural positions on the carbon chain. During the partial hydrogenation process, the bent *cis* isomer changes to the *trans* isomer forming a molecule that has a straight configuration, similar to saturated fatty acids. The straight configuration of *trans* unsaturated fatty acids enable the molecules to pack easily together resulting in a higher melting point with a longer shelf life and flavour stability. These more stable fats are used in margarines and shortenings (Marais, 2007).

Following the mentioned of regulations, industry practices regarding unsaturated fat hydrogenation changed over last 10 years. In practice, several temperature points exist near which critical effects in the reaction course are to be expected. For example, a low-temperature (120°C) hydrogenation may result in less than 20 % of trans acid (Andrzej and Jarosława, 2012).

However, the so obtained fats would not meet the requirements of baking, confectionery, and frying processes. Fats for baking require a long plastic range. One of the approaches is the so-called trans-suppressive hydrogenation in which the melting point is being approached gradually and, in consequence, the solid fat content or solid fat index curves are flatter than normal as required of a shortening or of baking fats. In that approach, a

fresh or nearly fresh nickel catalyst is used at the usual nickel/oil level (0.05–0.15 %), the pressure of up to 5 at and temperature up to 160°C. In case the texture of that fat does not meet the requirements, fully hardened vegetable oil (3–5 %) is being added. On the other hand, hydrogenation above 160°C renders best functional properties of confectionery fats due to an increased content of trans-isomers (Patterson, 2009). The principal structural changes of hydrogenated fats are as follows:

- Structural (positional) isomerization of FAs which involve the migration of double bonds from the most abundant Δ -9 or Δ -11 positions along the hydrocarbon chain of unsaturated FA.
- Geometrical isomerization in which the cis isomers of FAs (exclusively present in plant oils) are converted during hydrogenation to their trans analogs. The process is associated with structural (positional) isomerization.

1.4.3 Effect of frying:

Frying is a unit operation which is mainly used to alter the eating quality of a food. A secondary consideration is the preservative effect that results from thermal destruction of micro-organisms and enzymes, and a reduction in water activity at the surface of the food (or throughout the food, if it is fried in thin slices). When food is placed in hot oil, the surface temperature rises rapidly and water is vaporized as steam. The surface then begins to dry out in a similar way to that described during baking and roasting (Blumaenthal, 1991).

During frying, both water and water vapor are removed from the larger capillaries first, and replaced by hot oil. The temperature used for frying is determined mostly by economic considerations and the requirements of the product. At high temperatures (180–200°C), processing times are reduced and production rates are therefore increased. However, high temperatures also cause accelerated deterioration of the oil and formation of free fatty acids, which alter the viscosity, flavor and color of the oil and promote foaming. This increases the frequency with which oil must be changed and hence increases costs. A second economic loss arises from the vigorous boiling of the food at high temperatures which causes loss of oil by aerosol formation and entrainment in the product. Acrelein is a

breakdown product of oil, produced at high temperatures, which forms a blue haze above the oil and is a source of atmospheric pollution (Fellows, 2000).

Presence of moisture, proteins, fats, carbohydrates and oxygen in frying foods, causes reaction with oil to form a range of volatile carbonyls, hydroxy acids, keto acids and epoxy acids. These cause unpleasant flavours and darkening of the oil. The various breakdown products are classified as volatile decomposition products that has indicated up to 220 different components (Nielsen, 1993) and non-volatile decomposition products, that are formed by oxidation and polymerization of the oil and form sediments on the sides and at the base of the fryer (Fellows,2000).

1.4.4 Type of oil for frying:

Oils for commercial frying require stability related to the thermal deterioration processes of oxidation, hydrolysis, and polymerization. For consumer acceptance, the fatty acid composition of the oils needs to have 20% to 30% linoleic acid to produce a desirable full deep-fried flavor to the foods; however, higher levels of linoleic acid might introduce “off”-flavors from oxidation. For restaurant use, oils need to be stable because a long fry life is required and the oil has to withstand the high temperatures of commercial frying. Food manufacturers prefer stable oils that can also tolerate high temperatures and allow an extended shelf life for foods after they are packaged. Stable frying oils are characterized by increased amounts of oleic acid (preferably in the moderate range of 50% to 65%), decreased amounts of linoleic acid (preferably in the 20% to 30% range), and decreased amounts of linolenic acid (preferably no more than 3%). It has been common to acquire stable commercial frying oils by changing the fatty acid composition by partial hydrogenation. Potential alternatives to partially hydrogenated oils for commercial frying include naturally stable oils such as corn, cottonseed, palm, peanut, and rice bran and modified fatty acid oils such as mid-oleic corn, high-oleic/low-linolenic canola, high-oleic sunflower, mid-oleic sunflower, low-linolenic soybean, and mid-oleic/ low-linolenic soybean oils. In choosing trans fatty acid-free frying oils, consider the cost, availability, oxidative stability, functionality in terms of the appearance and texture, flavor, and nutrient composition of the option. Specifically, some of these oils such as animal fats and tropical

oils contain high amounts of saturated fats and should not be considered as replacements (Marais, 2007).

1.5 Literature Review

Ludger (2014) studied Fatty acid alterations in oils and fats during heating and frying. According to this study, " Oils and fats degrade during the frying process and many reactions with numerous fatty acid alteration products have been examined. The geometrical isomerisation of double bonds leads to the formation of trans fatty acids. At frying temperatures also conjugated double bond systems are detected. The reaction of oxygen with unsaturated fatty acids results in hydroperoxides, which immediately degrade in further radical reactions at frying temperature. A set of oxygenated fatty acids has been detected including epoxy-, keto- and hydroxyl fatty acids. Another route leads to β -scission at the carbonyl- or the alkyl side of the oxygen bearing carbon atom in the fatty acid chain. In this case short chain fatty acids, aldehydic, keto, and hydroxyl acids appear together with volatile compounds. Also the formation of cyclic and furan fatty acids was detected. As a reaction between fatty acids also dimeric and polymerised fatty acids can be observed. Taking into account the different amounts of these fatty acid degradation products the physiological relevance has to be discussed. Due to high concentrations of dimeric and polymerised molecules these substances can lower significantly the digestibility of fried foods, while oxidised fatty acid monomers are readily absorbed and raise concern about their effect on lipid metabolism. These two different effects of altered TAGs and fatty acids have to be considered separately".

Meiyan, et al (2014), studied The Formation of trans fatty acids during the frying of chicken fillet in corn oil. According to this study, " To assess effects of heated edible oils on intake of trans fatty acids (TFAs); the formation of TFAs in cooking conditions was investigated by a frying system model, in which chicken fillet was fried in a commercial corn oil at 170°C, for 12 frying cycles. The main TFAs detected in chicken fillet were trans C18:2 fatty acids (FAs) and trans C18:3 FAs, which exhibited no significant differences among the frying cycles. Besides, the content of trans C18:1 FAs were very low in all samples on different frying cycles. The intake of TFAs was estimated to be 0.06g/100g when chicken fillet fried in this process was consumed. These results suggest that an

ordinary frying process upon a commercial corn oil has little impact on the daily TFAs intake".

Changmo, et al (2013) studied The Mechanism of Formation of Trans Fatty Acids under Heating Conditions in Triolein. According to this study, To elucidate the relationship between heat-induced cis/trans isomerization and reaction temperature and energy in unsaturated lipids, we investigated the molecular mechanism of the heat-induced cis/trans isomerization of 18:1 isomers. Triolein (18:1,9c) was heated at two range temperatures (130, 160, 190, 220 °C and 135, 140, 145, 150, 155 °C) and analyzed by the gas chromatography (GC) method. When the heating temperature increased to 150 °C, the amount of trans 18:1n-9 changed from 0.0897 mg/g oil (1 h) to 0.1700 mg/g oil (3 h). This study shows that the cis to trans isomerization may occur at 150 °C".

Roman and Felix (2012) studied the Formation of Trans Fats During Food Preparation. According to this study, " Minimal changes were observed in the amount of trans fats during baking. Application of extreme temperatures during baking, which caused carbonization of the outer layer of products, yielded an insignificant increase in the amount of trans isomers. As with baking, stir-frying did not result in significant isomerization of the fatty acids, even when the oil was heated to 275° C and smoking heavily before the food was placed in it. Irrespective of the cooking procedure, linolenic acid was the most prone to isomerization with the highest amount of trans isomers formation".

Jun-Cai, et al (2012) studied The Effects of frying on the trans-fatty acid formation in soybean oils. According to this study, " In the present study, we demonstrated that increasing the number of frying cycles can cause an intensive increase in the concentration of TFAs in different types of soybean oil, but especially in PSBO".

Jun-Cai, et al (2011) studied The Assessment of trans fatty acids in edible oils in China. According to this study, " Trans fatty acid (TFA) is commonly present in edible oils. TFA has been proven to have adverse effects on blood lipids, including increasing the LDL-cholesterol concentration and decreasing the HDL-cholesterol concentration. The aim of our study was to determine the levels of TFA in edible oil samples consumed in Harbin, China. In this study, 93 samples of soybean oil (SBO) (n = 29), rapeseed oil (RSO) (n = 23), sunflower oil (SFO) (n = 22), and corn oil (CO) (n = 19) were analyzed between

October 2010 and January 2011, using a gas chromatograph (GC) with a flame ionization detector (FID). TFA (>2%) was detected in 17 (18%) samples, ranging from 0.14% to 4.76%. The overall TFA content was $1.15 \pm 0.12\%$ for SBO, $1.37 \pm 0.23\%$ for RSO, $1.41 \pm 0.10\%$ for SFO, and $2.01 \pm 0.24\%$ for CO. Trans C18:2 and C18:3 fatty acids were normally predominant in the investigated edible oils. The variance in the percentage of TFA in the edible oils probably resulted from differences in the quality, processing technique, and storage condition of the edible oils. The results indicated that, in China, TFA is widely present in edible oils at low levels. Therefore, it is important to assess the content of TFA in edible oils in China".

K.Cihelkova, et al (2009) studied The effect of high temperature on sun flower Oil Polyenoic Fatty Acid. According to this study, " Heat induced cis-trans isomerisation of sunflower oils depending on temperature, reaction time and original content of linoleic acid was investigated. The content of isomeric fatty acids was determined by gas chromatography and the content of polymers by gel permeation high-performance liquid chromatography. The content of trans fatty acids increased with time and with temperature and a rate of cis-trans isomerisation and polymerisation depends on the temperature according to Arrhenius equation. The content of polymers was significantly lower in sunflower oil with high content of oleic acid because of the low concentration of linoleic acid in oil. In both oils the content of conjugated linoleic acid initially increased depending on time and temperature, however after certain time the stationary state occurred. Polymerisation of polyenoic fatty acids takes place directly with heat induced cis-trans isomerization".

1.6 Health risks of man-made trans fatty acid

Depending on the human lipase enzyme that helps digest, transport, and process dietary lipids such as triglycerides, fats, and oils, lipase enzyme work only on the cis configuration and cannot metabolize a trans fatty acid (Eckel, et al, 2006). Trans fatty acids are well absorbed and incorporated into tissue lipids and similarly transported to other fatty acids to be distributed within the cholesterol ester, triacylglycerol, and phospholipid fractions of the lipoproteins. The ingestion of trans unsaturated fatty acids increase low-density lipoproteins (LDL) to a similar degree of that of saturated fatty acids, but also reduces high-density lipoproteins (HDL). Therefore, trans fatty acids are considered to be more harmful than saturated fatty acids (Eckel et al, 2006).

The primary health risk identified for trans-fat consumption is an elevated risk of coronary heart disease (CHD). (Esther et al, 2004). A comprehensive review of studies of trans fats was published in 2006 in the New England Journal of Medicine reports a strong and reliable connection between trans-fat consumption and CHD, concluding that "On a per-calorie basis, trans fats appear to increase the risk of CHD more than any other macronutrient, conferring a substantially increased risk at low levels of consumption from 1 to 3% of total energy intake (Mozaffarian et al ,2006).

1.7 Analytical method for determination cis and trans fatty acids

The Analytical method used to determine the Trans fatty acid and/or Especially Octadecenoic Acid (18:1) isomers group carried out according to literature states and official methods reported by the American Oil Chemists' Society (AOCS) and/or the Association of Official Analytical Chemists (AOAC). Some of these method used by high performance liquid chromatography (HPLC), Gas chromatography (GC), and Infrared (IR) Spectroscopy.

Nine Official methods regarding to determine Trans Fatty Acid in food (Pierre et al, 2007), Each of these methods has advantages and drawbacks as show in Table . official Method AOAC 996.06, official Method AOAC 994.14, official Method AOAC 965.34, official Method AOAC 2000.10, official Method AOAC 994.15, official Method AOAC 985.21, official Method AOCS Ce 1f-96, official Method AOCS Ce 1g-96, official Method AOCS Cd 14-95, official Method AOCS Cd 14d-99, official Method CEN EN ISO 15304: 2002. this methods recognized by national offices or international entity and validated by a published or reported collaborative study.

Table 1.2: The Name & Number Method, Analytical Tools and Advantages & Disadvantages of Nine Official methods regarding to determine Trans Fatty Acid in food

Name & Number Method	Analytical Tools	Advantages & Disadvantages
AOAC 996.06	GC	<ul style="list-style-type: none"> – Suitable for fat (total, saturated, and unsaturated). – It has not been validated by any collaborative studies for TFA. – overlapping of FA Peaks especially with medium-length 60m columns. – The methylation procedure does not guarantee in any way the integrity of CLA isomers.
AOCS Ce 1f-96	GLC	<ul style="list-style-type: none"> – overlapping of FA under isothermal conditions. – It is restricted to oils and pure fats, refined or partially hydrogenated, with simple initial FA compositions, and is not well adapted to complex fats such as milk fats for instance. – The use of 170–198 C isotherms is unsuitable for the analysis of short-chain FA. – The absence of transmethylation conditions does enable the effect on CLA isomers to be assessed. – The method does not mention the use of an internal standard to quantify the FA in weight terms. – No mention of any collaborative studies.
CEN EN ISO 15304:2002	GC	<ul style="list-style-type: none"> – Determination of TFA isomer content in vegetable fats and oils – the amount of TFA in refined oils as the sum of trans18:1, trans-18:2, and trans-18:3 (as FAME), expressed as a mass fraction of total FAME. – the amount of TFA in PHVO as the sum of all FAME containing a double bond, expressed as a mass fraction of total FAME. – it does introduce the use of long (100 m), highly polar columns and mentions the use of reference material and collaborative studies.

AOCS Ce 1g-96	Silver-ion exchange HPLC(Ag- HPLC)	<ul style="list-style-type: none"> – It is a practical recommendation for Ag HPLC fractionation using special columns packed with silver-loaded ion exchange resin. – This now appears more or less obsolete since new commercial columns such as ChromSpher Lipids® (Chrompack) have become available.
AOAC 994.14	IR spectrophotometric	<ul style="list-style-type: none"> – Isolated trans unsaturated FA content in partially hydrogenated fats. – This method presumes that elaidic acid is the main trans component of test fats. – It is applicable to foods containing more than 5% TFA and is unsuitable for samples containing more than 5% conjugated unsaturation. – The collaborative study organised by the AOAC was performed using PHVO only.
AOCS Cd 14-95 And AOCS Cd 14-96	IR spectrometric	<ul style="list-style-type: none"> – AOCS Cd 14-95 (official method) – AOCS Cd 14-96 (recommended practice) – The collaborative study was conducted on oil, fried oil, margarine and shortening only. – It is applicable to foods containing more than 5% TFA.
AOCS 965.34	IR spectrometric	<ul style="list-style-type: none"> – This method title states that it was developed for margarines and shortenings. – It is used for the accurate determination of isolated trans double bonds in natural or processed long chain acids, esters and triacylglycerols with trans content 0.5%. – It is unsuitable for fats and oils containing conjugated unsaturation over 5%. – This method assumes that the major component to be determined in test samples is methyl elaidate.
AOCS Cd14d-99 and AOAC 2000.10	IR spectroscopy	<ul style="list-style-type: none"> – to determine isolated trans double bond levels in both refined and hydrogenated vegetable oils.

		<ul style="list-style-type: none"> – Rapid determination of isolated trans geometric isomers in fats and oils. – quick, simple and easy to use since it requires little handling (no test sample extraction or dilution, no derivatisation), but it uses expensive sophisticated material.
AOAC 994.15	Capillary GC – IR spectrophotometric	<ul style="list-style-type: none"> – Total cis-and trans-octadecenoic isomers and general FA composition in hydrogenated vegetable oils and animal fats. – The total trans content is determined by IR spectrometry (% methyl elaidate vs. methyl oleate), and trans-18:2and trans-18:3isomers are determined by GC (highly polar, long capillary column). – This method is reported as being applicable to PHVO and terrestrial animal fats containing >5% TFA. – It is unsuitable for hydrogenated marine oils and partially hydrogenated fish oils and animal fats and dairy products.

1.8 problem

Depending on Health risks of man-made trans fatty acid, Many studies approved that hydrogenated fat, shortening and Margarine have trans fatty acids can and so adversely affect LDL and HDL cholesterol levels, and some data suggest that trans fatty acids adversely affect other outcomes.

No Palestinian standard deals with the amount of trans fats occurring with fats and oils used in frying process in Palestinian areas.

1.9 Hypothesis

- Partially hydrogenated fats e.g. margarine and shortenings contain variable amounts of trans fatty acids (especially the trans fatty acid elaidic acid t9 18:1, in addition to other trans fatty acids as t6, 7, 8, 10, 11 ,12, 13, 14, 15, 16).

- Frying process affects (increases) formation of trans fatty acids.
- Temperature of frying is one of the major factors in the formation of trans fatty acids.

1.10 Aim and objective of the study

The general aim of this research is to identify and quantify the amount of Trans fatty acid (represented as Elaidic Acid) in food containing unsaturated fatty acids (Ghee, Margarine, Corn oil and olive oil) under different frying temperatures and different times.

- To determine the factors affecting the formation of Trans Fatty Acid in different types of fats and oils..
- To standardize and optimize an analytical technique to identify and quantify the different cis and trans mono-unsaturated fatty acid isomers in frying fats and oil by HPLC.
- To determine if there are a Trans Fatty Acid in different types of fats and oils used in our food.

Chapter Two:

Material and Methods

2.1 introduction

To determine the factor affected the formation of trans fatty acid, samples from margarine, ghee, corn oil and olive oil were chosen. For each one, three samples types were chosen, no treatment, heat and extract types of fats and oil. Each one is applied to heat treatment at four different temperature and each heat treatment applied at four different times.

The percentage of each of oleic and elaidic acid was determined by HPLC. For each oil the oleic and elaidic acid were determined at different temperature and different time. The correlation between oleic and elaidic acid occurring in four difference oils determined and the correlation between heat and extract oil also determined.

To achieve the required accuracy and precision, each sample has been repeated for three times. Statistical analyses were applied to each result, average, standard deviation and range.

2.2 Fat and Oils:

- Margarine: "Atlas, vegetable margarine" contain Fat (60%), water, emulsifier (mono and diglyceride soya lecithin, Salt (0.4 %), preservative (potassium sorbate), acidity regulator (citric acid), vitamins (A and D), color (Beta-carotene), Flavors. Produced by BESLER Grad veKimya San, Turkey.

- Ghee: "AL-GHZAL, pure vegetable Ghee" contain refined Palm Oil, Vitamins (A (24 I.U/gm), D (3 I.U/gm)), Beta-carotene), Flavors. Produced by vegetable oils industries CO. Ltd, Palestine.
- Corn Oil: taken from local market.
- Olive Oil: taken from Nuba area olive tree, Hebron.

2.3 Chemicals and Reagents:

- Petroleum ether
- n-hexane
- Glacial acetic acid
- Acetonitrile HPLC grade
- Purified Water

2.4 Apparatus and Laboratory equipment:

- Fryer.
- Weighing balance.
- Soxhlet.
- Condenser.
- High Performance Liquid Chromatograph (HPLC) (Elite Lachrom and D-7000 HPLC) with UV detector and supported with autosampler and column oven and data system.
- Laboratory Glass ware (Volumetric flasks, measuring cylinders, beakers, volumetric pipettes and graduated pipettes).
- Laboratory Equipment's.
- Disposable syringe 5 ml.
- syringe filter – Nylon 66- pore size 0.45 μ m, diameter 25 mm.
- Magnetic stirrer.
- Vacuum pump and filtration assembly.
- Thermal balance.
- 100 ml Dark Glass tubes.

2.5 Fat and Oil samples and sample handling:

Margarine, Ghee, Corn Oil and Olive Oil were purchased from local market in Palestine. All of these oils are not expired and stored few days in dark and dry place at room temperature until used.

Potato was also purchased from local market, and stored not more than one day in room temperature until used.

