Studies on the Physico-chemical Properties and Pharmacology of Heated Edible Oils

by

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A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in the Department of Chemistry

Khulna University of Engineering & Technology
Khulna-9203, Bangladesh

December, 2016

Declaration

This is to certify that the thesis work entitled "**Studies on the Physico-chemical Properties and Pharmacology of Heated Edible Oils**" has been carried out by Md. Raju Ahmad in the Department of Chemistry, Khulna University of Engineering & Technology, Khulna, Bangladesh. The above thesis work or any part of this work has not been submitted anywhere for the award of any degree or diploma.

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ACKNOWLEDGEMENT

At first, I would like to acknowledge my debt of honor to the Almighty Allah, for his enabling me the strength and opportunity to accomplish this research work successfully.

I would like to express my best indebtedness and deepest thanks to my honorable supervisor **Prof. Dr. Mohammad Hasan Morshed,** Department of Chemistry, Khulna University of Engineering & Technology, Khulna, Bangladesh, for his enthusiastic guidance, imperative supervision, co-operation, invaluable suggestions and constant encouragement throughout this research work.

I am pleased to express my gratitude to **Prof. Dr. Md. Abdul Motin,** Head, Department of Chemistry, Khulna University of Engineering & Technology, Khulna, Bangladesh, for providing me all necessary laboratory requirements and facilities, expert guidance, suggestions and never ending inspiration throughout the research.

I should take this opportunity to express my sincere thanks to **Prof. Dr. Mohammad Abu Yousuf**, Department of Chemistry, Khulna University of Engineering & Technology, Khulna, Bangladesh, for his encouragement, expert guidance and co-operation throughout this research work.

I am highly grateful and greatly indebted to **Prof. Dr. Apurba Kumar Roy**, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi, Bangladesh, for his kind permission of Lab facilities and expert guidance.

I am extremely thankful to **Prof. Dr. Md. Saiful Islam**, Department of Chemistry, Shahjalal University of Science and Technology, Sylhet, Bangladesh, for helping me to analyze the IR spectra.

I would like to express my special thanks to the **Director**, Islami Bank Hospital, Khulna, Bnagladesh, for his kind permission of lab facilities.

I wish to pay also my profound gratitude to **Dr. Md Shawkat Ali Laskar**, Managing Director, CT Imaging Center, Khulna, Bangladesh, for helping me to analyze histopathological section of organs.

I am also obliged to **all teachers** of this department for their valuable advice and moral support. I am also expressing my thanks to **all the stuffs** of this department.

I wish to express my heartful obligation to my beloved and respected **parents,** my **brothers** and **sister** for their moral and financial support, enthusiastic inspiration, constant encouragement and never ending affection in my education.

Lastly I also express my heartful thanks to all my friends and class fellows specially **Md. Abdur Rahim, Prodip Kumar Das** and **Maksuda Khanam.** All of them helped me according to their ability.

Md. Raju Ahmad

Abstract

The present study was carried out to evaluate the physico-chemical properties like iodine value, acid value and compare FT-IR spectra of unheated and heated edible oils, the effects of consumption of heated edible oils diet on blood lipid profile, biochemical parameters and hematological profile of rabbits. On the other hand, the present study aimed to observe the histological change in rabbit liver, kidney, heart, lung, brain and spleen tissues after feeding heated edible oils. The experimental data showed that the lowest iodine value and the highest acid value were observed in 20 hours heated palm oil (18.02±0.05), (7.01±0.03) and soybean oil (83.06±0.08), (5.68±0.05) respectively. The spectra of unheated and 2, 10, 20 hours heated palm and soybean oils showed very similar FT-IR spectra in this study. The body weight of experimental rabbit 20 hours heated palm oil diet group P3 (821.57±3.29 gm) and similar soybean oil diet group S3 (730.50±2.20 gm) were higher than that of control group. Total white blood cell (WBC) of 2 hours heated palm oil diet group P1 ($9.5x10^3$±$0.16x10^3$ blood cell/µl), red blood cell (RBC) of P3 group (6.58±0.03 m/ µl), platelet count (PC) of 10 hours heated palm oil diet group P2 (593±2.44 thousand/µl) were higher than that of fresh normal rabbits. Creatinine of S3 group (1.1±0.08 mg/dl), serum glutamic pyruvic transminase (SGPT) of P3 group (81±2.16 U/L), serum glutamic oxaloacetic transminase (SGOT) of P3 group (147±0.82 U/L) and uric acid of P2 group (1.9±0.08 mg/dl) were higher than that of control group. The total cholesterol (TC) of P3 group (158±2.16 mg/dl), high density lipoprotein (HDL) of P3 group (57±1.63 mg/dl), low density lipoprotein (LDL) of P3 group (81±1.41 mg/dl) and triglyceride (TG) of P3 group (176±0.82 mg/dl) were found higher than that of untreated rabbits. Histopathological study of liver, kidney, heart, lung, brain and spleen of the heated edible oil treated rabbits showed abnormalities as compared to control group organs. Consumption of heated edibles oil may occur infection, stress, allergy, hepatic damage, anemia, vascular disease, renal system damage, liver cirrhosis, pulmonary infarction, coronary artery disease of the consumers. Liver, heart, kidney, lung, brain, spleen and muscles may be damaged by chronic consumption of heated edible oils.