Pretreatment of potato: Washed, peeled, drained them, potato were cut into 2 cm diameter thickness and 4 cm long tube slice were prepared.

Frying: 3 Liters oil was placed into fryer (Horng Yun Steel Factory, Yon Lin, Taiwan), and 1 kg tube slices potato (2 cm D * 4 cm long) was fried at 4 different temperatures (120°C, 150 C,190°C and 250°C) for different times (10 min, 30 min, 60 min and 180 min).

Extraction: Fat and oil samples were extracted from Potato using the Soxhlet. The potato fries were dried to few minutes, the samples extracted by Soxhlet petroleum ether for 3 hours. After extraction the petroleum ether was evaporated and collected again by condensor.

A total of 230 different samples were collected (192 fats and oils sample and 38 control samples), each sample was filled in dark glass bottle (100ml). Every 50 sample were packaged in closed cartons, cooled and stored in dark and dry place before analysis by HPLC.

2.6 Analytical Procedure

2.6.1 HPLC Condition:

Mobile phase was prepared by mixing 800ml Acetonitrile HPLC grade with 200ml Purified Water and 1.0ml of glacial acetic acid was added. The Mobile phase was filtered by using 0.45µm microporous membrane filter and degassed by sonication, to avoid column blockage by any particulate matters and to prolong pump and column life. The mobile phase was then left few minutes to reach a room temperature.

The column utilized for separation was C18 (150 mm long x 4.0 mm inner diameter) with particle size of 5 µm. The flow rate was 2ml/ minute and injection volume was 50 µl. The temperature of autosampler was 15°C, while the temperature of column was 25°C. The wavelength of UV detector was 205 nm, and the run time was 30 minutes.

2.6.2 Preparation of samples solution:

Samples and control samples were prepared by dissolving 1g oil in 50 ml of n-hexane. The samples and control samples solution were then shaken well and filtered by using Disposable syringe 5 ml and syringe filter – Nylon 66- pore size 0.45µm, diameter 25 mm.

The samples and control samples were used within 24 hours, and protected from light and kept at 20 °C.

2.6.3 Preparation of Standard solutions:

Mixture of Oleic and Elaidic Standard solution was prepared by dissolving of 10 mg of each standard in 100ml n-hexan to get a solution with 100 ppm concentration of both oleic and elaidic acid.

As for samples solution, the standard solution was then shaken well and filtered by using Disposable syringe 5 ml and syringe filter – Nylon 66- pore size 0.45µm, diameter 25 mm. The standard solution was used within 24 hours, and protected from light and kept at 20°C.

2.7 Calculations

$$\text{Elaidic Fatty Acid (100ppm)} = \frac{A_{sa}(t)}{A_{st}(t)} \times \frac{C_{st}}{C_{sa}} \times 1/100$$

$$\% \text{ ElaidicFattyAcid} = \text{ElaidicFattyAcid (100ppm)} \times 10000$$

Where :

Asa(t) : Area under Peak of Elaidic sample solution

Ast(t) : Area under Peak of Elaidic standard solution

Csa : Concentration of sample solution

Cst : Concentration of standard solution

$$OleicFattyAcid(100ppm) = \frac{Asa(c)}{Ast(c)} \times \frac{Cst}{Csa} \times 1/100$$

% Oleic Fatty Acid = Oleic Fatty Acid (100 ppm) × 10000

Where:

Asa(c) : Area under Peak of Oleic sample solution

Ast(c) : Area under Peak of Oleic standard solution

Csa : Concentration of sample solution

Cst : Concentration of standard solution

Chapter Three:

Results and Discussion

3.1: Evaluation of standard samples and control samples

3.1.1 Evaluation of standard samples:

The standard peak identification of each oleic and elaidic standard was based mainly on their retention times. The standards were injected separately and mixed. The retention times for oleic standard as show in Fig. 3.1, and elaidic standard as show in Fig. 3.2 were 14.85 and 17.60 minutes respectively.

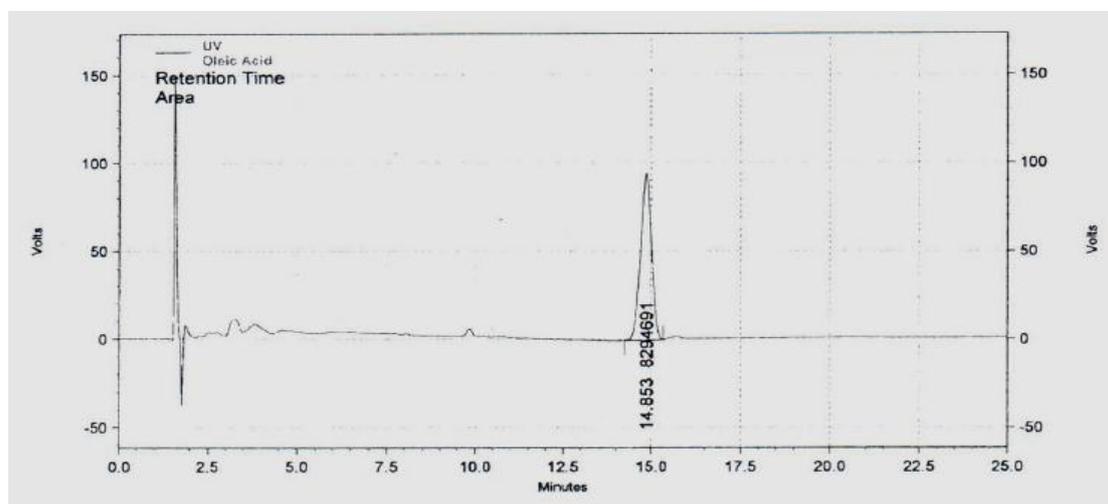


Fig. 3.1: Chromatogram of oleic standard

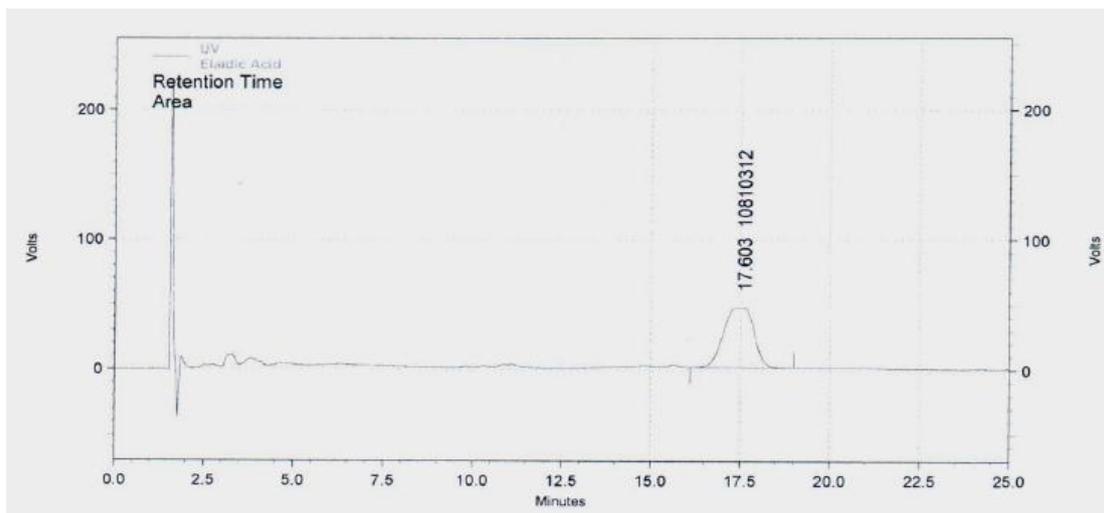


Fig. 3.2: Chromatogram of elaidic standard

After injection of oleic acid standard and elaidic acid standard separately, mixture of the two standards was injected as show in Fig. 3.3, and the peaks were found to be good separated with retention times of 14.83 and 17.29 minutes respectively.

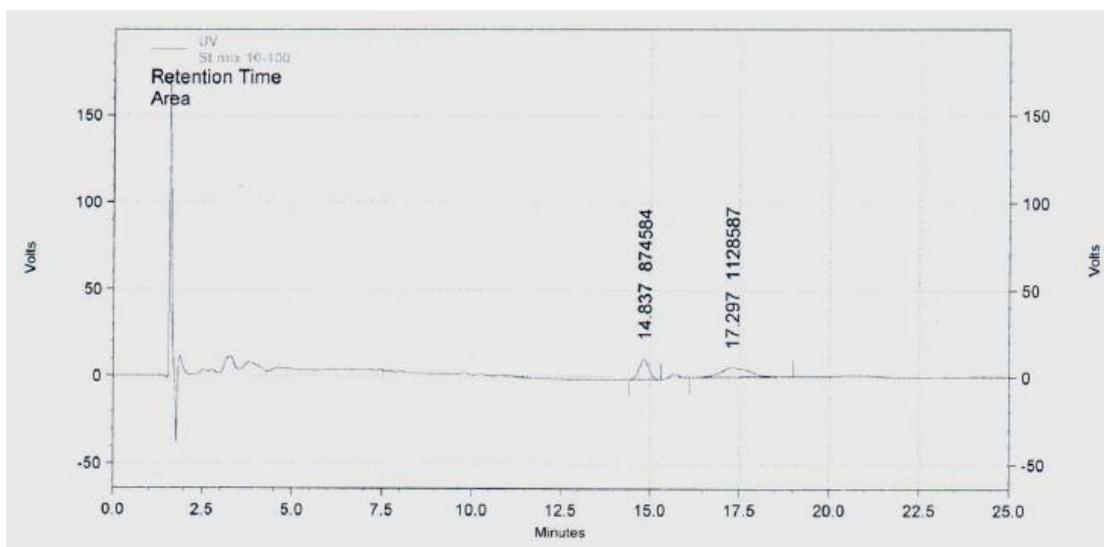


Fig. 3.3: Chromatogram of mix oleic and elaidic Standard : tow Peaks separation at 14.83 and 17.29 minutes.

3.1.2 Evaluation of control samples:

Control samples from each studied fat and oil were analyzed for elaidic acid content before applying heat treatment to be as a baseline as compared to studied treatments.

The control samples include raw margarine, raw ghee, raw corn oil, severe heating corn oil, applied to 220°C for 24 hours, olive oil, severe heating olive oil, applied to

220°C for 24 Hours, restaurant corn oil used in broasted chicken frying from Abu-Dis area and restaurant corn oil used in broasted chicken frying from Ramalla area.

The elaidic acid in control samples as show in table (3.1) appear in raw margarine, raw ghee, sever heating corn oil, sever heating olive oil and corn oil used in broasted chicken Restaurant. While the raw corn oil and raw olive oil elaidic acid content was not detected (0%).

Table 3.1: percentage of elaidic acid and oleic acid in control samples

Control Samples	Elaidic %			Oleic %		
	Range	Mean	±SD	Range	Mean	±SD
Margarine Not treatment <9>*	0 - 1.41	0.75	0.5	0.89 - 7.91	3.42	2.27
Ghee Not treatment <5>	0 - 1.09	0.38	0.45	1.74 - 7.03	3.82	2.36
Corn Oil Not treatment <4>	0 - 0	0	0	1.19 - 4.51	27.61	16.53
Corn Oil Severe Heating <4>	0.36 - 1.32	0.78	0.4	32.35 - 46.98	40.97	6.17
Olive Oil Not treatment <5>	0 - 0	0	0	60.19 - 75.67	69.53	6.21
Olive Oil Severe Heating <3>	0.58 - 4.17	2.63	1.85	26.41 - 31.22	28.9	2.42
Resturant Oil "brostad" Abu Dis <4>	0 - 1.77	0.85	0.78	77.62 - 99.00	90.14	10.61
Resturant Oil "brostad" Ramalla <4>	0 - 1.00	0.32	0.46	1.60 - 10.99	4.1	4.59

<x>*: number of samples studied

Elaidic acid in most samples of raw margarine and raw ghee present is due to hydrogenation (Marais, 2007; Andrzej and Jarosława, 2012).

Investigation of the four different samples of corn oil and five different samples of olive oil didn't contain elaidic acid.

Sever heating of four different samples of corn oil and three different samples of olive oil were heated to 24 hours at 220°C showed different percentages of elaidic acid.

Corn oil obtained from restaurants also showed different percentage of elaidic acid, all samples taken after been used in chicken frying because they were exposed to heating at 220°C in addition to pressure.

Kiritsakis (1998) showed that olive oil has no trans fatty acids, and Sean, et al. (1994) showed that vegetable oils often don't contain trans fatty acid. Meiyang, et al. (2014) showed that trans C18:1 (elaidic) were very low in all samples on different frying cycles during the frying of chicken fillet in corn oil at 170°C, for 12 frying cycles.

Ludger (2014) showed that oils and fats degrade during frying and many reactions with numerous fatty acid alteration products have been examined. The geometrical isomerisation of double bonds leads to the formation of trans fatty acids.

Jun-Cai, et al. (2011) recorded $2.01 \pm 0.24\%$ trans fatty acid in 19 corn oil samples analyzed by gas chromatograph (GC) with a flame ionization detector (FID).

All samples above (Table 3.1) were done as a baseline to draw plan work of temperature and times cycle treatment.

3.2: Evaluation of fat and oil samples

3.2.1 Evaluation of factors affecting the percentage of elaidic acid using partial regression analysis:

Five factors affecting percentage of elaidic acid were highlighted in this study (type of fat and oil, percentage of oleic acid in every fat and oil type, temperature treatment, time of heating, sample type (heat or extract)).

Table 3.2: Ridge regression analysis results to analyze the relationships between elaidic % and the studied factors (type of fat and oil, temperature treatment, time of heating, sample type (heat and extract) and oleic %)).

Coefficients						
Independents	Unstandardized Coefficients	Standardized Coefficients		df	F	Sig.
	Beta	Beta	Bootstrap (1000) Estimate of Std. Error			
Type of fat and Oil	0.1551	0.322	0.011	3	796.027	0.000
Oleic %	-0.0025	-0.118	0.007	1	300.514	0.000
Temp	-0.0004	-0.061	0.013	2	21.404	0.000

Time	-0.0003	-0.033	0.016	4	4.639	0.001
Extract	0.0197	0.030	0.011	2	7.500	0.001
Dependent Variable: Elaidic %						
Regression Constant = 0.00597						
F-ANOVA=28.631, Sig.=0.000, R Square=0.424						

As shown in table 3.2, regarding the type of fat and oil, the regression results show that there is statistically significant relationship between type of fat and Oil and elaidic% at 0.05 level since the sig. =0.000, it is <0.05, which means that the type of oil affects on elaidic% levels.

Regarding the percentage of oleic acid, the regression analysis results reveal that there is negative statistically significant relationship between oleic% and elaidic% at 0.05 level since Beta=-0.0025 and the sig.=0.000<0.05.

Regarding the temperature treatment, the regression results show that there is negative statistically significant relationship between temperature and elaidic %at 0.05 level since Beta=-0.0004 and the sig.=0.000<0.05.

Regarding the time of heating, the regression results show that there is negative statistically significant relationship between time of heating and elaidic% at 0.05 level since Beta=-0.0003 and the sig.=0.001<0.05.

Finally, regarding the extract type, the regression results show that there is statistically significant relationship between type extract type and elaidic% at 0.05 level since the sig.=0.001<0.05 which means that the extract affects on percentage of elaidic.

The R Square=0.424 means that our factors (type of fat and oil, temperature treatment, time of heating, extract type and oleic%) explain the variation in the elaidic% by 42.4% and the remaining variation can be explained by other unstudied factors.

The F-ANOVA=28.631 is significant(Sig.=0.000<0.05) means that our regression model is appropriate to use in study the relationship between factors (type of fat and

oil, oleic percentage, temperature treatment, time of heating and extract type) and elaidic percentage and the regression model fits the data will.

The most effective factor was the type of fat and oil (Standard Beta=0.322), the next was oleic% with (Standard Beta=-0.118), the next was the temperature treatment with (Standard Beta=-0.061), the next was the time of heating with (Standard Beta=-0.033), and the last effective factor was the Extract with(Standard Beta=0.030).

3.2.1.1 Evaluation of type of fat and oil factor:

In our study, samples from four, two solids (margarine and ghee), and two liquids (olive oil and corn oil) fats and oils are evaluated.

Based on the results of control samples, solid samples contained elaidic acid while the elaidic acid does not appear in the raw liquid samples.

Table 3.3: descriptive statistics results for the differences in elaidic % levels due to the type of fat and oil

Type of fat and oil	N	Mean	Std. Deviation	Minimum	Maximum
Margarine	150	0.770601	0.4150834	0.0000	1.6218
Ghee	156	0.615415	0.7339128	0.0000	2.5685
Olive Oil	162	0.000000	0.0000000	0.0000	0.0000
Corn Oil	160	0.000000	0.0000000	0.0000	0.0000
Total	628	0.336935	0.5446891	0.0000	2.5685

Table3.4: one way anova test results to analyze the differences in elaidic% levels due to the fat and oil type

Source Of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.863	3	25.621	146.461	.000
Within Groups	109.159	624	0.175		

Source Of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.863	3	25.621	146.461	.000
Within Groups	109.159	624	0.175		
Total	186.022	627			

as show in table 3.4, the statistically significant differences in elaidic% due to the type of fat and oil at 0.05 level (Sig.=0.000<0.05). According to the tukey post hoc multiple comparisons test, margarine have elaidic% higher than ghee, olive oil and corn oil with significant levels, also the ghee have elaidic % higher than olive oil and corn oil with significant levels.

Table 3.5: tukey hsd multiple comparisons post hoc tests

(I) Fat and Oil	(J) Fat and Oil	Mean Difference (I-J)	Sig.
Margarine	Corn Oil	0.7706013*	0.000
	Ghee	0.1551866*	0.007
	Olive Oil	0.7706013*	0.000
Ghee	Corn Oil	0.6154147*	0.000
	Margarine	-0.1551866*	0.007
	Olive Oil	0.6154147*	0.000
Olive Oil	Corn Oil	0.0000000	1.000
	Ghee	-0.6154147*	0.000
	Margarine	-0.7706013*	0.000
Corn Oil	Ghee	-0.6154147*	0.000
	Margarine	-0.7706013*	0.000
	Olive Oil	0.0000000	1.000

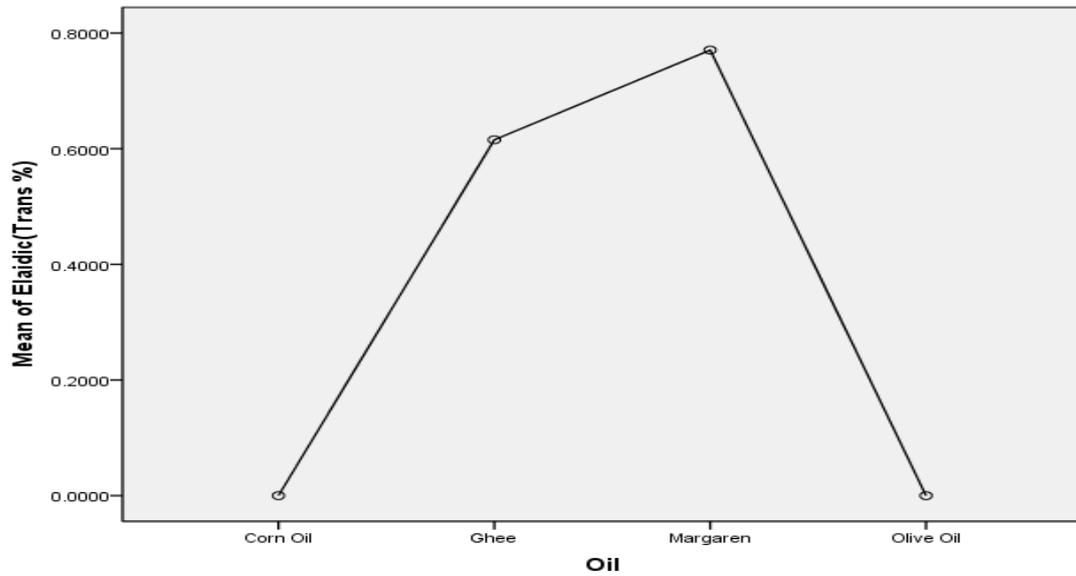


Fig. 3.4: Mean Plots: Effect of type of fat and oil on the percentage of elaidic acid

The means plot Fig. 3.4 exhibited that margarine have the highest elaidic %, it have higher than the ghee, while the olive oil and corn oil did not show any percentage of elaidic acid.

3.2.1.2 Evaluation of percentage of oleic acid factor:

According to the Fig. 3.5, it exhibited that a negative linear relationship may exist between oleic % and elaidic %. That mean if the percentage of elaidic acid increase, the percentage of oleic acid decrease.

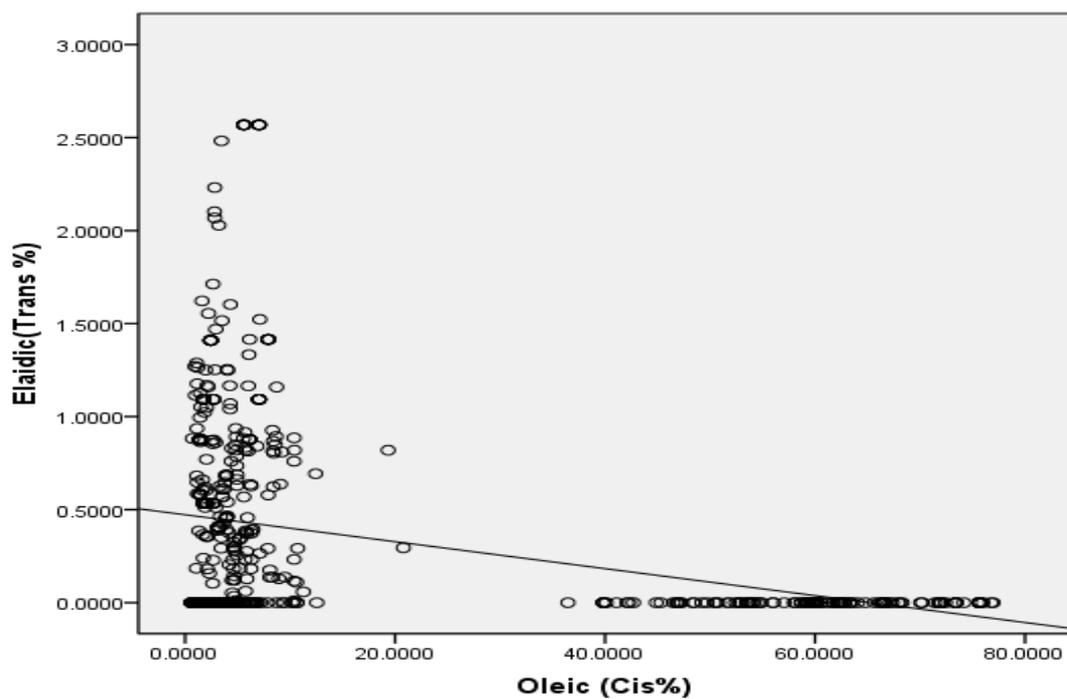


Fig. 3.5: scatter plot: correlation between oleic acid and elaidic acid

3.2.1.3 Evaluation of temperature treatment factor:

Table 3.6: descriptive statistics results for the differences in elaidic % levels due to the temperature treatment

Temperature (°C)	N	Mean	Std. Deviation	Minimum	Maximum
Room Temp	240	0.410350	0.6588228	0.0000	2.5685
120	100	0.375175	0.5859775	0.0000	2.2315
150	96	0.285229	0.3290940	0.0000	1.1675
190	96	0.300109	0.4655903	0.0000	2.4824
250	96	0.202093	0.3816421	0.0000	1.6218
Total	628	0.336935	0.5446891	0.0000	2.5685

Table 3.7: one way anova test results to analyze the differences in elaidic % levels due to temperature treatment

Source Of Variation	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3.572	4	0.893	3.049	0.017
Within Groups	182.450	623	0.293		
Total	186.022	627			

Table 3.7, shows that there are statistically significant differences in elaidic acid due to the temperature treatment at 0.05 P level (Sig.=0.017<0.05). According to the tukey post hoc multiple comparisons test, the elaidic % at room temperature was higher than that at 250°C temperature with significant level.

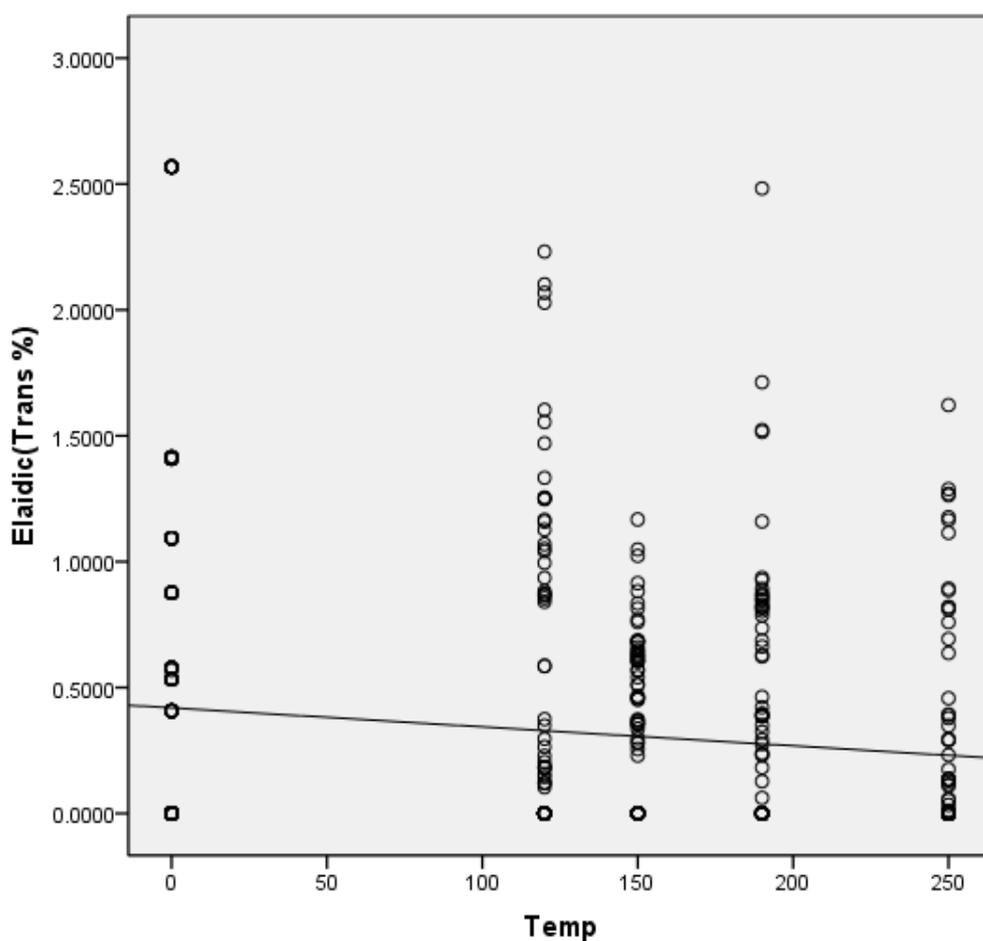


Fig. 3.6: scatter plot: correlation between elaidic acid and temperature treatment

As show in Fig 3.6, it exhibited that a negative linear relationship may exist between temperature treatment and Elaidic acid.

Table 3.8: tukey hsd multiple comparisons post hoc tests

(I) Temp	(J) Temp	Mean Difference (I-J)	Sig.
Room Temperature (RT)	120	0.0351750	0.982
	150	0.1251208	0.311
	190	0.1102406	0.443
	250	0.2082573*	0.013
120	0	-0.0351750	0.982
	150	0.0899458	0.772
	190	0.0750656	0.868
	250	0.1730823	0.167
150	0	-0.1251208	0.311
	120	-0.0899458	0.772
	190	-0.0148802	1.000
	250	0.0831365	0.825
190	0	-0.1102406	0.443
	120	-0.0750656	0.868
	150	0.0148802	1.000
	250	0.0980167	0.719
250	0	-0.2082573*	0.013
	120	-0.1730823	0.167
	150	-0.0831365	0.825
	190	-0.0980167	0.719

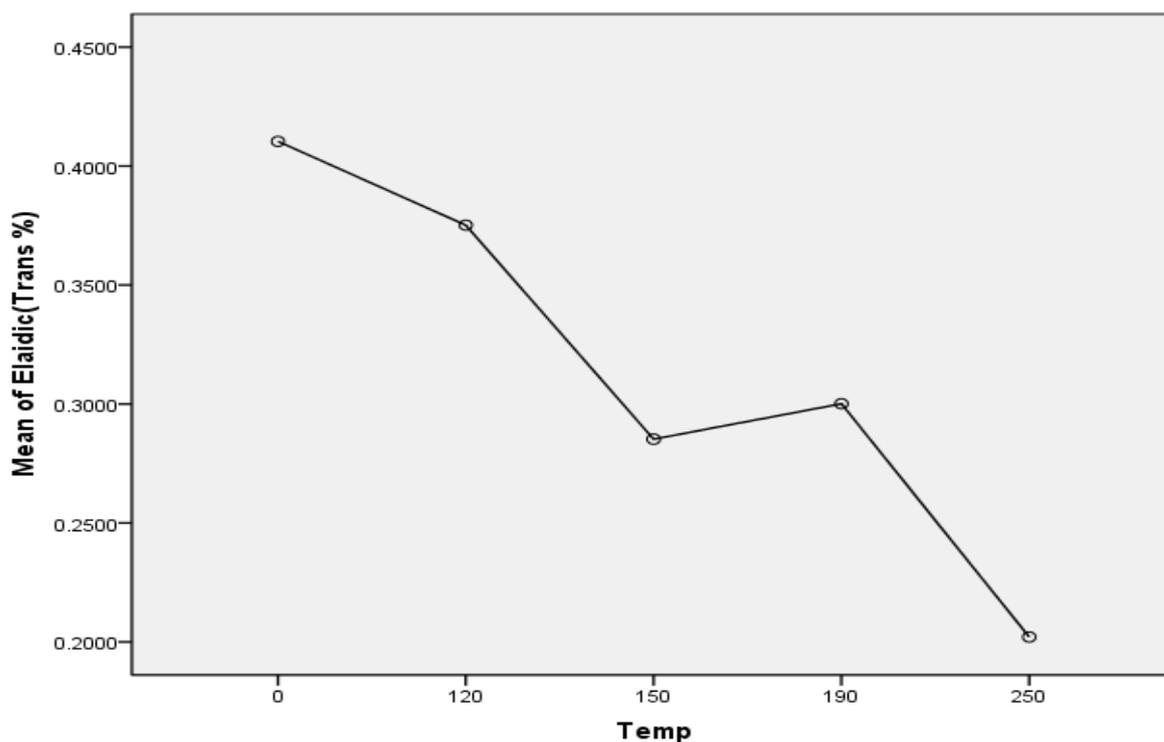


Fig.3.7:Means Plots: correlation between elaidic acid and temperature treatment

As show in Means plot Fig 3.7, it exhibited that Elaidic acid levels decrease at 150°C then increase at 190°C then Sharp fall at 250°C.

3.2.1.4 Evaluation of time of frying factor:

Table 3.9: descriptive statistics results for the differences in elaidic acid levels due to the time of frying

Time(Minutes)	N	Mean	Std. Deviation	Minimum	Maximum
0	240	0.410350	0.6588228	0.0000	2.5685
10	97	0.319013	0.4882330	0.0000	2.4824
30	98	0.295209	0.5380014	0.0000	2.2315
60	97	0.295868	0.4208103	0.0000	1.5227
180	96	0.255593	0.3558695	0.0000	1.1652
Total	628	0.336935	0.5446891	0.0000	2.5685

Table 3.10: one way anova test results to analyze the differences in elaidic acid levels as affected with time of frying

Source Of Variation	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.294	4	0.574	1.945	.101
Within Groups	183.728	623	0.295		
Total	186.022	627			

Table 3.10 shows that there are no statistically significant differences in elaidic acid due to the time of frying at $P0.05$ level($Sig.=0.101>0.05$).

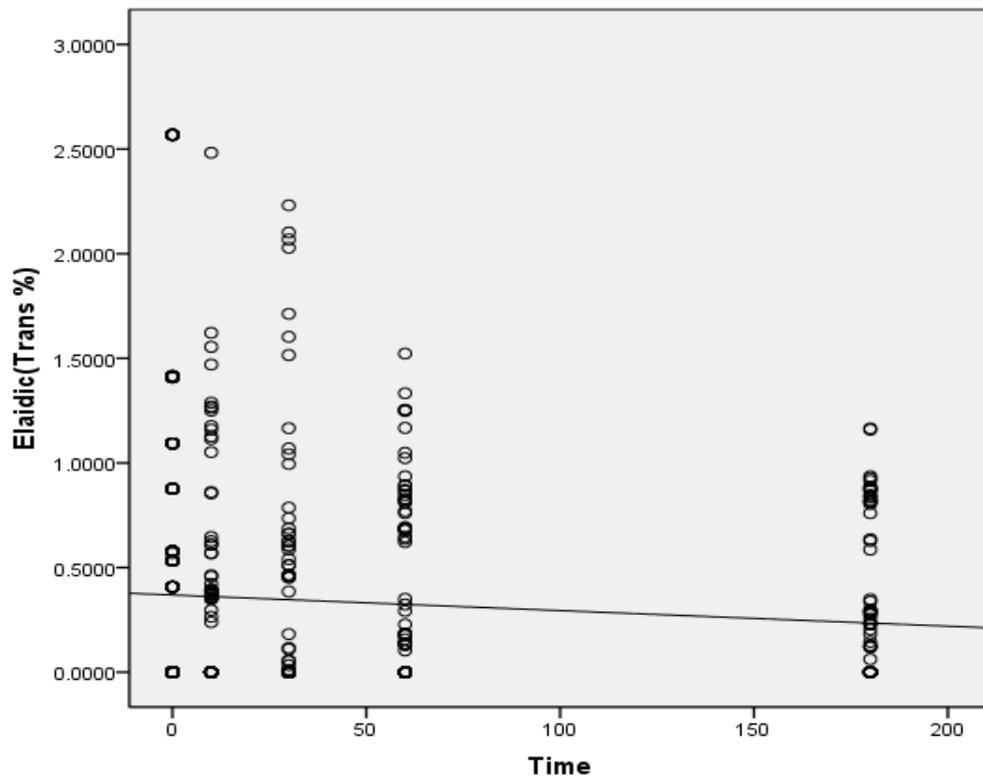


Fig.3.8: scatter plot : correlation between elaidic acid and time of frying

According to the Scatter Plot fig 3.8, it exhibited that a negative linear relationship may exist between time of frying and elaidic acid concentration.

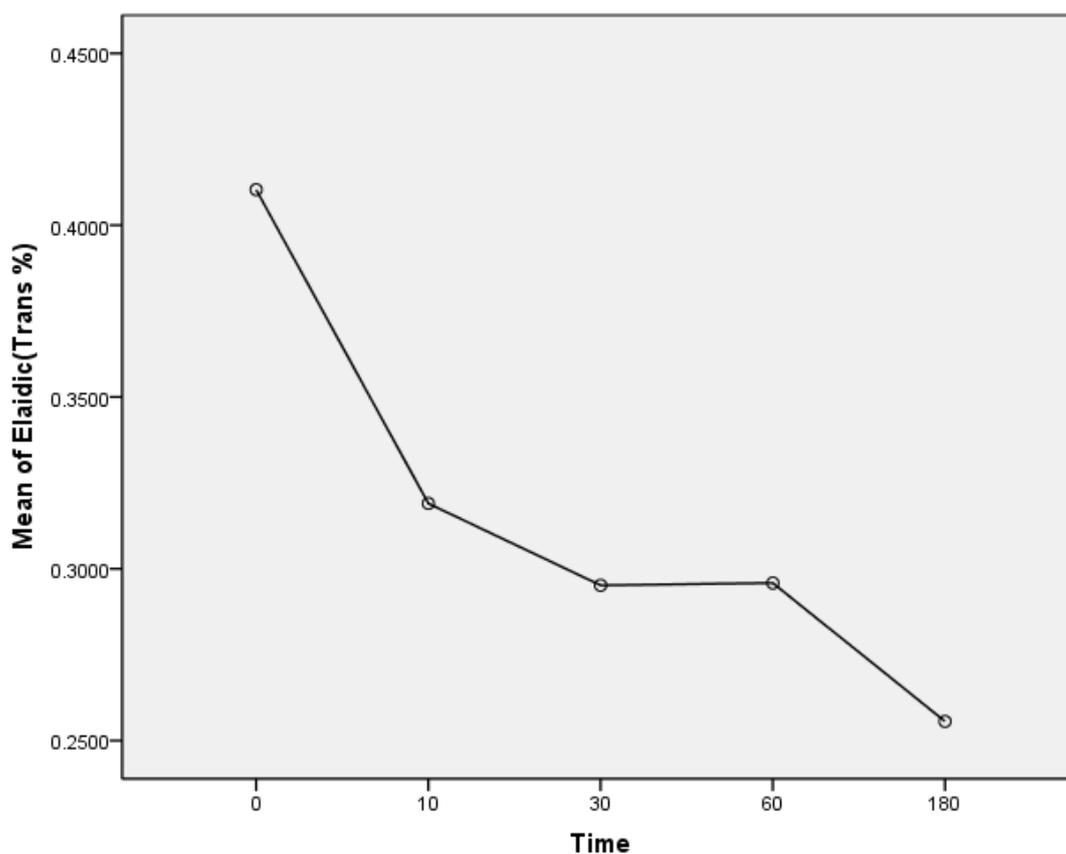


Fig.3.9: means plot : correlation between elaidic acid and time of frying

As show in means plot Fig. 3.9, elaidic acid levels decrease at 10min, 30min , it is stable between 30 min and 60 min, then decrease again at 180 min.

3.2.1.5 Evaluation of extract type factor :

Table 3.11: descriptive statistics results for the differences in elaidic acid levels due to the extract type

Extract type	N	Mean	Std. Deviation	Minimum	Maximum
Extract type	194	0.302860	0.4796227	0.0000	2.4824
Heat type	194	0.280186	0.4303511	0.0000	2.2315
Not treatment samples	240	0.410350	0.6588228	0.0000	2.5685
Total	628	0.336935	0.5446891	0.0000	2.5685

Table 3.12: one way anova test results to analyze the differences in elaidic acid levels due to the extract type

Source Of Variation	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.144	2	1.072	3.643	0.027
Within Groups	183.879	625	0.294		
Total	186.022	627			

Table 3.12 shows that there are statistically significant differences in elaidic acid due to the extract type at P 0.05 level (Sig.=0.027<0.05). According to the tukey post hoc multiple comparisons test, not treatment samples have elaidic acid higher than heat type samples with significant level.

Table 3.13: tukey hsd multiple comparisons post hoc tests

(I) Extract	(J) Extract	Mean difference (I-J)	Sig.
Extract	Heat	0.0226737	0.911
	No treatment	-0.1074902	0.101
Heat	Extract	-0.0226737	0.911
	No treatment	-0.1301639*	0.035
No treatment	Extract	0.1074902	0.101
	Heat	0.1301639*	0.035

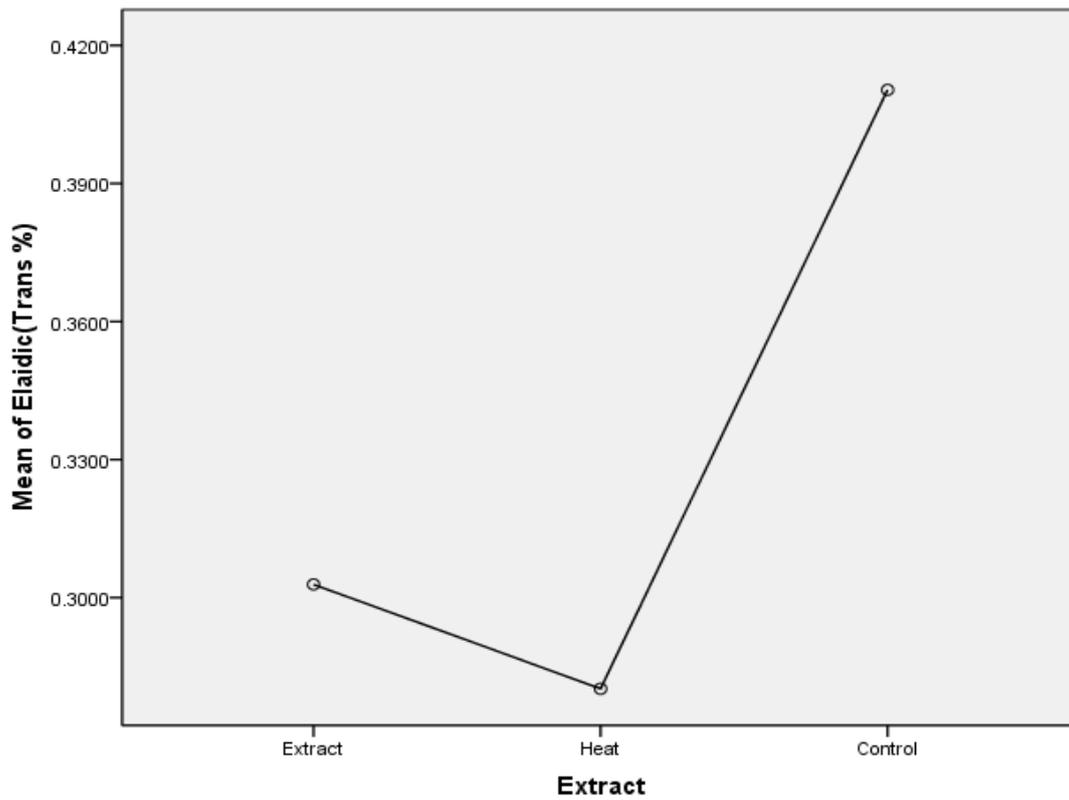


Fig. 3.10: means plot : correlation between elaidic fatty acid and extract type

The means plot show in Fig 3.10 exhibited that elaidic acid levels from the no treatment are higher than elaidic acid levels from the heat type and the extract type treatments.