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Chapter I

Introduction

1.1 Edible oil

Edible oils are a source of lipids and triacylglycerol. Edible oils known as vegetable oils that used for cooking. They come from various plants and seeds like soybean, sunflower, palm etc. Edible oils play a vital role in human life. Edible oils are vital constituent of our daily diet, which provide energy, essential fatty acids and serve as a carrier of fat soluble vitamins [1] They are liquid at room temperature [2]. Edible oils are sometimes added during the preparation of processed foods. They are also used to fry foods and to make salad dressing. Although some oils that contain saturated fat, such as palm oil [3]. There is a wide variety of edible oils from plant sources such as palm oil, soybean oil. Edible Oil can be flavoured with aromatic foodstuffs such as herbs, chillies or garlic. Edible Oils derived from plants are rich in unsaturated fatty acids such as linoleic, alpha linolenic and arachidonic. Unsaturated fatty acids can prevent occurrence arteriosclerosis (narrowing of blood vessels). However, besides its function is beneficial for health, oils are often also causes various health problems it can cause various problems. This assumption is not entirely wrong, and not entirely correct. Edible oil is oil that is used as a medium for frying. Edible oil is important food ingredient. Oil calories in addition to providing the greatest value among other nutrients, oil can provide a savory flavor, texture and appearance of food becomes more attractive and the surface which dry.

1.2 Palm oil

Palm oil is one of the common edible oil. It derived from the mesocarp of the fruits of the palm tree (*Elaeis guineensis*). Basically, there are two main products of the palm oil industry – palm oil and palm kernel oil. P.E. Ebong et al. [4] described that the two types of palm oil commonly used for culinary purposes are fresh and thermally oxidized oils. Palm oil contains a few highly saturated vegetable fats and is semi-solid at room temperature. On the other

hand, palm oil is plant based product that contains very little cholesterol described by Behrman, E. J. et al. [5]. Palm oil is 50% saturated, while palm kernel oil is 81% saturated respectively reported by Harold McGee [6]. Palm oil mainly contains palmitic acid (16-carbon), which is a saturated fatty acid and dietary intake of saturated fat increases serum cholesterol proposed by Keys-Anderson equation [7]. Palm oil is composed of saturated and unsaturated fatty acids in almost equal proportions. Sambanthamurthi R. et al. [8] reported the approximate concentration of fatty acids remain in palm oil.

Table1.1 : The approximate concentration of fatty acids remains in palm oil

Type of fatty acid	Status	No of carbon atom	Percentage
Myristic acid	saturated	C14	1%
Stearic acid	saturated	C18	5%
Palmitic acid	saturated	C16	44%
Oleic acid	monounsaturated	C18	39%
Linoleic acid	polyunsaturated	C18	11%

1.3 Soybean oil

Soybean oil is one of the most widely consumed cooking oils. It extracted from the seeds of the soybean (*Glycine max*). Soybean oil is mostly used in processed foods, salad dressings and snack foods, and many restaurants used soybean oil for establishment of fast food [9]. Soybean oil's stability to oxidation also is limited by its content of linolenic acid. Recent decades have witnessed numerous attempts to manipulate the fatty acid composition of soybean oil to help it compete better in various uses, but the cost of growing, segregating, and testing special varieties and resistance to genetically modified oils have limited the appeal of these altered varieties [10]. The approximate concentration of fatty acids remains in soybean oil investigated by Lvanov et al. [11].