3.2.2 Evaluation of elaidic acid in margarine:

The percentage of elaidic acid in both types of margarine samples ((heat type sample that was take directly from fryer) and (extract type sample that was extract margarine from potato that was frying in margarine fat)) were evaluated , two types of margarine treated at 120°C, 150°C, 190°C and 250°C for different time intervals.

3.2.2.1 Evaluation of elaidic acid in heat type of margarine

Fig. 3.11, show four different heat treatment applied in heat type (temperature and time) of margarine, the result show differ value in percentage of elaidic acid. According to regression analyses, results show that percentage of elaidic acid in heat margarine treated at 120°C did not influence all different time intervals. That's mean the structural (positional) isomerization and geometrical isomerization in which the cis isomers of fatty acids in margarine be stables and not effected by applied 120°C heat treatment.

Changmo, at al. (2013), showed that the cis to trans isomerization may occur at 150°C. This result corresponds with many of the researchers, who used a frying temperatures lower than 150°C.

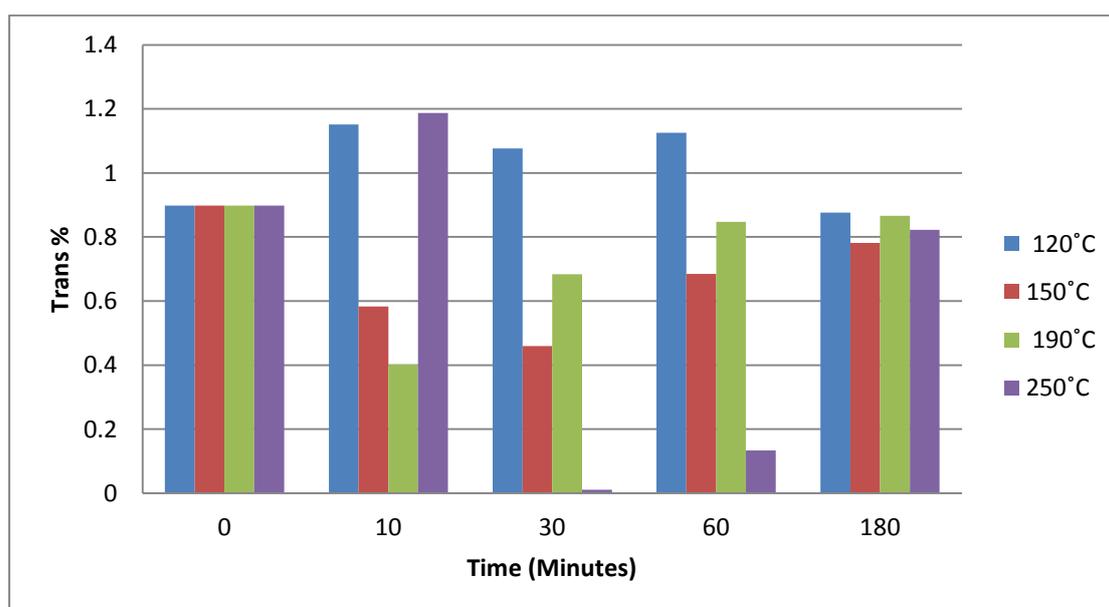


Fig. 3.11: Effect of heating on the percentage of elaidic acid for margarine sample treated at four different temperatures (120°C, 150°C, 190°C and 250°C)

At 10 min of heating, the obtained result showed that the lowest percentage of elaidic acid at 190°C, and the highest percentage of elaidic was recorded at 250°C. At 30 min and 60 min the lowest percentage of elaidic acid react at 250°C, and the highest percentage of elaidic reacted at 120°C.

At 180 min for all thermal treatments applied showed that the percentage of elaidic acid went to the approximate values, which is almost to the extent of the value of elaidic acid at margarine that not treatment.

According the analyses of model summary and parameter Estimates in SPSS, the elaidic acid in heat type of margarine treated at 150°C, 190°C and 250°C showed that there is cubic nonlinear significant relationship between percentage of elaidic acid and time of heating.

At 150°C, as show Fig.3.12, the percentage of elaidic acid decrease at 10 min and 30 min, then increased at time of heating increased.

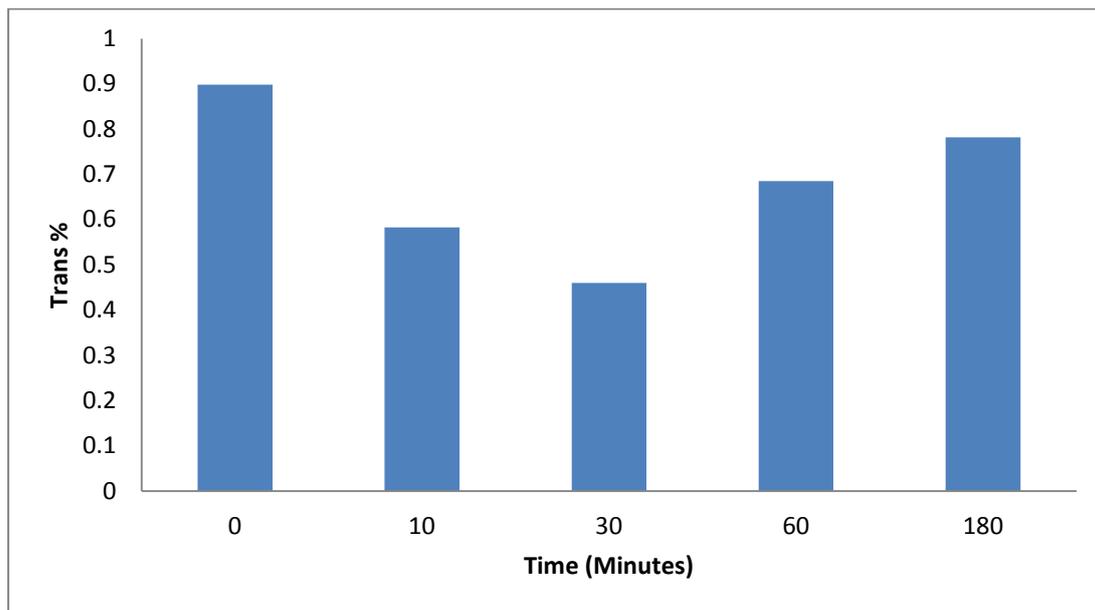


Fig. 3.12:Effect of heating on the percentage of elaidic acid for margarine sample treated at 150°C

Fig.3.13, show that increasing of the percentage of elaidic acid with time of heating interval increase in heat type of margarine treated at 190°C, at 180 min the percentage of elaidic acid was more stably.

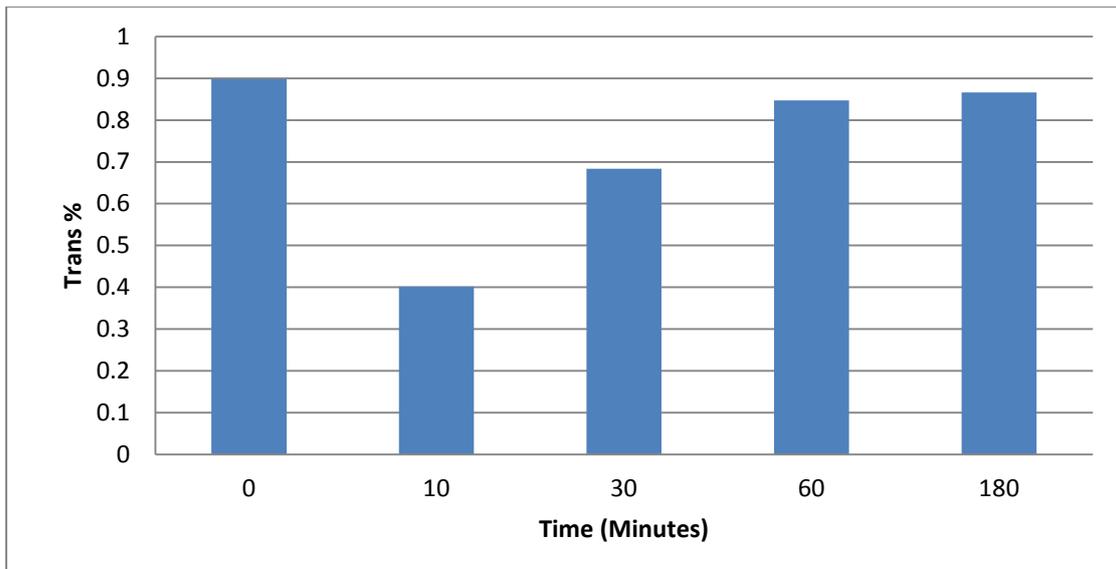


Fig. 3.13:Effect of heating on the percentage of elaidic acid for margarine sample treated at 190°C

Fig. 3.14, the highest percentage of elaidic acid appeared at 10min and the lowest percentage appeared at 30min, however after 30min the percentage of elaidic acid increased with time of heating increased.

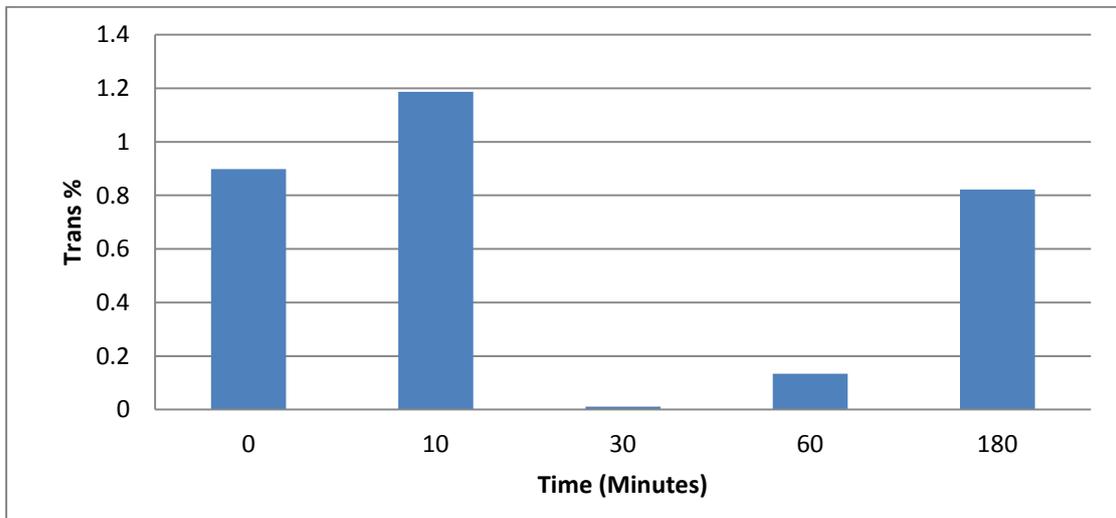


Fig. 3.14:Effect of heating on the percentage of elaidic acid for margarine sample treated at 250°C

By Comparing the different treatment temperature on the elaidic acid content of heated margarine, results showed the highest influence effected of elaidic acid at 250°C, the highest amount were observed after heating at 250°C for 10min, where the lowest amount were observed at 250°C for 30min.

The dependence of the degree of fat and oil heat treatment temperature is in agreement with other researchers including K.Cihelkova, et al. (2009) who showed that the content of trans fatty acids increased with time and with temperature and a rate of cis-trans isomerisation and polymerization depends on the temperature according to Arrhenius equation. Roman and Felix (2012) reported that insignificant increase in the amount of trans isomers were applied of extreme temperatures during baking.

3.2.2.2 Evaluation of elaidic acid in extract type of margarine:

Fig. 3.15, show the percentage of elaidic acid in extract type of margarine treated at 120°C, 150°C, 190°C and 250°C for different time intervals, according to regression analyses results and the analyses of model summary and parameter estimates SPSS to elaidic acid in extract type of margarine show that the percentage of elaidic treated at 120°C did not effect at any different time intervals, but the percentage of elaidic acid treated at 150°C, 190°C and to a less extent at 250°C showed that there is cubic nonlinear significant relationship between percentage of elaidic and time of heating.

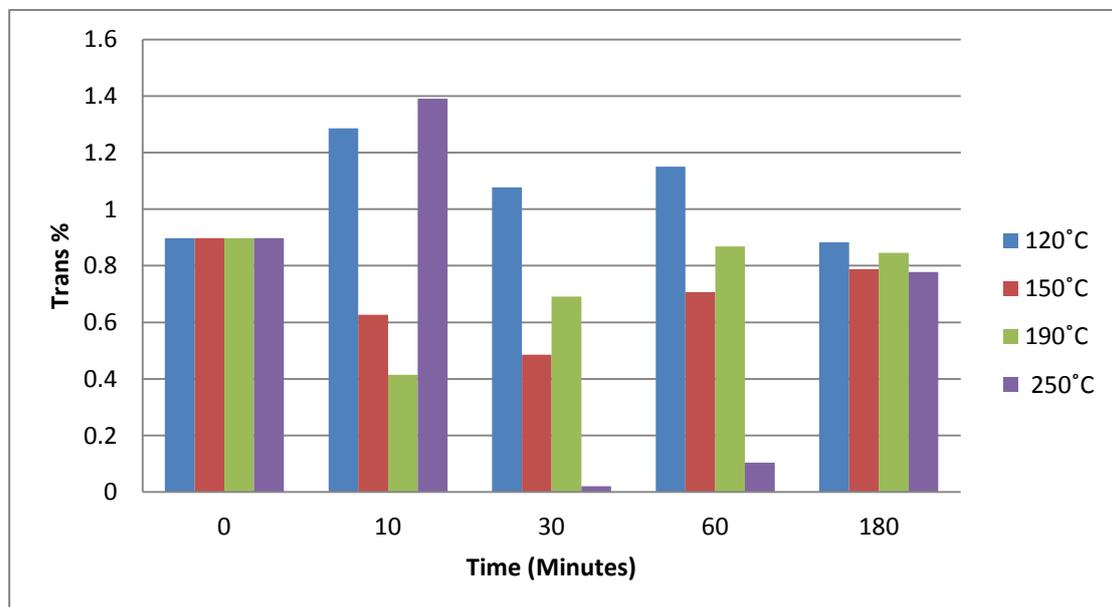


Fig.3.15:Effect of heating on the percentage of elaidic acid for extract margarine sample at four difference temperature (120°C, 150°C, 190°C and 250°C)

At 150°C, the obtained results show that the percentage of elaidic decrease at 10min and 30 min, then increased at time of heating increased.

At 190°C, the obtained results show increase of the percentage of elaidic acid with time of heating interval increase, at 180minthe percentage of elaidic acid was more stably.

At 250°C, the obtained results show the highest percentage of elaidic acid appeared at 10min and the lowest percentage appeared at 30min, however after 30min the

percentage of elaidic acid increased with time of heating increased. by Comparing the different treatment temperature on the elaidic acid content of extract type of margarine result showed the highest influence effected of elaidic acid at 250°C.

The percentage of elaidic acid in extract type of margarine showed the same value in heat type of margarine at all heat treatment applied, while the accurate different between heat type and extract type obtained in the next section.

3.2.2.3 Correlation between elaidic acid in heat type and extract type of margarine:

Fig. 3.16 shows the correlation between elaidic acid as affected by heat type and extract type of margarine treated at 120°C for different time intervals, the obtained results show slightly difference in percentage of elaidic acid at 10 min.

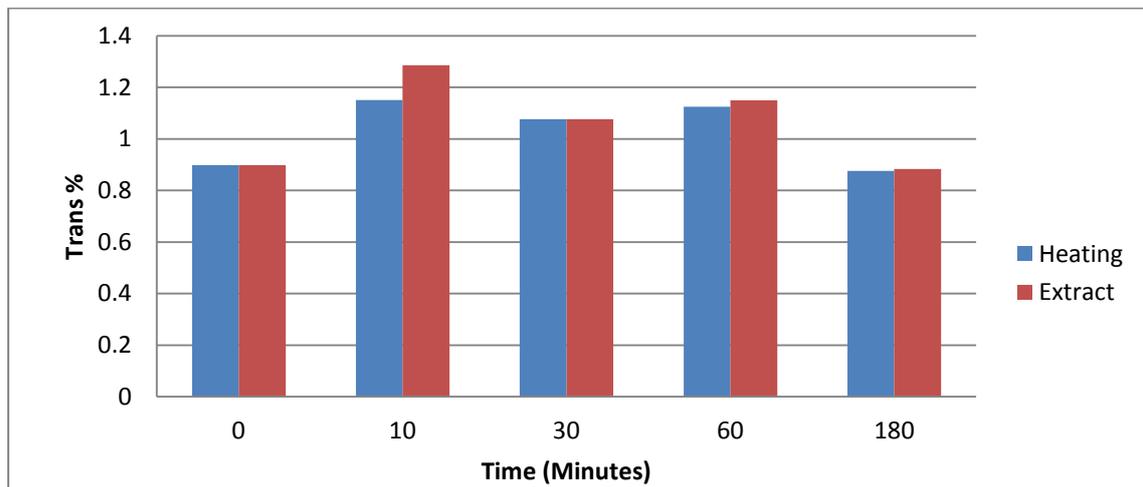


Fig.3.16:Correlation between elaidic acid in heat type and extract type of margarine treated at 120°C

Fig. 3.17, show the correlation between elaidic acid in heat type and extract type of margarine treated at 150°C for different time intervals, the obtained results show slightly difference appeared at 10min, 30min and 60min between heat type and extract type of margarine.

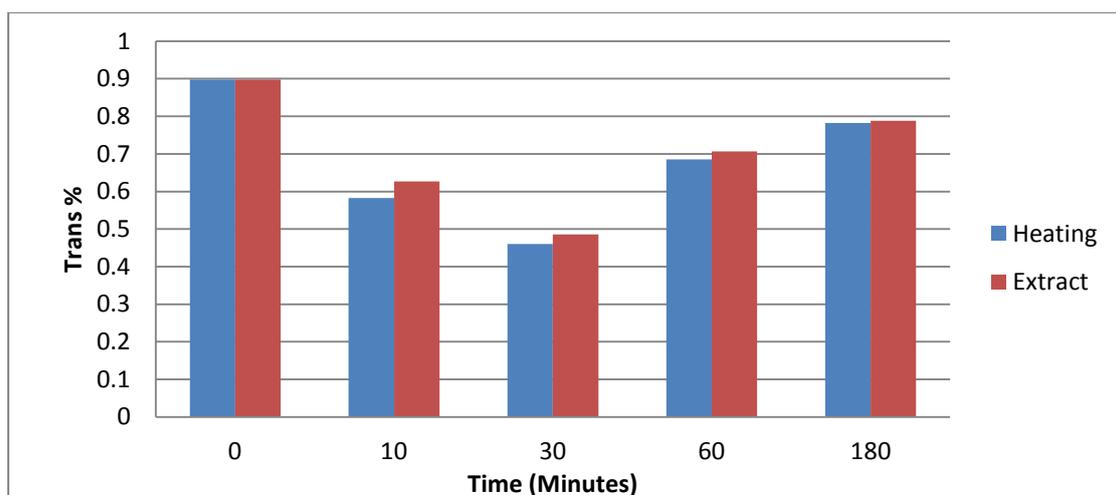


Fig.3.17:Correlation between elaidic acids in heat type and extract type of margarine treated at 150°C

Fig.3.18, the correlation between elaidic acid in heat type and extract type of margarine treated at 190°C for different time intervals, the obtained results show it is no significant difference appeared between heat type and extract type of margarine heated at 190°C for different time intervals.