Table1.2: The approximate concentration of fatty acids remains in soybean oil

Type of fatty acid	Status	No of carbon atom	Percentage
Stearic acid	saturated	C18	4%
Palmitic acid	saturated	C16	10%
Oleic acid	monounsaturated	C18	23%
Alpha-linoleic acid	polyunsaturated	C18	12%
Linoleic acid	polyunsaturated	C18	51%

1.4 Heated edible oil

Edible oils are one of the main constituents of the diet used for cooking purposes [1]. Edible oil is an essential ingredient both in single kitchen and restaurant kitchen. During a cooking process, oil gets heated up to different temperatures. More heating oil produced smoking point. Oil starts to smoke when it is over-heated. If continuing to cook something in the same oil, the food product will taste poorly. Heated oil lost its characteristics. Physical appearance changed when edible oil heated in more time [12]. Choe E and Min DB [13] investigated that more heating oil causes several oxidative and thermal reactions like oxidation, hydrolysis and polymerization and the reactions depend on some factors such as replenishment of fresh oil, frying conditions, original quality of frying oil, food materials and oxygen concentration. During cooking process, oils cause several reactions that changed in the physicochemical, nutritional properties of the oil reported by Che man and Jasvir [14]. However, more heating changes the flavor, stability and quality of the oil by the reactions [13].

Hydro peroxides and aldehydes are formed during the heated oil is heated at high temperatures and preparing food by heated oil are absorbed these toxic products and eventually into the gastrointestinal tract after ingestion [15]. In addition, Leong XF et al. [16] reported that long-term ingestion of foods prepared using more heated oil could severely

compromise one's antioxidant defense network, leading to pathologies such as hypertension, diabetes and vascular inflammation. The tendency of repeated use of more heated oil leads to serious health abnormalities like as histological abnormalities [17-19].

1.5 Physico-chemical Properties

Physico-chemical properties of edible oil means density, boiling point, saponification value (SV), iodine value (IV), acid value (AV) etc. There are many tests designed to measure physical and chemical properties of oils and a part from the purpose of quality control, tests are carried out to find out the origin and properties of a given oil, to know whether the oil will meet the set specifications which include the mentioned qualities and to get technical information and determine the availability of contaminants [20]. These tests give technicians much valuable information to enable them assess given oil by considering the earlier mentioned factors [21]. Physico-chemical parameters of edible oil helped to investigate the quality of oils [22]. The physio-chemical properties of oils are amongst the most important properties that determine the quality and help to describe the present condition of oils [23].The chemical and physical properties of edible oils depend primarily on composition and temperature [20]. Heated oil changes the physico-chemical properties of the oil [1].

1.5.1 Iodine value

Oil is a mixture of triglyceride. Triglycerides are made up of three fatty acids linked to glycerol by fatty acyl esters. Fatty acids are long chain hydrocarbons with carboxyl groups (COOH groups). These fatty acids can be classified into saturated or unsaturated based on the number of double bonds present in the fatty acid. Saturated fatty acids contain only single bond between the carbon atoms and are tend to be solids at room temperature. Unsaturated fatty acids contain double bonds between the carbon atoms in addition to the single bonds present in the fatty acid chain. They are likely to exist as liquids at room temperature. The double bonds present in the naturally occurring unsaturated fats are in the cis form. Trans fatty acids are associated with health problems and cardiovascular diseases.

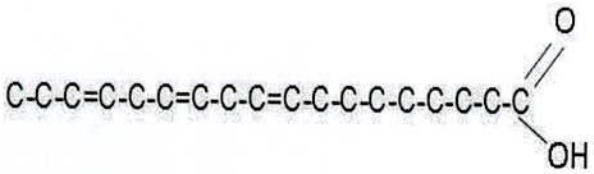


Figure 1.1: Unsaturated fatty acid

Unsaturated fatty acids can be converted into saturated by the process of hydrogenation. Depending upon the degree of unsaturation, the fatty acids can combine with oxygen or halogens to form saturated fatty acids. So it is important to know the extended to which a fatty acid is unsaturated. There are different methods for checking the unsaturation level in fatty acids, one among them is by determining the iodine value of oils. Iodine value or number is the number of grams of iodine consumed by 100g of oil. A higher iodine value indicates a higher degree of unsaturation [24].

The iodine value in chemistry is the mass of iodine in grams that is consumed by 100 grams of a chemical substance. Iodine numbers are often used to determine the amount of unsaturation in fatty acids. This unsaturation is in the form of double bonds, which react with iodine compounds. The higher the iodine number, the more C=C bonds are present in the oil. Oils rich in saturated fatty acids have low iodine numbers, while oils rich in unsaturated fatty acids have high iodine numbers [25]. Test method for measurement of iodine value of fats and oils stipulated in the below quoted standards is generally practiced by Wijs Method using monobasic iodine, however, Hanus Method is demonstrated here using iodine bromide [26-27]. The iodine value of fatty sample is calculated from titration volume of sodium thiosulfate. Fatty acids react with a halogen [iodine] resulting in the addition of the halogen at the C=C double bond site. In this reaction, iodine monobromide reacts with the unsaturated bonds to produce a di-halogenated single bond, of which one carbon has bound an atom of iodine.