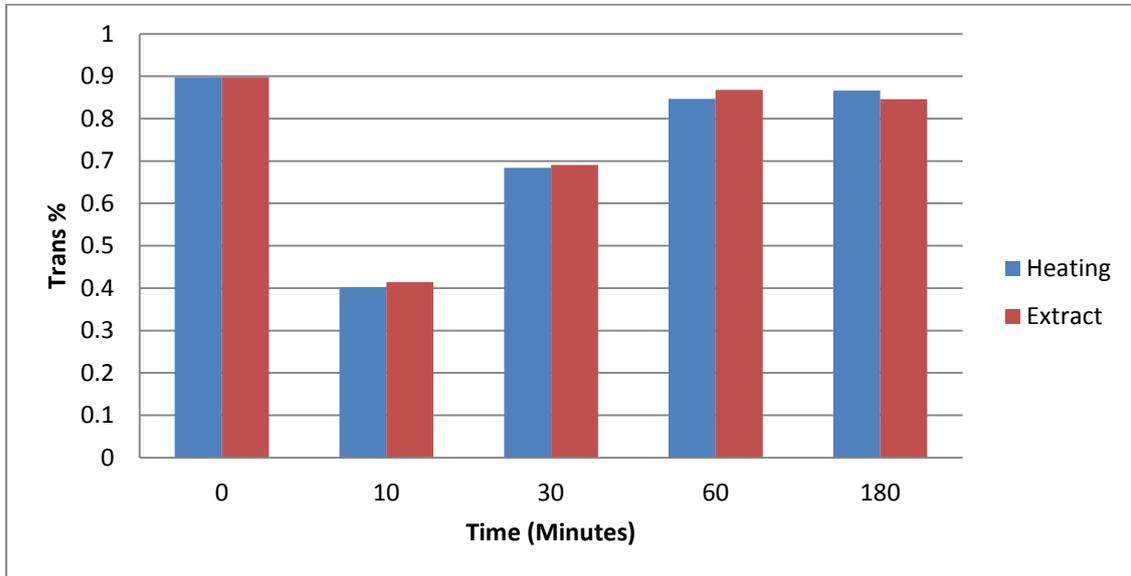


Fig.3.18: Correlation between elaidic acids in heating type and extract type of margarine at 190°C

Fig.3.19 show the correlation between elaidic acids in heating type and extract type of margarine at 250°C for different time intervals, the obtained results show difference at 10min and slightly difference at 60min and 180min.

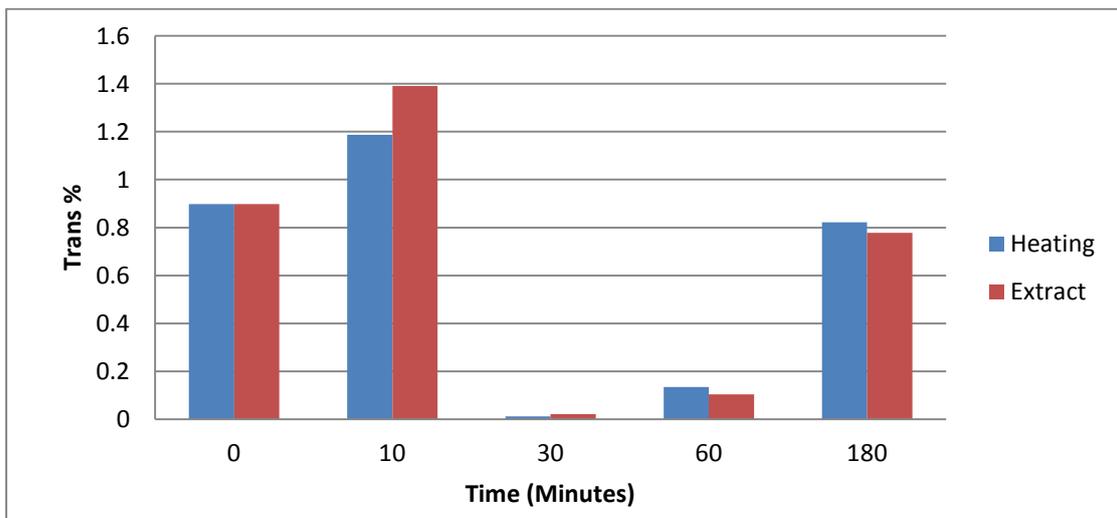


Fig.3.19: Correlation between elaidic acid in heat type and extract type of margarine at 250°C

Fig. 3.20 show the correlation between the percentage of elaidic acid obtained from heat type and extract type of margarine treated at four different heat treatment for different time interval, tow type (heat and extract) margarine show the same value except some of points appear more influence of extract type margarine, it is due to influence of heat applied in condensation and soxhselt heat in extraction process.

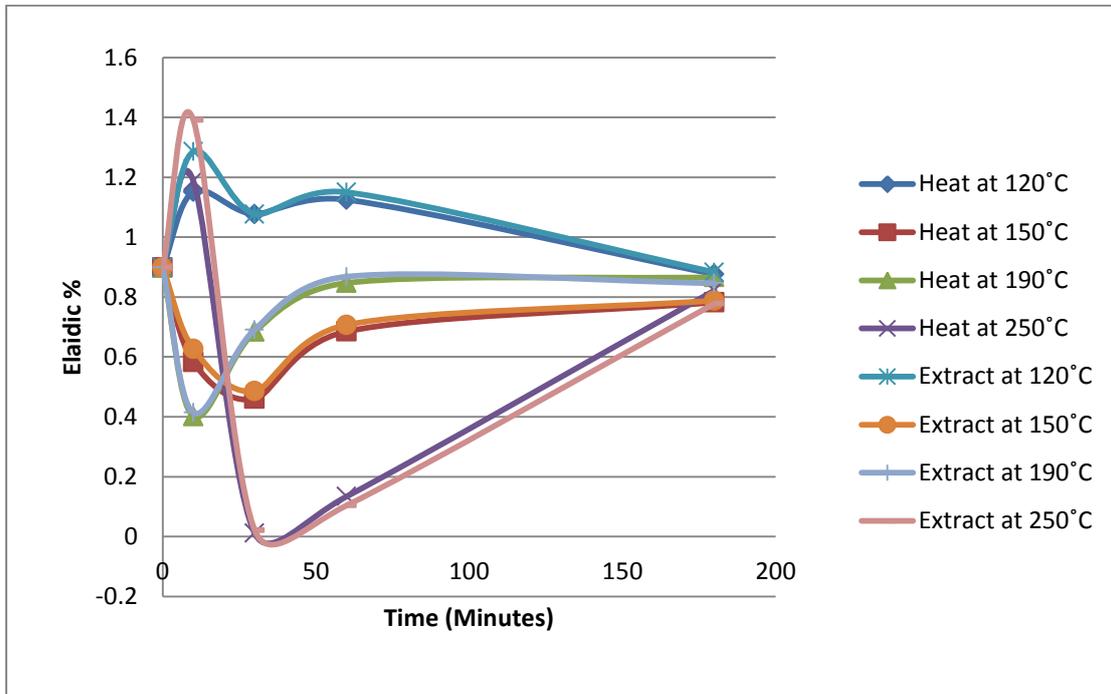


Fig.3.20: Correlation between elaidic acid in heat type and extract type of margarine at four different temperature (120C°, 150C°, 190C° and 250C°)

3.2.2.4 Correlation between elaidic acid and oleic acid in heat type and extract type of margarine:

The obtained results show opposite relation between elaidic acid and oleic acid in heat type (as show in Fig. 2.21) and extract type (as show in Fig. 3.22) of margarine treated at 120°C for different time intervals, if the oleic increase, the elaidic decrease at all points obtained.

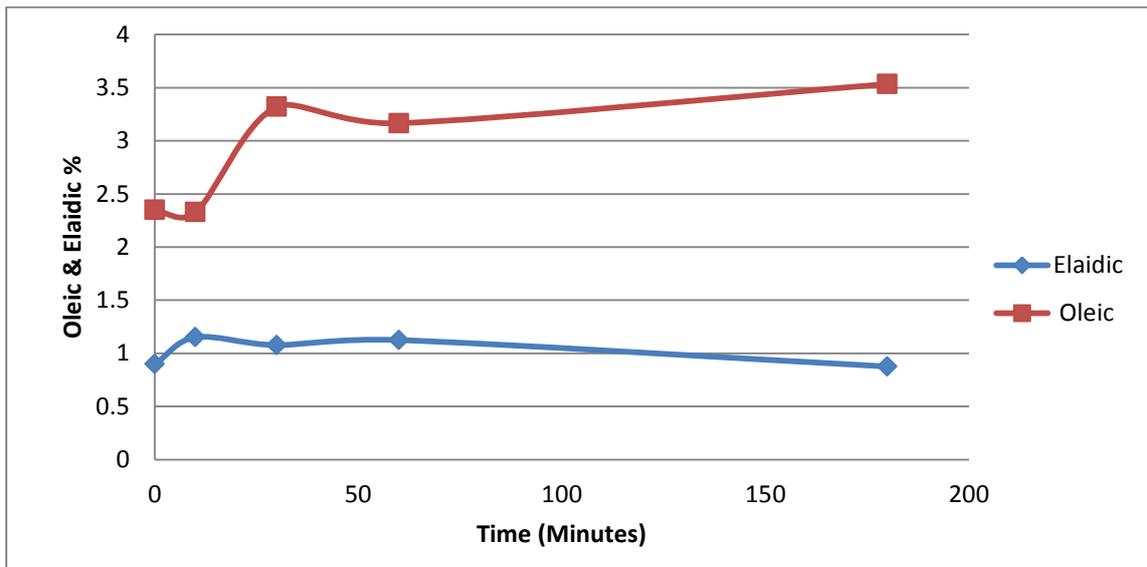


Fig.3.21: Correlation between elaidic acid and oleic acid in heat type of margarine treated at 120°C

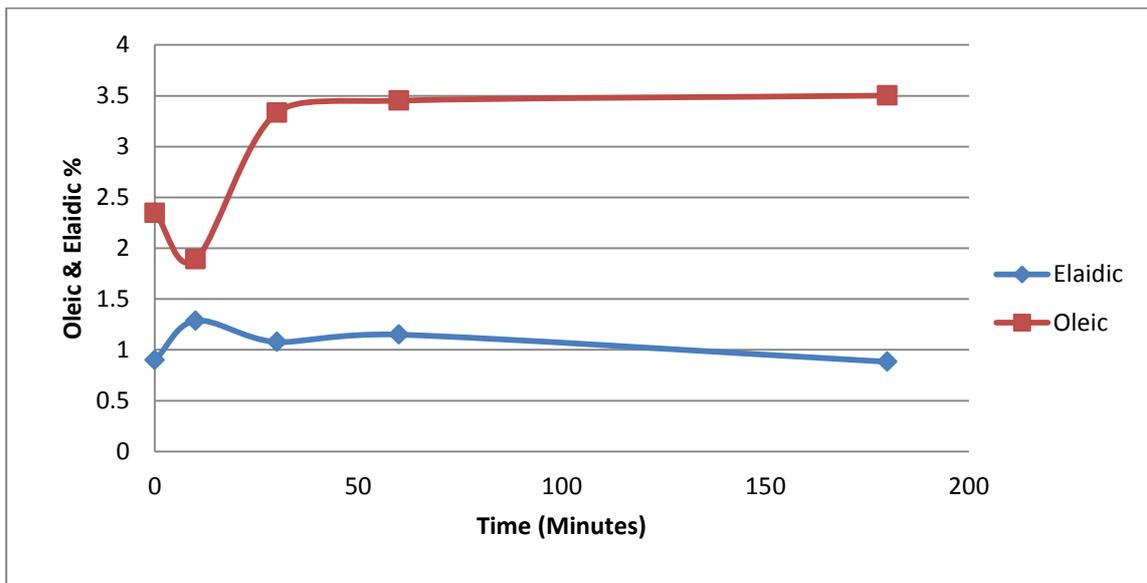


Fig.3.22: Correlation between elaidic acid and oleic acid in extract type of margarine treated at 120°C

Fig.3.23 show relation between elaidic acid and oleic acid in heat type of margarine treated at of 150°C for different time intervals, at 10, 30 and 60 min showed opposite relation between oleic and elaidic, but at 180min the percentage of both oleic and elaidic increased.

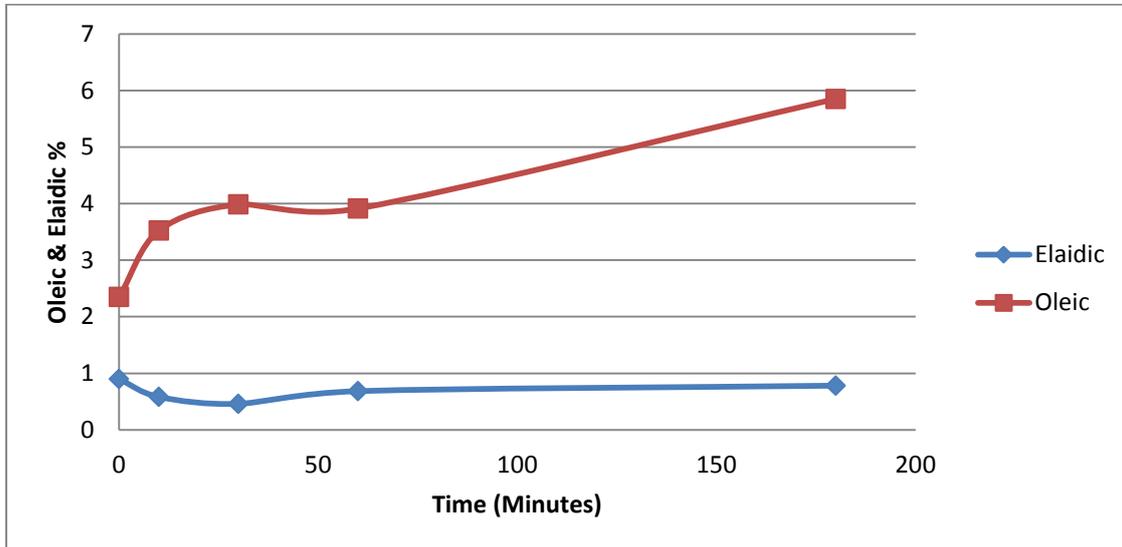


Fig.3.23: Correlation between elaidic acid and oleic acid in heat type of margarine treated at 150°C

Fig. 3.24 show relation between elaidic acid and oleic acid in extract type of margarine treated at of 150°C for different time intervals, the opposite relation appeared at 10, 30 and 60 min, but at 180min the percentage of both oleic and elaidic increased.

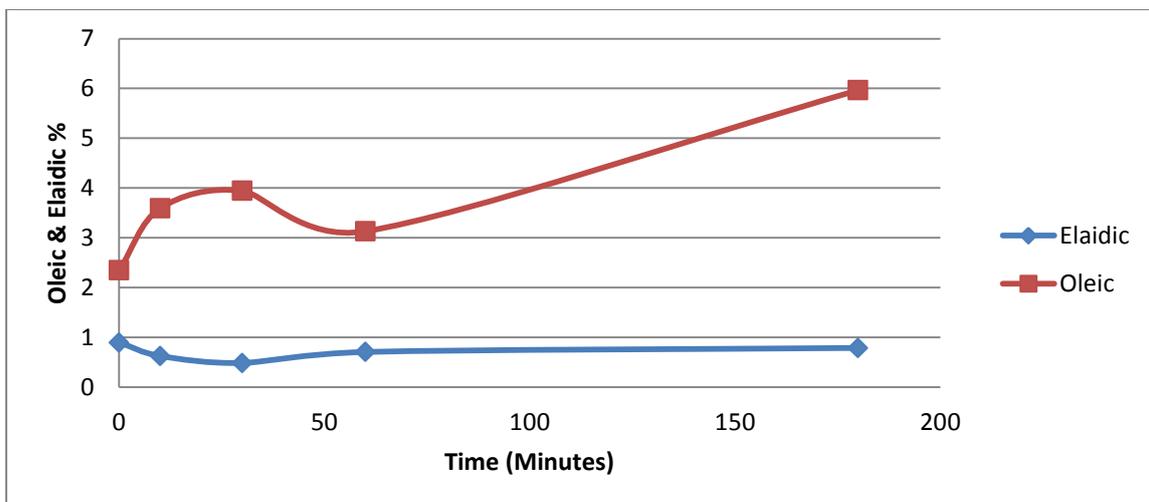


Fig.3.24: Correlation between elaidic acid and oleic acid in extract type of margarine treat at 150°C

Fig. 3.25 show relation between elaidic acid and oleic acid in heat type of margarine treated at 190°C for different time intervals, the opposite relation appeared at 10, 30 and 60 min appeared, at 180min the percentage of oleic increase while the elaidic did not affected.

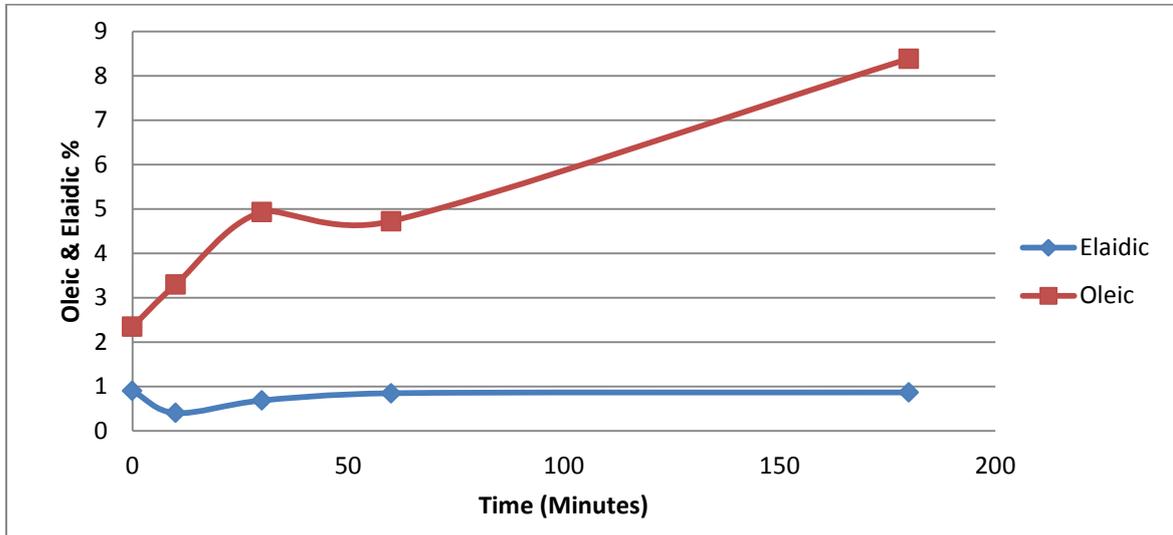


Fig.4.3.25: Correlation between elaidic acid and oleic acid in heat type of margarine treat at 190°C

Fig. 3.26 shows opposite relation between elaidic acid and oleic acid in extract type of margarine treated at 190°C for different time intervals, the opposite relation appeared at 10, 30 and 60 min, but at 180min the percentage of oleic increase while the elaidic did not affected.

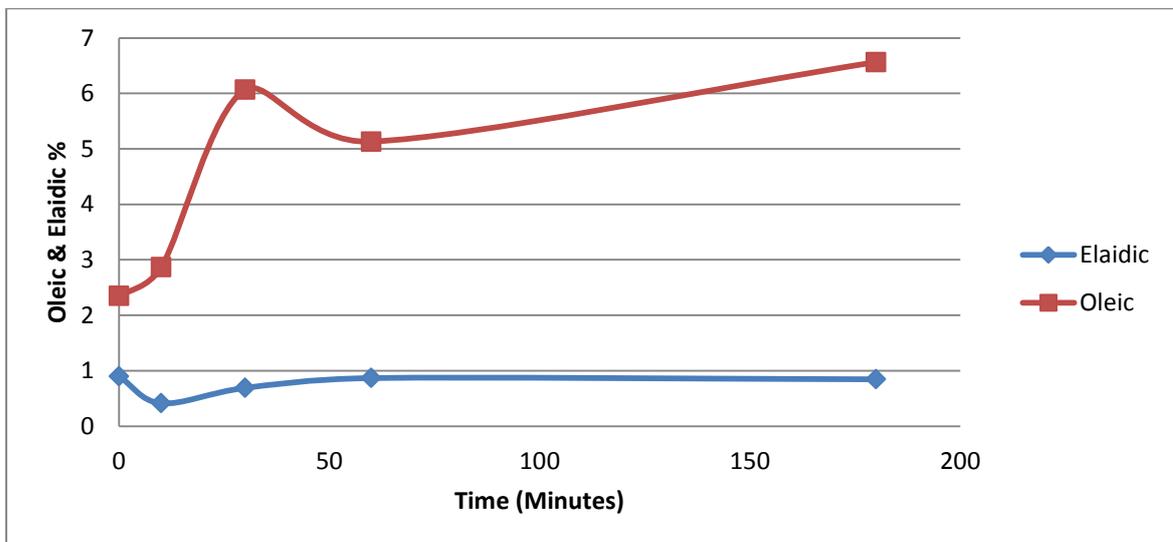


Fig.3.26: Correlation between elaidic acid and oleic acid in extract type of margarine treat at 190°C

Fig. 3.27 show relation between elaidic acid and oleic acid in heat type of margarine treat at 250°C for different time intervals, the opposite relation appeared at 10 and 30, but at 60 and 180min the percentage of both oleic and elaidic increased.

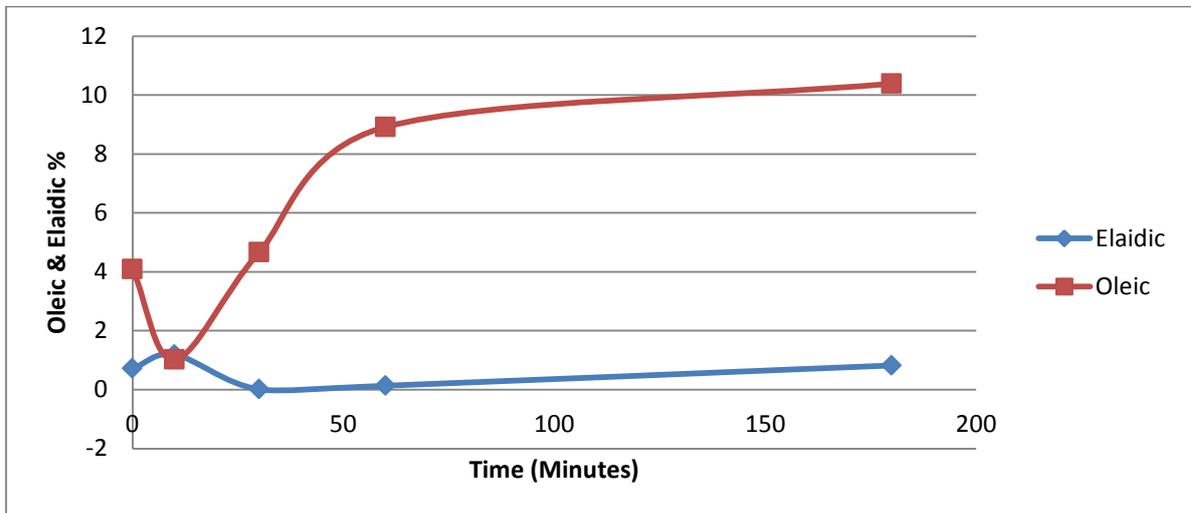


Fig.3.27: Correlation between elaidic acid and oleic acid in heat type of margarine treat at 250°C

Fig. 3.28 show relation between elaidic acid and oleic acid in extract type of margarine treated at 250°C for different time intervals, opposite relation appeared at 10 and 30min, but in 60 and 180min the percentage of both oleic and elaidic increased.

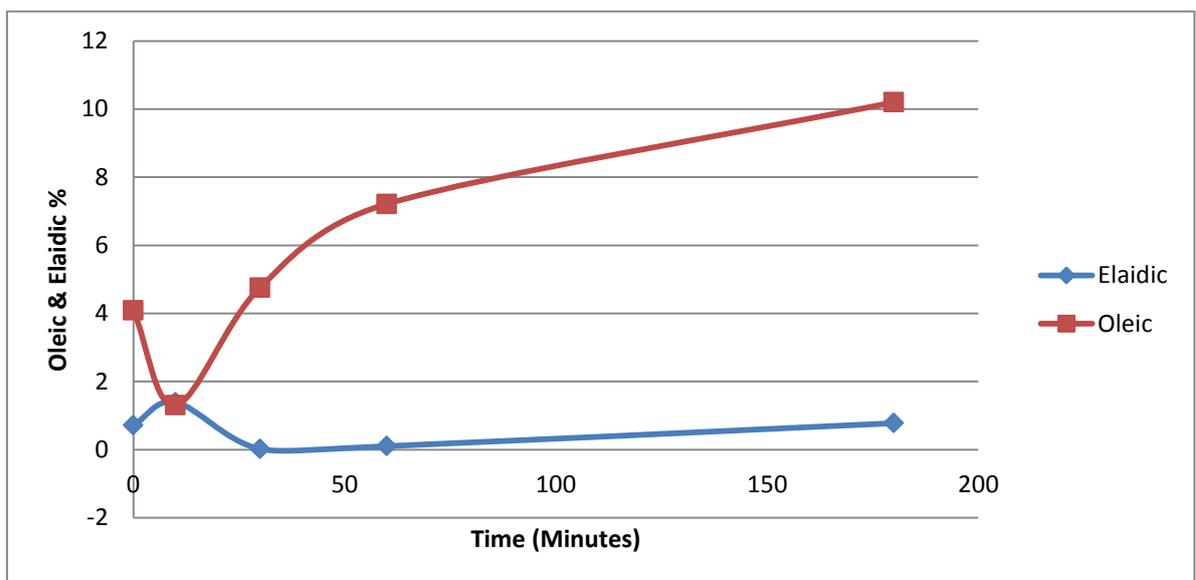


Fig.3.28: Correlation between elaidic acid and oleic acid in heat extract of margarine treat at 250°C

3.2.3 Evaluation of elaidic acid in ghee:

3.2.3.1 Evaluation of elaidic acid in heat type of ghee:

Fig. 3.29 show influence of percentage of elaidic acid in heat type of ghee by applied four different heat treatment (120°C, 150°C, 190°C and 250°C) for different time intervals (10min, 30min, 60min and 180min). In each heat treatment the result show differ value in percentage of elaidic acid. As show in Fig.3.29 the highest influence of percentage of elaidic acid appeared at 150°C° for 60min, and the lowest amount of percentage of elaidic showed at 250°C° for 30min.

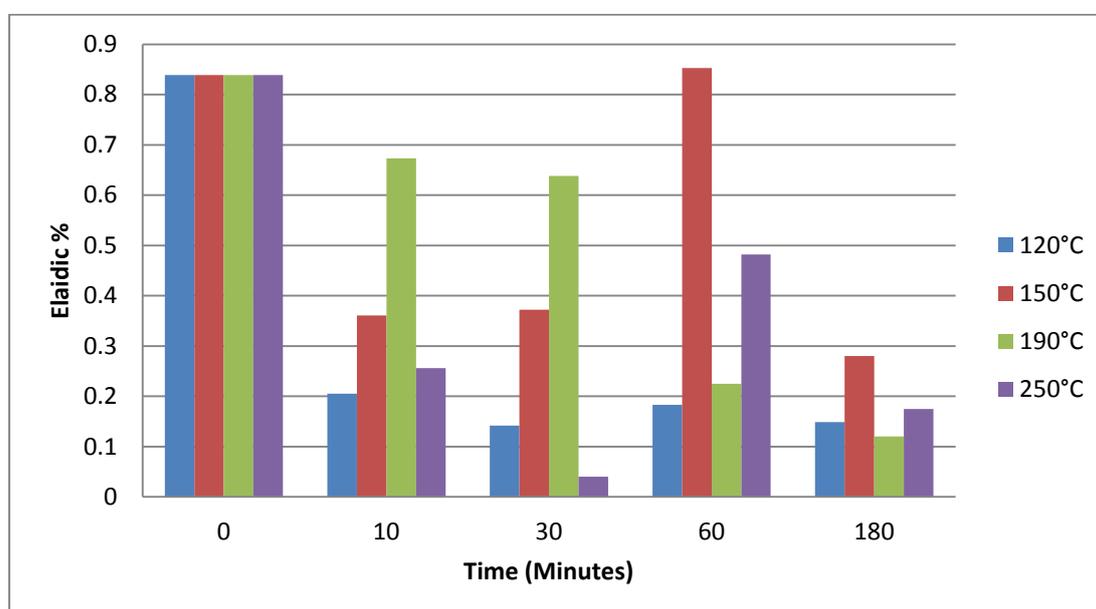


Fig. 3.29: Effect of heating on the percentage of elaidic acid in heat type of ghee sample treated at four different temperature (120°C, 150°C, 190°C and 250°C)

According to regression analyses, the obtained result at 120°C for different time intervals show no significant difference in the percentage of elaidic acid in heat type of ghee treated at 120°C for different time intervals.

According to the analyses of model summary and parameter estimates in SPSS, the elaidic acid in heat type of ghee treated at 150°C showed that there is cubic nonlinear significant relationship between percentage of elaidic and time of heating. Fig. 3.30 show the percentage of elaidic acid in heat type of ghee treated at 150°C for different time intervals, the percentage of elaidic acid showed highest value at 60min.

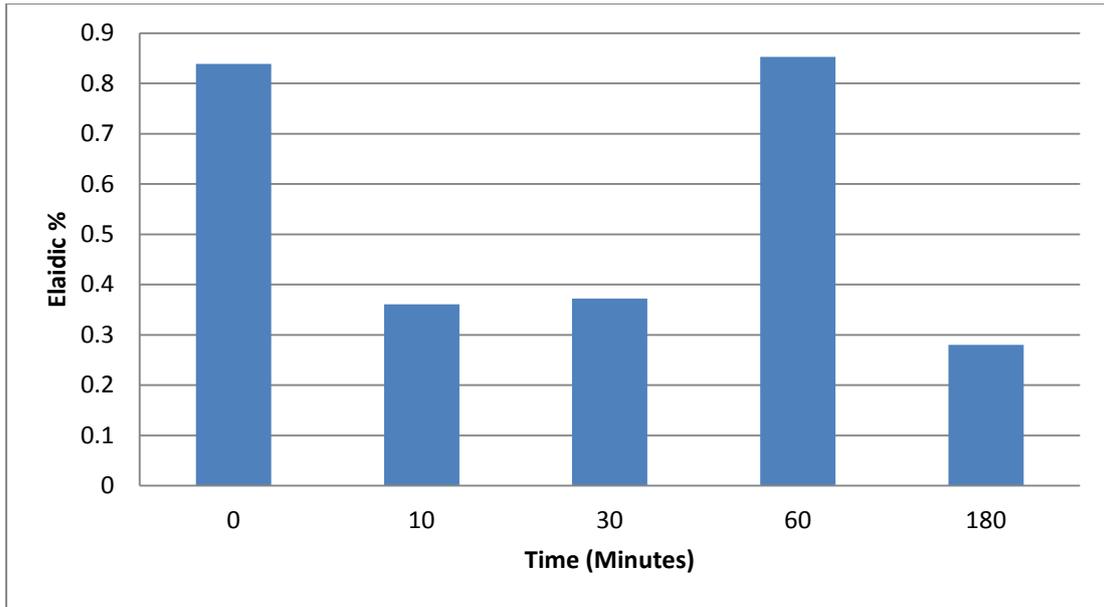


Fig.3.30: Effect of heating on the percentage of elaidic acid in heat type of ghee treated at 150°C

Fig. 3.31 show the percentage of elaidic acid in heat type of ghee treated at 190°C for different time intervals, according to the fig 3.31, it exhibited that a negative linear relationship may exist between the percentage of elaidic acid and time of frying. If the time of heating increase at 190°C, the percentage of elaidic acid decrease.

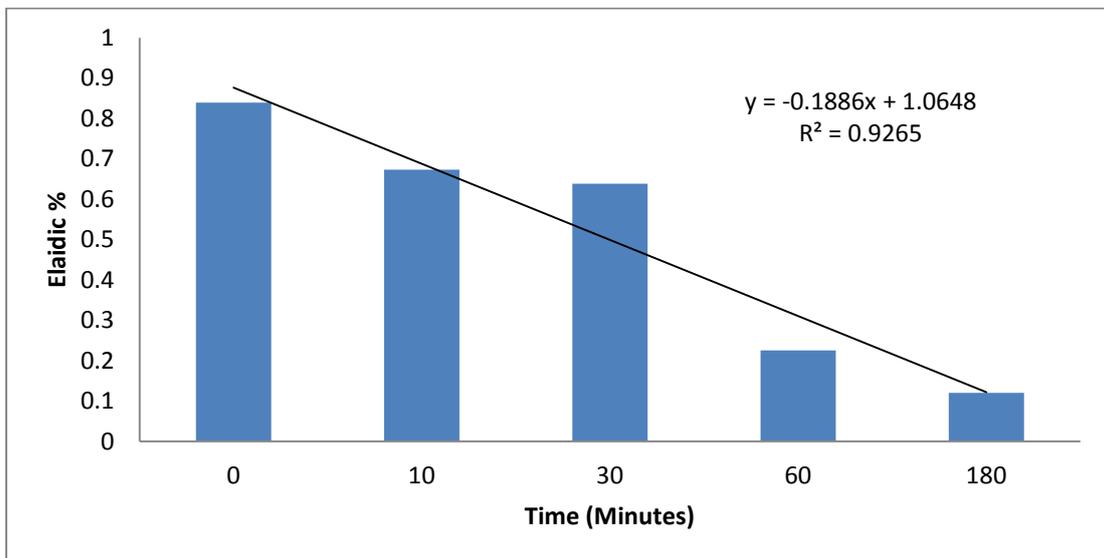


Fig. 3.31:Effect of heating on the percentage of elaidic acid in heat type of ghee sample treated at 190°C

Fig. 3.32 show the percentage of elaidic acid in heat type of ghee treated at 250°C for different time intervals, as show in fig, the results was oscillatory, the highest percentage of elaidic acid occurred at 60 min, where the lowest percentage of elaidic acid occurred at 30min, according to the analyses of model summary and parameter estimates and regression analyses in SPSS the results was oscillatory and no relationship exist between the percentage of elaidic acid and time of frying.

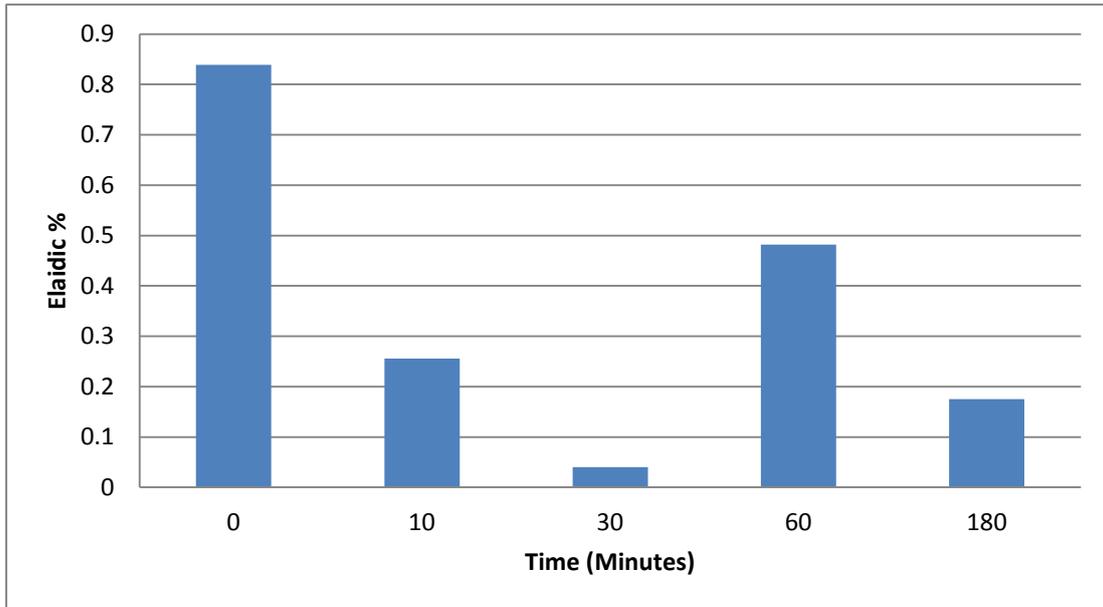


Fig. 3.32: Effect of heating on the percentage of elaidic acid in heat type of ghee sample treated at 250°C

3.2.3.2 Evaluation of elaidic acid in extract type of ghee:

Fig. 3.33 show influence of percentage of elaidic acid in extract type of ghee by applied four different heat treatment (120°C, 150°C, 190°C and 250°C) for different time intervals (10min, 30min, 60min and 180min). In each heat treatment the result show differ value in percentage of acid. the highest influence of percentage of elaidic acid appeared at 150C° for 60min, while the lowest amount of percentage of elaidic showed at 250C° for 30min.

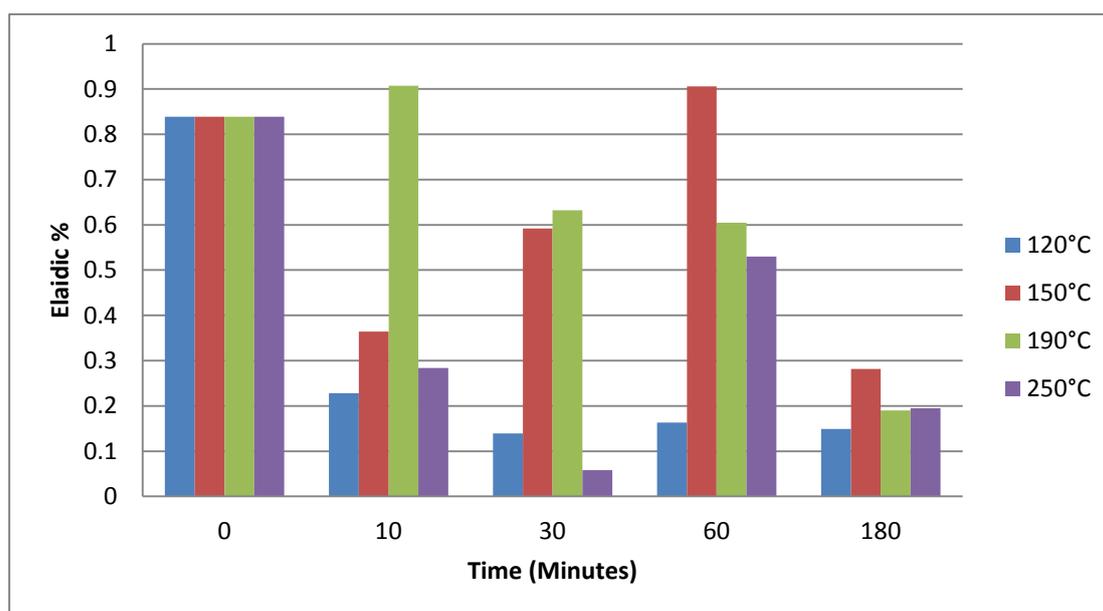


Fig. 3.33: Effect of heating on the percentage of elaidic acid in heat type of ghee sample treated at four different temperature (120°C, 150°C, 190°C and 250°C)

At 120°C for different time intervals, the percentage of elaidic acid in extract type of ghee and according to regression analyses, results show that no significant difference in the percentage of elaidic acid.

According to the analyses of model summary and parameter estimates in SPSS, the elaidic acid in extract type of ghee treated at 150°C showed that there is cubic nonlinear significant relationship between percentage of elaidic and time of heating.

At 190°C for different time intervals the percentage of elaidic acid in extract type of ghee treated exhibited that a negative linear relationship may exist between the percentage of elaidic acid and time of frying. If the time of heating increase at 190°C, the percentage of elaidic acid decrease.

the percentage of elaidic acid in extract type of ghee treated at 250°C for different time intervals show the highest percentage of elaidic acid occurred at 60min, where the lowest percentage of elaidic acid occurred at 30min, however and according to the analyses of model summary and parameter estimates and regression analyses in SPSS the results was oscillatory and no relationship exist between the percentage of elaidic acid and time of frying.

3.2.3.3 Correlation between elaidic acid in heat type and extract type of ghee:

Fig. 3.34 show the relation between elaidic acid in heat type and extract type of ghee treated at 120°C for different time intervals, there are slightly difference in percentage of elaidic acid at 10 min and 60 min.

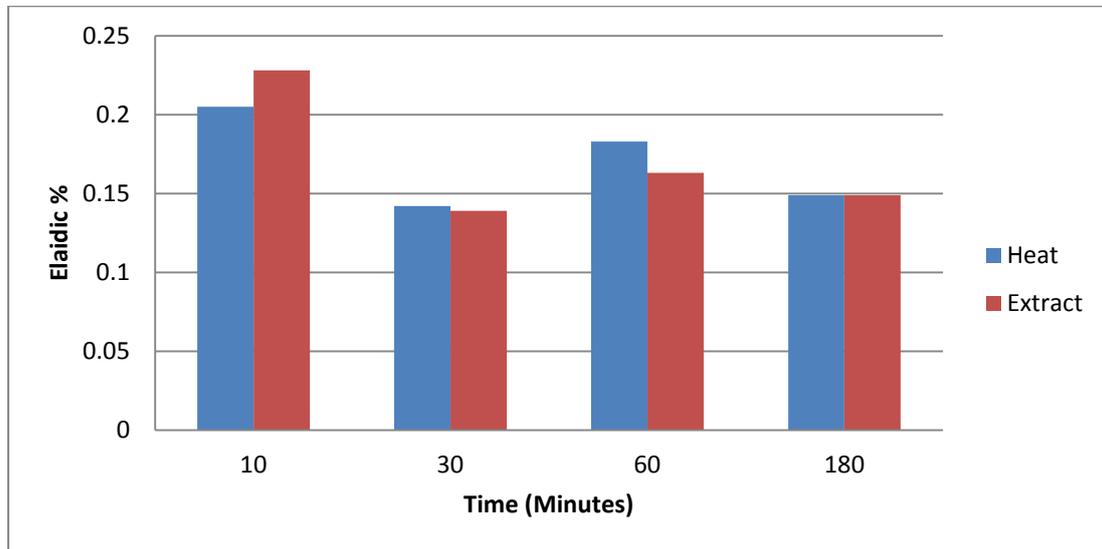


Fig.3.34: Correlation between elaidic acid in heat type and extract type of ghee treated at 120°C

Fig. 3.35 show the relation between elaidic acid in heat type and extract type of ghee treated at 150°C for different time intervals, the percentage of elaidic acid show difference at 30min while slightly difference 60min occurred.