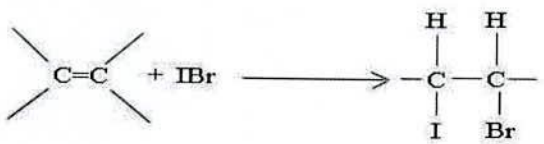


Figure 1.2: Reaction of fatty acid and halogen

After the reaction is complete, the amount of iodine that has reacted is determined by adding a solution of potassium iodide to the reaction product.

$$IBr + KI \text{ ----------> } KBr + I_2$$

This causes the remaining unreacted IBr to form molecular iodine (I_2). The liberated I_2 is then titrated with a standard solution of 0.1N sodium thiosulfate.

$$I_2 + 2\ Na_2S_2O_3 \text{ ----------> } 2\ NaI + Na_2S_4O_6$$

Saturated fatty acids will not give the halogenation reaction. If the iodine number is between 0-70, it will be a fat and if the value exceeds 70 it is an oil. Starch is used as the indicator for this reaction so that the liberated iodine will react with starch to give purple coloured product and thus the endpoint can be observed [24]. Iodine value of edible oil [28-31] are shown in table 1.3

Table 1.3: Iodine value of some edible oil

Edible oil	Iodine value
Palm	44 – 51
Soybean	120 – 136
Mustard	96 – 112
Olive	80 – 88
Coconut	7– 12

1.5.2 Acid value

Acid value is an important indicator of edible oil quality. The acid value is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance [32-33]. The acid value may be overestimated if other acid components are present in the system, *e.g.* amino acids or acid phosphates. The acid value is often a good measure of the breakdown of the triacylglycrols into free fatty acids, which has an adverse effect on the quality of many lipids. It is a relative measure of rancidity

as free fatty acids are normally formed during decomposition of oil glycerides. The value is also expressed as percent of free fatty acids calculated as oleic acid [23]. Acid values are used to measure the extent to which glyceride in the oil has been decomposed by lipase and other actions such as light and heat [34].

The majority of national and international standards for acid value determination in edible oils are based on the acid-base titration techniques in non-aqueous solvents [32-33].

1.6 Pharmacology study

Pharmacology is the branch of medicine and biology concerned with the study of drug action [35] where a drug can be broadly defined as any man-made, natural, or endogenous (from within body) molecule which exerts a biochemical and/or physiological effect on the cell, tissue, organ, or organism (sometimes the word pharmacon is used as a term to encompass these endogenous and exogenous bioactive species). More specifically, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function.

The biochemical parameters which are related to liver functions such as SGOT, SGPT, SALP and Lipid Profile which are related to kidney functions such as serum level of creatinine and uric acid are determined. Serum levels of these parameters change with the pathological changes of these organs. In case of hepatic necrosis, cirrhosis and obstructive jaundice, the serum level of SGOT and SGPT may increase up to 200 IU/L. If a drug possesses any effect on liver and kidney, several pathological changes may occur and ultimately serum level of these parameters will be altered.

Edible oil appears to enhance the recovery from hepatic damage induced by carbon tetrachloride [36]. On the other hand, Long-term ingestion of foods prepared using reheated oil could severely compromise one's antioxidant defense network, leading to pathologies such as hypertension, diabetes and vascular inflammation [16]. Heated oil has effect of aorta. The

ingestion of fresh palm oil may have a protective effect on the aorta but such a protective action may be lost when the palm oil is repeatedly heated [37].

1.7 Aim of the Present Study

Erum Z et al. [1] investigated the effect on the use of edible oil for repeated frying as it ultimately changes the physicochemical, nutritional and sensory properties of the oil. In Bangladesh, maximum restaurants and street food corners use more times heated edible oils. We know the reports on the physico-chemical and pharmacological properties of multi heated oils are limited in Bangladesh. The present study has been undertaken to investigate the physico-chemical properties of multi time heated edible oils and pharmacology of those. The objectives of the research work is the analysis of the physcio-chemical and pharmacological properties of heated edible oils.

The specific aims are:

i) to analyze the physico-chemical properties of the multi heated edible oils

ii) to investigate the effects of heated oils on hematological and biochemical parameters of animal

iii) to observe the organs tissue damaging of animal after feeding the heated oil.