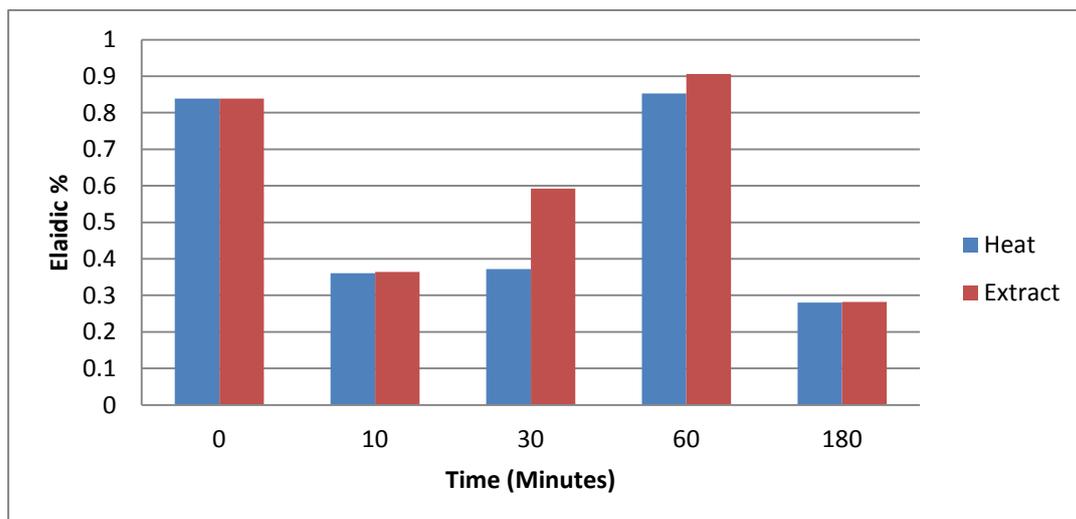


Fig. 3.35: Correlation between elaidic acid in heat type and extract type of ghee treated at 150°C

Fig. 3.36 show the relation between elaidic acid in heat type and extract type of ghee treated at 190°C for different time intervals, the percentage of elaidic acid show difference at 10min and 60min while slightly difference at 180 min occurred.

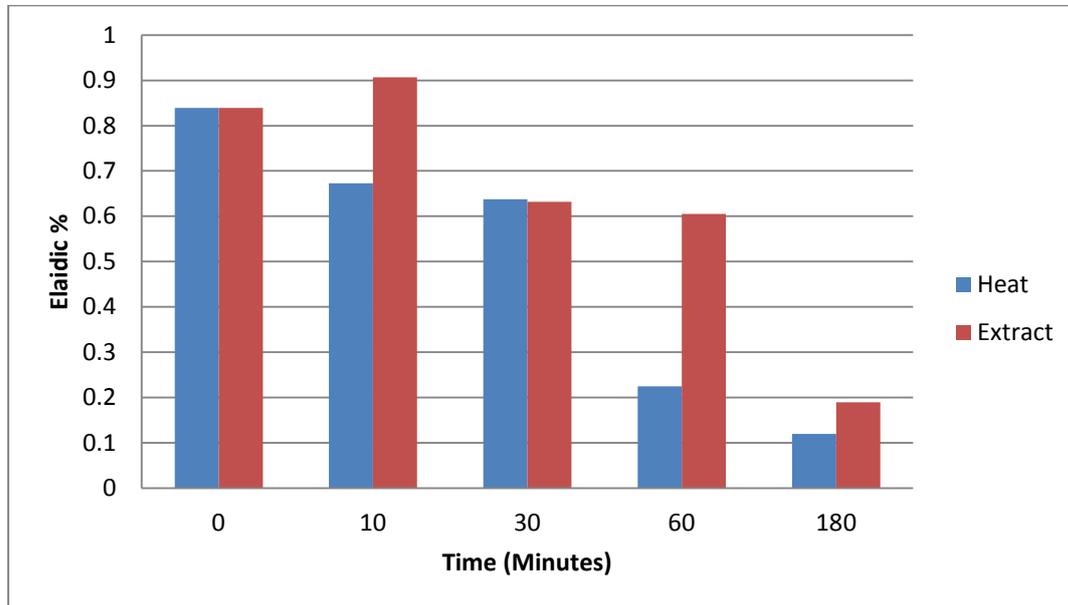


Fig. 3.36: Correlation between elaidic acid in heat type and extract type of ghee treated at 190°C

Fig. 3.37 show the relation between elaidic acid in heat type and extract type of ghee treated at 250°C for different time intervals, the percentage of elaidic acid show slightly difference at all time obtained.

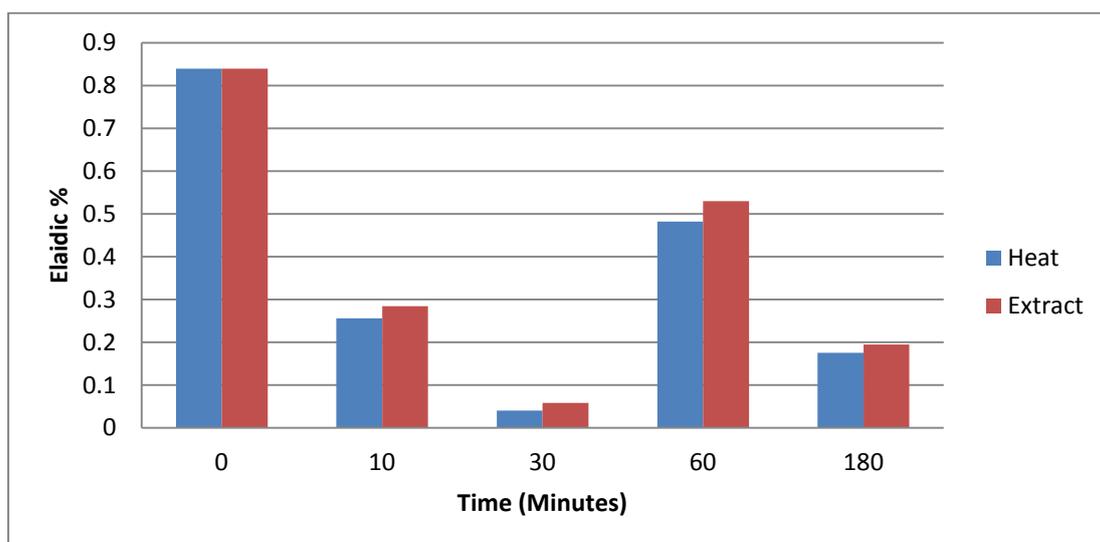


Fig. 3.37: Correlation between elaidic acid in heat type and extract type of ghee treated at 250°C

Fig. 3.38 show the percentage of elaidic acid obtained from heat type and extract type of ghee at all heat treatment applied for different time intervals, the percentage of elaidic are the same in heat type and extract type except some of points appear more influence of extract type of ghee due to influence of heat applied in condensation and soxhselt heat in extraction process.

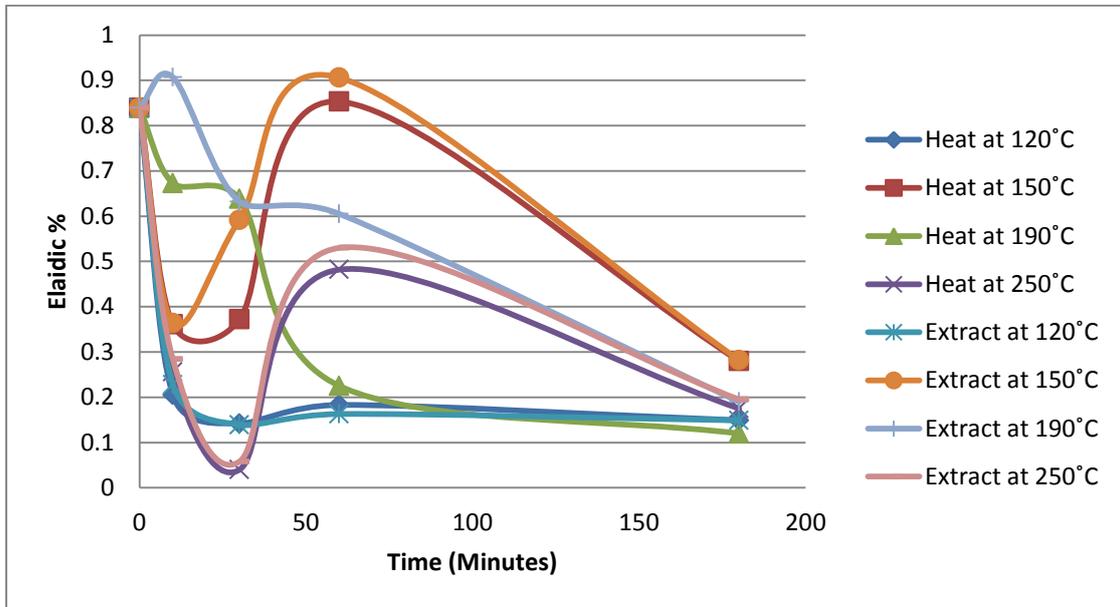


Fig.3.38: Correlation between elaidic acid in heat type and extract type of ghee treated at four different temperature (120°C, 150°C, 190°C and 250°C)

3.2.3.4 Correlation between elaidic acid and oleic acid in heat type and extract type of ghee:

Fig. 3.39 show opposite relation between elaidic acid and oleic acid in heat type of ghee treated at 120°C for different time intervals, if the oleic acid increase, the elaidic acid decrease and opposite.

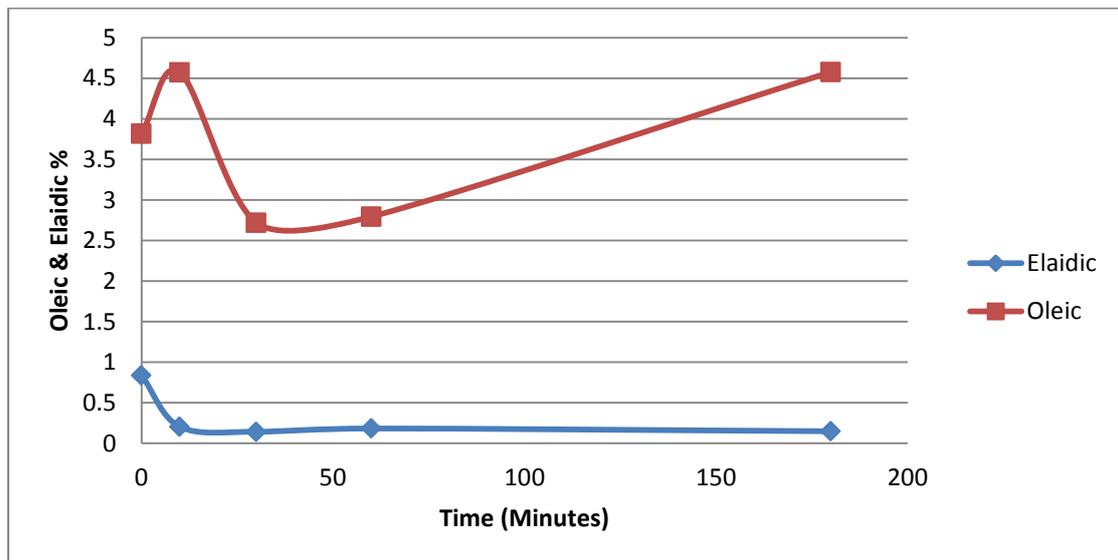


Fig.3.39: Correlation between elaidic acid and oleic acid in heat type of ghee treated at 120°C

Fig. 3.40 show opposite relation between elaidic acid and oleic acid in extract type of ghee treated at 120°C for different time intervals, if the oleic acid increase, the elaidic acid decrease and opposite.

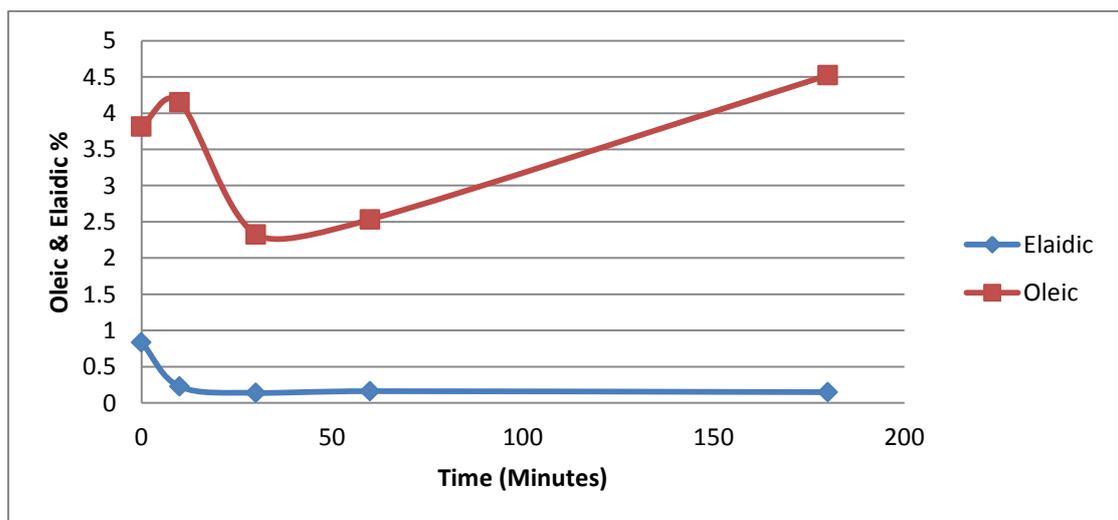


Fig.3.40: Correlation between elaidic acid and oleic acid in extract type of ghee treated at 120°C

Fig. 3.41 show opposite correlation between elaidic acid and oleic acid in heat type of ghee treated at 150°C for different time intervals, if the oleic increase, the elaidic decrease and opposite.

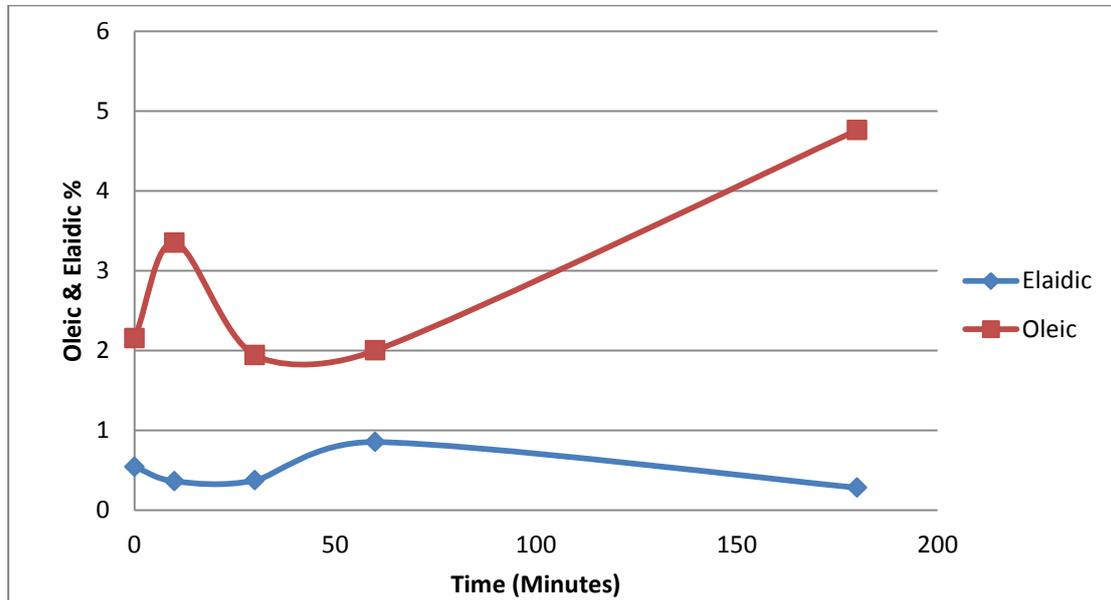


Fig. 3.41: Correlation between elaidic acid and oleic acid in heat type of ghee treated at 150°C

Fig. 3.42 show opposite relation between elaidic acid and oleic acid in extract type of ghee treated at 150°C for different time intervals, if the oleic increase, the elaidic decrease and opposite.

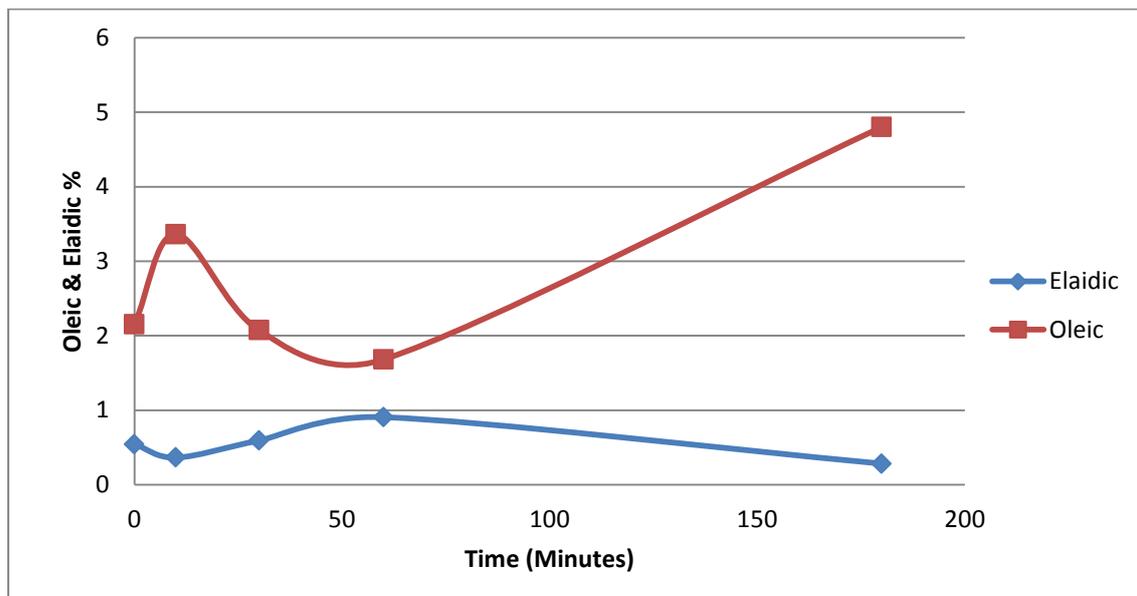


Fig. 3.42: Correlation between oleic elaidic acid and oleic acid in extract type of ghee treated at 150°C

Fig. 3.43 show opposite correlation between elaidic acid and oleic acid in heat type of ghee treated at 190°C for different time intervals, if the oleic increase, the elaidic decrease and opposite.

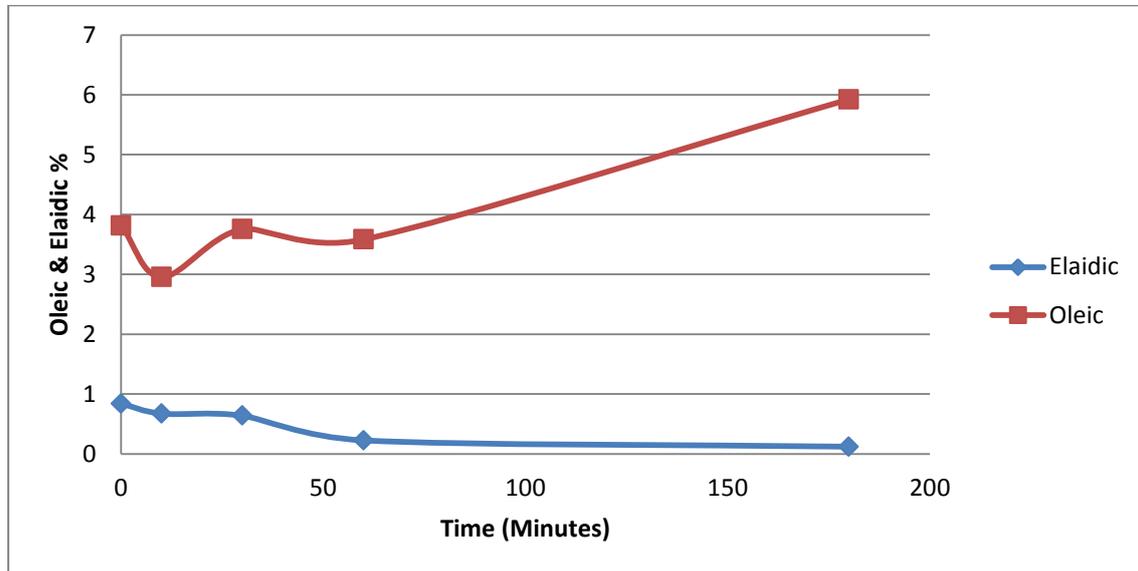


Fig.3.43: Correlation between elaidic acid and oleic acid in heat type of ghee treated at 190°C

Fig. 3.44 show opposite correlation between elaidic acid and oleic acid in extract type of ghee treated at 190°C for different time intervals, if the oleic increase, the elaidic decrease and opposite.

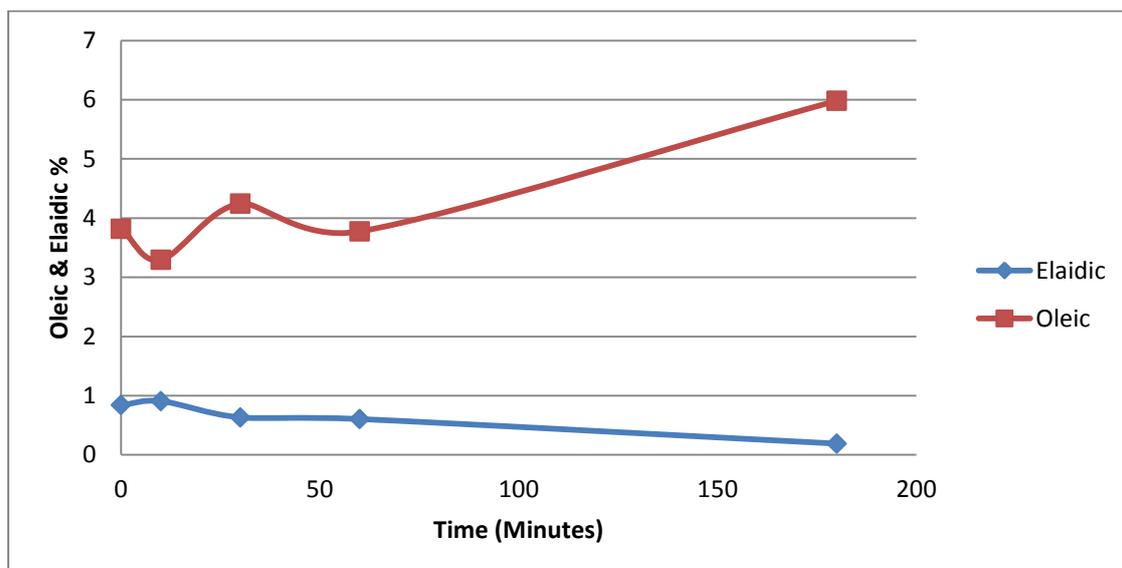


Fig. 3.44: Correlation between elaidic acid and oleic acid in extract type of ghee treated at 190°C

Fig. 3.45 show opposite correlation between elaidic acid and oleic acid in heat type of ghee treated at 250°C for different time intervals, if the oleic increase, the elaidic decrease and opposite.

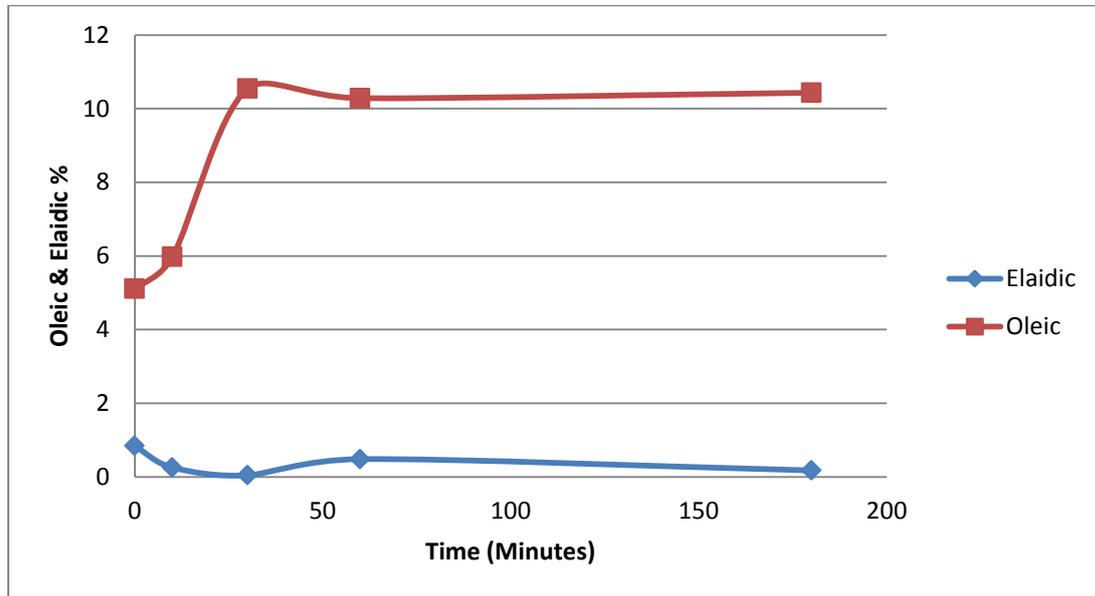


Fig. 3.45: Correlation between elaidic acid and oleic acid in heat type of ghee treated at 250°C

Fig. 3.46 show opposite correlation between elaidic acid and oleic acid in extract type of ghee treated at 250°C for different time intervals, if the oleic increase, the elaidic decrease and opposite.

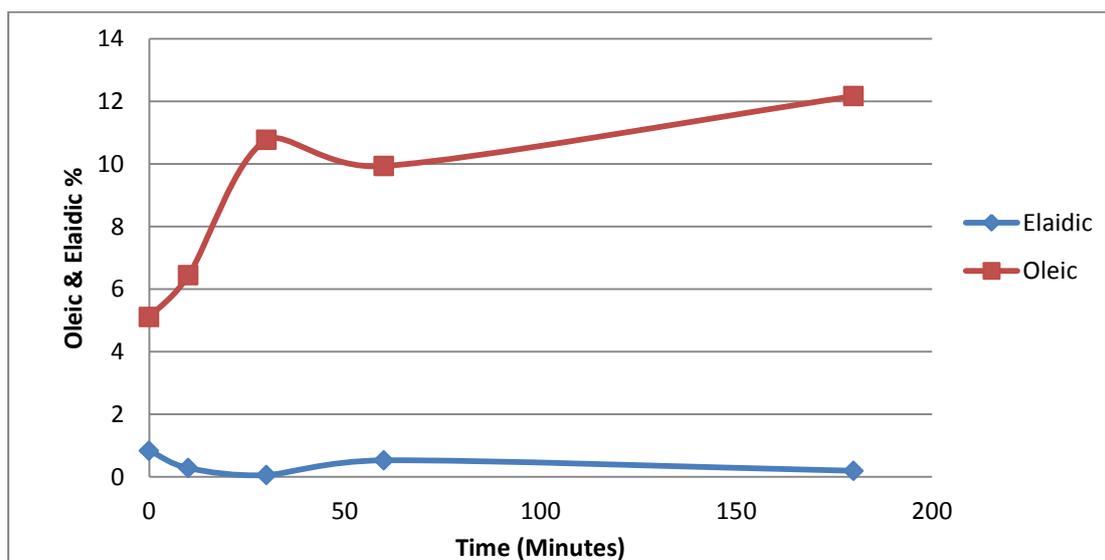


Fig. 3.46: Correlation between elaidic acid and oleic acid in extract type of ghee treated at 250°C

3.2.4 Evaluation of elaidic acid in liquid oils:

Tow liquid oils chosen in this study are olive and corn oil, the obtained result show that there is no elaidic acid appeared at four different heat treatment applied (120°C,150°C,190°C and 250°C) for different time intervals (10 minutes, 30 minutes, 60 minutes and 180 minutes) in each oil.

Elaidic acid did not appear in olive oil and corn oil at all heat treatments applied, but the corn oil used in brostad chicken Restaurant and the severe heating of olive oil and corn oil that treated at 220°C for 24 hours appeared elaidic acid in all samples analyses. In this research, we evaluated the elaidic type of trans acid, however another type of industrial trans may be appeared such as non-conjugated linoleic acid.

Juhee Song, et al. (2015), showed that Among cooking methods, stir-frying increased trans fat in corn oil whereas baking, pan-frying, and frying procedures did not make changes in trans fat content compared to untreated corn oils.

Eunckand David(2006) showed that the trans formation from cis configuration in unsaturated lipids is inevitable steps during autoxidation. That is mean our heat treatment did not reach oxidation in both olive oil and corn oil. They also showed that Chlorophylls and phenolic compounds decrease the autoxidation of oil, and carotenoids, tocopherols, and phospholipids demonstrate both antioxidant and prooxidant activity depending on the oil system. In photosensitized oxidation chlorophyll acts as a photosensitizer for the formation of $^1\text{O}_2$; however, carotenoids and tocopherols decrease the oxidation through $^1\text{O}_2$ quenching.

Diraman and Hisil (2002) showed that elaidic acid found as 1.36% in corn oil sample that exposed to microwave radiation at 700 Watt for 15 min.

Gamel, et al. (1999) showed that elaidic acid and linoleic were increased with frying time increased in olive oil and sunflower oil treated at 180°C with 24 hr. they also showed that the rosemary additives (extracts) alone and in combination with BHA decreased the level of trans fatty acids (mainly elaidic acid), while the addition of olive vegetable water did not have any effect.

Chapter Four:

Conclusions and Recommendations

On the basis of result and discussion the following conclusions and Recommendations can be drawn:

4.1 Conclusions:

- This study demonstrated that the major trans (elaidic acid) and cis (oleic acid) can be identified and qualified by High Performance Liquid Chromatography (HPLC) with UV detector (wavelength 205 nm) and column (C18 (150 mm long x 4.0 mm inner diameter)).
- The results of different margarine and ghee analyzed, all of results had elaidic acid before and after heat treatment while corn oil and olive oil were free from elaidic before and after heat treatments.
- The results of severe heating of liquid oils (olive and corn oil) in control samples showed elaidic acid, while severe heating of olive oil had more elaidic acid than severe heating of corn oil.
- Broasted oils in control samples showed elaidic acid due to pressure in broasting in addition to heating.
- The results obtained from control samples indicated that, in Palestine, elaidic acid is widely present in our food at low levels. Therefore, it is important to assess the content of it in our food.
- Five factors affect the formation of elaidic acid (type of fat and oil, oleic%, temperature of heating, time of heating and extract type) with $R^2 = 0.424$ while the remaining variation can be explained by other unstudied factors.

- The most effective factor affecting elaidic acid formation was the type of oil, oleic %, temperature treatment, time of heating and the lowest effective factor was the extract type.
- margarine have elaidic% higher than ghee, while olive oil and corn oil not show any percentage of elaidic acid.
- The highest influence on the percentage of elaidic acid in heat and extract type of margarine occurred at 250°C.
- The percentage of elaidic acid in margarine and ghee did not affected by treated at 120°C at all different time intervals.
- The percentage of elaidic acid in margarine treated at 150°C, 190°C and 250°C showed that there is cubic nonlinear significant relationship between percentage of elaidic acid and time of heating.
- The percentage of elaidic acid in ghee treated at 150°C showed that there is cubic nonlinear significant relationship between percentage of elaidic acid and time of heating.
- The percentage of elaidic acid in ghee treated at 190°C showed that there is a negative linear relationship between percentage of elaidic acid and time of heating.
- The percentage of elaidic acid in ghee treated at 250°C showed oscillatory results between percentage of elaidic acid and time of heating.
- The percentage of elaidic acid in liquid oil (olive and corn) treated at 120°C, 150°C, 190°C and 250°C didn't show any affect in percentage of elaidic acid with time of heating.
- The correlation between heat type and extract type in all fat and oil samples treated showed that no significant difference in the percentage of elaidic acid except slightly difference in some points that showed more influence of extract type, its due to effects of condensation and extract heat during extraction process.
- The correlation between oleic acid and elaidic acid in all fat and oil samples treated under the same condition showed the opposite relation between oleic acid and elaidic acid except margarine samples treated for 180 mins at (150°C, 190°C and 250°C).

4.2 Recommendations

4.2.1 To the Palestinians' institution:

- Margarine and Ghee had Elaidic trans so It shall to issued standard deals with the amount of trans fats occurring with Margarine and Ghee used in local market in Palestinian areas.
- Recommended to Fat and oil factory to modification processing in margarine and Ghee making.

4.2.2 To the consumer:

- Keep margarine and Ghee consumption as low as possible by limiting foods that contain them or to by used them during food processing.
- Cook and bake with vegetable oils (such as Corn oil) instead of solid fats (margarine and Ghee).

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العوامل المؤثرة على تشكيل الدهن المتحول (الترانس) في الأغذية المحتوية على الدهون غير

المشبعة أثناء المعاملات الحرارية.

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المشرف الرئيس: د. إبراهيم عفانة.

المشرف المساعد: د. جهاد عبادي.

ملخص

تحتوي الأغذية التي نتأولها على الدهون المتحولة (الترانس) والموجودة في أغذيتنا إما طبيعياً أو صناعياً، ويمكن تمييز الدهون المتحولة الصناعية على أنها تحتوي على مجموعة المثيلين بين الروابط الغير مشبعة. وتختلف الدهون المتحولة الطبيعية في الخصائص الفسيولوجية والحيوية عن الدهون المتحولة الصناعية والتي جاءت من صنع من خلال عدة عوامل مثل الهدرجة أو التسخين. وتأتي خطورة الدهون المتحولة الصناعية على أنها تعمل على تصلب الشرايين وأمراض القلب. وتشمل الدهون المتحولة إما على رابطة أحادية غير مشبعة أو أكثر من رابطة في الدهون غير المشبعة.

تهدف هذا الدراسة إلى تحديد العوامل التي تؤثر في تشكيل الأحماض الدهنية المتحولة وذلك من خلال العمليات الحرارية والتي يتعرض لها غذائنا والذي يحتوي على الدهون غير المشبعة. حيث تم تقسيم البحث إلى قسمين رئيسيتين: احتوى القسم الأول على عينات مرجعية تم اختيارها ودراستها بشكل مستقل وذلك لمعرفة ما إذا كانت الزيوت والدهون الموجودة بالسوق الفلسطيني تحتوي على الدهون المتحولة (الترانس) أم لا؟. واحتوى القسم الثاني على عينات من أربع أنواع من الدهون والزيوت (اثان منها دهون صلبة وهما المرجرين والسمنة واثان منها زيوت سائلة وهي زيت الزيتون وزيت الذرة). حيث تم معاملة كل دهن أو زيت على أربع درجات حرارية مختلفة ولمدة أربع أوقات مختلفة لكل معاملة حرارية. وتم الحصول على نوعين من العينات وهما العينة الأولى والتي تعرضت للمعاملة المطلوبة وتم سحبها من المقلاة مباشرة والعينة الثانية والتي تحتوي على الزيت المستخرج من البطاطا المقلية، هذا وتم تخزين هذه العينات في علب زجاجية معتمه سعتها 100 مل لحين تحليلها. وتم تحديد النسبة المئوية لكل من الحمض الدهني أليديك (ترانس) والحمض الدهني أولييك (سس) بواسطة جهاز HPLC مع الجهاز الكاشف UV. وتم بعد ذلك دراسة العلاقة بين الحمض الدهني أليديك (ترانس) مع الحمض الدهني أولييك، بالإضافة إلى دراسة العلاقة ما بين الزيت التي تم تسخينه وسحبه من المقلاة مباشرة مع الزيت الذي يتم تسخينه واستخراجه من البطاطا المقلية.

بعد تحليل النتائج التي تم الحصول عليها من العينات المرجعية والبالغة 38 عينة على جهاز HPLC وباستخدام الكاشف UV، أظهرت النتائج بعد تحليل 9 عينات من المرجرين احتوائه على حمض أليديك بنسبة (0.75%) وبمدى (0% - 1.41%)، كما أظهرت النتائج احتواء السمن بعد تحليل 5 عينات على حمض أليديك بنسبة (0.38%) وبمدى (0% - 1.09%)، كما أظهرت النتائج احتواء زيت الذرة والذي تعرض للتسخين الحاد وبعد فحص 4 عينات على حمض أليديك بنسبة (0.78%) وبمدى (0.36% - 1.32%)، كما أظهرت النتائج احتواء زيت الزيتون والذي تعرض للتسخين الحاد وبعد فحص 3 عينات على حمض أليديك بنسبة (2.63%) وبمدى (0.58% - 4.17%)، كما أظهرت النتائج احتواء عينات الزيت وعددها 4 عينات والتي جمعت من مطاعم البروستيد من منطقة أبو ديس على حمض أليديك بنسبة (0.85%) وبمدى (0% - 1.77%)، كما أظهرت النتائج احتواء عينات الزيت وعددها 5 عينات والتي جمعت من مطاعم الروستيد من منطقة رام الله على حمض أليديك بنسبة (0.32%) وبمدى (0% - 1.00%)، وأخيرا وبعد تحليل 5 عينات لزيت الزيتون الخام و4 عينات لزيت الذرة الخام لم تظهر أي نسبة مئوية لهذه الزيوت على حمض أليديك.

أظهرت النتائج التي تم الحصول عليها من عينات الدهون والزيوت تأثر الحمض الدهني أليديك بالعوامل الخمسة والتي تم اختيارها للدراسة وهي (نوع الدهون والزيوت، درجة الحرارة، زمن التسخين، والاستخراج، النسبة المئوية للحمض الدهني لأولييك). وكانت نتيجة معامل التحديد $R^2 = (0.424)$ هذا يعني أن العوامل الخمسة والتي تم اختيارها تؤثر بنسبة 42.4% على تشكيل الحمض أليديك وان هناك عوامل أخرى تؤثر بنسبة 67.4% لم يتم اختيارها في هذه الدراسة.

توصلت الدراسة إلى أن أكثر العوامل الخمسة تأثيرا على تشكيل الحمض الدهني أليديك كان لنوع الدهون والزيوت، نسبة الحمض الدهني لأولييك، درجة الحرارة، الوقت واقل العوامل تأثيرا هو نوع العينة الاستخراج.

أظهرت النتائج التي تم الحصول عليها في المرجرين عدم تأثر حمض أليديك عند معاملته على درجة 120 درجة مئوية ولمدة أوقات مختلفة، أما عند تعرضه لدرجات 150 درجة مئوية، 190 درجة مئوية و 250 درجة مئوية ولأوقات مختلفة أظهرت النتائج علاقة تكعيبية غير خطية بين النسبة المئوية لحمض أليديك ووقت التسخين.

أظهرت النتائج التي تم الحصول عليها في السمن المهدرج عدم تأثر حمض أليديك عند تعرضه لدرجة 120 درجة مئوية ولأوقات مختلفة. أما عند تعرضه لدرجة 150 درجة مئوية ولأوقات مختلفة أظهرت

النتائج أن هناك علاقة تكعيبية بين نسبة حمض أليدك ووقت التسخين. وعند تعرضه لدرجة 190 درجة مئوية ولأوقات مختلفة أظهرت النتائج إمكانية وجود علاقة خطية سالبة بين نسبة حمض أليدك ووقت التسخين. أما عند تعرضه لدرجة 250 درجة مئوية ولأوقات مختلفة أظهرت علاقة متذبذبة في نسبة حمض أليدك ووقت التسخين.

أظهرت النتائج أن العينات التي تم استخراجها من البطاطا المقلية من نفس الزيت وعينات الزيت التي تم أخذها مباشرة من المقلية تحتوي على نفس نسبة حمض الأليدك إلا في بعض النقاط التي أظهرت تأثير أكبر للعينات التي تم استخراجها وذلك بسبب تعرضها للحرارة أثناء عملية الاستخراج. أظهرت النتائج وجود علاقة عكسية بين حمض أوليك وحمض أليدك في كل من المرجرين والسمن المهرج والذان تعرضا لمعاملات حرارية مختلفة ولأوقات مختلفة باستثناء المرجرين وعند تعرضه لمدة 180 دقيقة ولدرجات حرارة مئوية (150، 190 و 250).