CHAPTER II

Literature Review

2.1 Edible oil and its uses

Lipids and triacylglycerol naturally occur in oils and fats. Their chemical composition contains saturated and unsaturated fatty acids and glycerides. Edible oils are vital constituents of our daily diet, which provide energy, essential fatty acids and serve as a carrier of fat soluble vitamins [1, 38]. Soybean oil (*Glycine max)* has saturated fat, monounsaturated fat and polyunsaturated fat. It is a source of complete protein [39]. Palm oil has saturated fat, specifically monounsaturated oleic acid, tocotrienol, phytosterols and glycolipids and it contains very little cholesterol [40-41]. There are a number of edible oils uses in Bangladesh such as Soybean oil, Palm oil, Mustard oil and so on. Those oils are using in cooking and frying food. Palm oil is most commonly used in hotel and restaurant in Bangladesh for cooking and frying food because palm oil is cheaper than other vegetable oil in Bangladesh [42-44].

2.2 Multi time heated oil and its physico-chemical change

Deep frying is one of the most common methods used for the preparation of food. Multi times frying causes several oxidative and thermal reactions which results the changes in the physico-chemical, nutritional and pharmacological properties of the oil. During deep frying different reactions depend on some factors of fresh oil such as frying condition, original quality of frying oil and decrease in their oxidative stability. During deep fat frying, fats and oils are continuously or repeatedly heated at high temperatures for prolonged periods in the presence of air. This leads to a variety of chemical reactions which can be categorized as hydrolysis, oxidation, and polymerization of the triacylglycerol molecule [45]. During heat treatment, a progressive decrease in unsaturation was observed in all oils by measurement of iodine value [45]. The acid value of the different oils subjected to microwave heating at different time intervals, increased generally by time of heating [46].

2.3 Multi time heated oil and its effect on health

Atmospheric oxygen reacts instantly with lipid and other organic compounds of the oil to cause structural degradation in the oil which leads to loss of quality of food and is harmful to human health. Therefore, it is essential to monitor the quality of oil to avoid the use of degraded oil. Feeding experiments in various animal species and humans have highlighted the beneficial role of fresh palm oil to health which includes reduction in the risk of arterial thrombosis and atherosclerosis, inhibition of cholesterol biosynthesis and platelet aggregation and reduction in blood pressure [47]. Fresh palm oil has no deleterious effects on blood pressure and cardiac tissue but prolonged consumption of repeatedly heated palm oil may result in an increase in blood pressure level with necrosis of cardiac tissue [48]. Izaki Y et al. [49] reported that consumption of oxidized oil caused liver dysfunction. Acceleration of fatty streak formation had been reported in rabbits fed oxidized lipid [50]. Consumption of thermally oxidized palm oil diets had deleterious effects on biochemical indices in rats investigated by Ayodeji Osmund Falade et al. [51]. Chronic consumption of repeatedly heated vegetable oils could be detrimental to health. It was shown to demonstrate genotoxic and preneoplastic change in the rat liver [52]. It also impaired fluid and glucose intestinal absorption in rats [53].

2.4 Effects on hematological and biochemical parameters

Haematological investigations provide information on the general state of blood and the reticulendothelial system. WBC, RBC, Hb, ESR etc. are the hematological parameters. The hematological system is a major organ system. Changes in hematological parameters may occur as a consequence of other systemic diseases [54]. Mesembe et al. [55] had earlier reported that thermally oxidized palm oil diet resulted in anemia because hemoglobin concentrations were decreased. Hussein S. Gumaih [56] reported that the hematological indices were significantly decreased in five and ten times repeated heat of edible oil.

Soybean and palm oil are the leading source of the world supply of oils and fats [57]. Studies have reported that soybean and palm oils reduce both total and LDL cholesterol

concentrations and increase HDL-C concentration [58-60]. Palm oil reduces the blood levels of total cholesterol, triglycerides, LDL-cholesterol, thrombotic eicosanoids (oxygenated metabolites of polyunsaturated omega-6 fatty acid and omega-3 fatty acid) implicated in several pathophysiological processes of the cardiovascular system [61]. In addition, heated palm and soybean oil caused an increase in serum LDL-cholesterol and caused transient changes in lipid profiles [62].

CHAPTER III

Methodology

3.1 Materials, chemicals and experimental animals

Edible oils were collected from local market. Sodium hydroxide (NaOH), Iodine (I_2) , Bromine (Br_2), Sodium thiosulphate ($Na_2S_2O_3$), Potassium iodide (KI), Chloroform ($CHCl_3$), Acetic acid (CH_3COOH) and Ethanol (CH_3CH_2OH) marketed by Sigma-Aldrich, India. All reagents were of analytical grade and obtained from local suppliers. Twenty one healthy and mature rabbits (640–775g) were taken from local market, Khulna, Bangladesh.

3.2 Heating process

A known amount (2 L) of each of refined soybean and palm oils were separately placed in a saucepan and heated by electric heater which contains 1500 watt. The heating process was conducted for 2 hours day^{-1}. This process was repeated for 10 consecutive days. Total continuous heating period was 20 hours. The oil samples were left to cool down then stored at room temperature for chemical analysis and biological evaluation.

3.3 Iodine value (IV) measurement:

Reagents:

1. 0.1 N Sodium thiosulphate ($Na_2S_2O_3$)
2. Chloroform ($CHCl_3$)
3. Hanus solution (I_2 + CH_3COOH+ Br_2)
4. 10% Potassium iodide (KI)
5. Starch

Procedure:

0.5 g each of the oil samples/oil and additives mixture was dissolved in 100 ml of chloroform contained in a 500 ml conical flask. 25 ml of Hanus solution was added into each flask, corked and allowed to stand for 30 minutes in the dark. A blank test was carried out without the samples using exactly the same quantity of chloroform and hanus solution, stoppered, kept for the same length of time. When the reaction was completed, 10 ml of 10% potassium iodide solution and 50ml of distilled water was added to each flask mixed by gentle shaking. The content of the flask was titrated with 0.1 N $Na_2S_2O_3$ to pale yellow before the addition of 2 ml of starch indicator. The titration continued until the blue-black color was completely discharged. The iodine value was calculated from the equation,

$$\text{IV} = \frac{(V_2 - V_1) \times 0.127 \times N}{\text{W}} \times 100$$

Where, V_2 is the quantity of sodium thiosulphate used for blank, V_1 is the quantity of thiosulphate for sample, N is the normality of thiosulphate solution, W is the weight of the oil sample and 127 is the molecular weight of iodine.

3.4 Acid value (AV) measurement:

Reagents:

1. 0.01 N Potassium hydroxide (KOH)
2. Chloroform ($CHCl_3$)
3. Phenolphthalein

Procedure:

2 g each of the different oil samples were weighted and were added to 25 ml of $CHCl_3$ in different conical flasks. Two drops of phenolphthalein was then added to the mixture. A similar titration was performed without the sample to determine the blank and titration was

carried out with 0.01 N potash until the color change occurred in the different conical flasks. The acid value was calculated from the equation,

$$AV = (V_2 - V_1) \times N \times \frac{56.1}{W}$$

Where, V_2 is the volume of titrant (ml) consumed by the oil sample, V_1 is the volume of titrant (ml) consumed by 1 ml of solution at the equivalent point, N is the normality of potassium hydroxide and 56.1 is the molecular weight of KOH, W is the weight of the oil sample in grams.

3.5 Evaluation by FT-IR

FT-IR spectra of oil samples before and after heating were recorded with the help of a Fourier Transform Spectroscopy Model I-R Prestige 21 Shimadzu. It is used to study the saturation and unsaturation composition of heated and unheated oils at room temperature for monitoring the oxidation process in oils. The spectra are recorded from 4000 to 400cm^{-1}, the number of scans being 256 at a resolution of 4cm^{-1}. Scan speed is 0.20cm/s. Data were recorded from department of chemistry, Shahjalal University of Science & Technology.

3.6 Experimental rabbit grouping

Twenty one rabbits were divided equally into seven group; with three rabbits per group and given treatment as follows: (i) control group; (ii) mixed diet with 2 hours heated palm oil (P1); (iii) mixed diet with 10 hours heated palm oil (P2); (iv) mixed diet with 20 hours heated palm oil (P3); (v) mixed diet with 2 hours heated soybean oil (S1); (vi) mixed diet with 10 hours heated soybean oil (S2); (vii) mixed diet with 20 hours heated soybean oil (S3).

3.7 Maintenance of the rabbit and diet

Rabbits kept in stainless steel cages at room temperature. They had access to tap water ad libitum and were administered test diet/day. Control group fed only mixed diet and fresh

water. On the other hand, P1, P2, P3, S1, S2, S3 group fed mixed diet with heated oil respectively. Mixed diet and heated oil ratio were 85:15.

Table 3.1: Diet for the rabbit

Composition	Amount
Grass	25 gm
Vegetables	25 gm
Wheat	10 gm
Rice	25 gm
Sample oil	15 gm

3.8 Study design

The rabbits were maintained for 1 week before to treatment and continued up to the end of the experiment. Treatment duration was 28 days. Body weight was checked every day using the weight measuring machine. After 28 days of study, the rabbits were sacrificed under chloroform anaesthesia, blood were collected and the heart, lungs, liver, spleen, kidney, brain were taken out. Biochemical and hematological profiles were measured and histopathological observation was made in the course of study.

3.9 Monitoring the hematological profiles

The hematological profiles of the experimental rabbit were done to check the hematological abnormalities after administration of the heated oil. For this purpose, the following parameters were observed: total RBC count, total WBC count, differential count of WBC, Platelet count, Hemoglobin estimation, ESR (Erythrocytes Sedimentation Rate). The hematological parameters were performed in the Biochemistry lab, Islami Bank Hospital, Khulna.

3.9.1 Blood sampling

Blood was drawn from the tail veins of all rabbit from individual groups before the commencement of sample oil administration. Blood smears were made on glass slides and stained with leishmen reagent to perform TC, DC and platelet counts. With the help of capillary tubes blood was drawn from each rabbit to estimate the hemoglobin percentage by Van Kampen-Zijlstra's method.

3.10 Monitoring the biochemical profiles

The biochemical profiles of the experimental rabbit were done to check the biochemical abnormalities after administration of the heated oil. For this purpose, the following parameters were observed: Serum glutamate-oxalo-acetate transaminase (SGOT), Serum glutamate-pyruvate transaminase (SGPT), Serum alkaline phosphatase (SALP), Serum Creatinine, Random plasma glucose (RBS), lipid profile [triglyceride, total cholesterol (TC), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL)], Creatinine and Uric acid. The biochemical parameters were performed in the Biochemistry lab, Islami Bank Hospital, Khulna.

3.10.1 Collection of serum

Blood samples were collected from the Jugular vein at fasting state. The blood was collected in plastic centrifuge tubes. These were then allowed to clot at 40°C for 4 hours. After clotting, the blood samples were centrifuged at 4000 ppm for 15 minutes using a WIFUNG centrifuge LABO-50M. The clear straw color serum was then collected in vials with pasteur pipette and stored at -20°C.

3.11 Histopathological procedure

Histopathological study of liver, kidney, heart, lung, brain and spleen were performed to observe any changes in the cellular structures (degradation and regeneration) of the rabbit receiving heated edible oil for 28 consecutive days with respect to control group.

Histopathological examination was performed in the Genetics and histology laboratory, Department of Biotechnology and Genetics Engineering, Rajshahi University, Rajshahi.

3.11.1 Collection and processing of the tissues

The liver, kidney, heart, lungs, brain and spleen were collected from different groups of treated and control rabbits. After sacrificing them at 28th day of observation, the tissues were sliced into pieces, each measuring a few millimeters of thickness. The sliced tissues were then immersed in 10% buffered formalin and used for histopathological study.

3.11.2 Preparation of 10% buffered formalin

Table 3.2: Preparation of 10% buffered formalin

37-40% formalin	100 ml
Distilled water	900 ml
Sodium Phosphate (monobasic)	4 gm
Sodium Phosphate (dibasic)	6.5 gm

The above ingredients were mixed thoroughly, preserved in an air tight container split in plastic jar @ 250ml/jar.

3.11.3 Chemicals required

(i) Alcohol (50%, 70%, 80%, 95% & absolute)
(ii) Chloroform
(iii) Paraffin
(iv) Xylene
(v) Distilled water
(vi) Hematoxylin
(vii) Acid alcohol
(viii) Ammonium water
(ix) Eosin

3.11.4 Histopathological examination procedure

Fixed tissue sections were processed for paraffin-embedding, sectioning [63] staining with Hematoxylin & Eosin stain.

3.11.5 Processing of tissues

The formalin fixed tissues were properly cropped. The tissues were washed overnight under running tap water to remove formalin. The tissues were dehydrated in ascending grades of alcohol.

Table 3.3: Time required for dehydrating tissues

50% alcohol	1 hour
70% alcohol	1 hour
80% alcohol	1 hour
95% alcohol	1 hour

The tissues were cleared in 10% buffered formalin for two changes in chloroform, 1.5 hours in each. The tissues were embedded with melted paraffin wax in two changes, 1.5 hours in each. Paraffin block containing tissue pieces were made using templates. The tissues were sectioned with a microtome at 5 μm thickness, allowed to spread on warm water bath containing a small amount of gelatin & taken on oil and grease free glass slides. The slides were air dried and kept in cool place until staining.

3.11.6 Staining procedure

3.11.6.1 Preparation of Harris hematoxylin solution

Table 3.4: Preparation of Harris hematoxylin solution

Hematoxylin crystals	5 gm.
Alcohol (100%)	50 ml
Ammonium or potassium alum	100 ml
Distilled water	100 ml
Mercuric oxide (red)	2.5 gm

The hematoxylin was dissolved in the alcohol and the alum in the water by the aid of heat. Two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added slowly and reheated to simmer unit it become dark purple. The solution was then removed from heat immediately and plunged the vessel into basin of cold water until become cool. Immediately before using, 2-4 ml of glacial acetic acid was added per 100ml of solution to increase the precision of the nuclear stain. Before using prepared solution was filtered and kept in the dark [63].

3.11.6.2 Preparation of eosin solution (1% stock alcoholic eosin)

Table 3.5: Preparation of eosin solution

Eosin R, water soluble	1gm
Distilled water	20 ml
Dissolved and 95% alcohol	80 ml

Table 3.6: Working eosin solution

Eosin stock solution	1part
Alcohol, 80%	3 parts

Immediately before use 0.5 ml of glacial acetic acid was added to every 100ml of stain & stirred.

3.11.6.3 Routine Hematoxylin & Eosin staining procedure

The tissue sections were deparaffinized in 3 changes of Xyline (3 minutes in each). Rehydrations of the sectioned tissues were done through descending grades of alcohol.

3.11.7 Histopathological studies and photomicrograph

The tissues were examined and photomicrographs were taken in the Genetics and histology laboratory, Department of Biotechnology and Genetics Engineering, Rajshahi University, Rajshahi.

Flow chart for histopathology:

Fixation in neutral buffered formalin

⬇

Trimming (0.5 cm thick)

⬇

Overnight washing (8-12 hours)

⬇

Dehydration in ascending grades of alcohol

(50%, 1 hours; 70%, 1 hours; 80%, 1 hours; 90%, 1 hours; 100%: 2 changes, 1 hours in each)

⬇

Clearing in chloroform/ Xylene

(2 changes, 1 hour and 30 minutes in each)

⬇

Embedding in paraffin (3 hours)

⬇

Blocking with paraffin

⬇

Sectioning on a Microtome

⬇

Staining with Hematoxylin & Eosin

⬇

Examination under light microscope

Flow chart for Hematoxylin & Eosin staining:

95% alcohol for 2 minutes

↓

80% alcohol for 2 minutes

↓

70% alcohol for 2 minutes

↓

Distilled water for 5 minutes

↓

Staining with Harris Hematoxylin for 15 minutes

↓

Washing in running tap water for 15 minutes

↓

Differentiated in acid alcohol: 2 to 4 dip (1 part HCl in 99 parts 70% alcohol)

↓

The tissue sections were then washed in tap water for 5 minutes.

↓

Blue in ammonia water (2-3 quick dips)

↓

The sections were then stained with eosin for 1 minute.

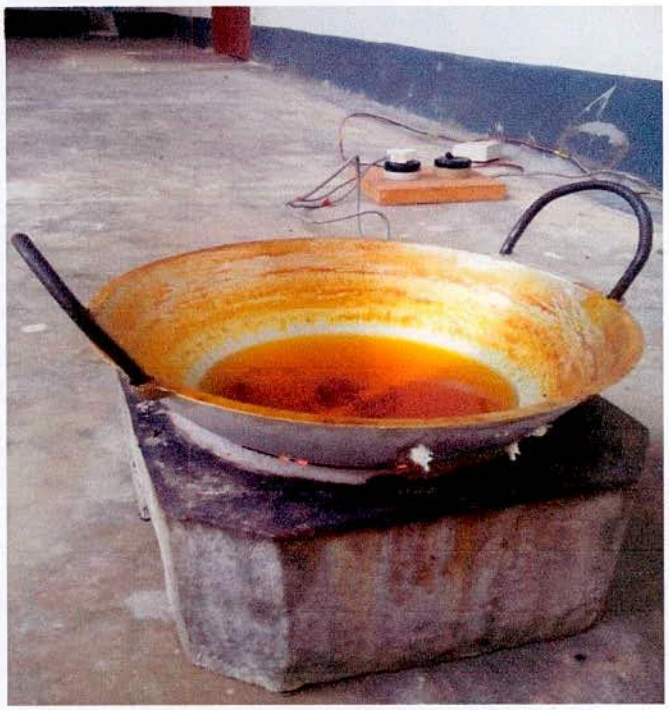
↓

Differentiation & dehydration in 95% alcohol: 3 changes (1 dips in each).

↓

The stained sections were then cleaned by 3 changes in Xyline, 5 minutes in each. Finally the sections were mounted with coverslip using DPX (a mixture of distyrene, a plasticizer, and xylene)

(a)



(b)

Figure 3.1: Heated (a) palm oil and (b) soybean oil

Unheated oil

2 hours heated oil

10 hours heated oil

20 hours heated oil

Figure 3.2: Samples for physical and pharmacological study

Figure 3.3: Experimental rabbits

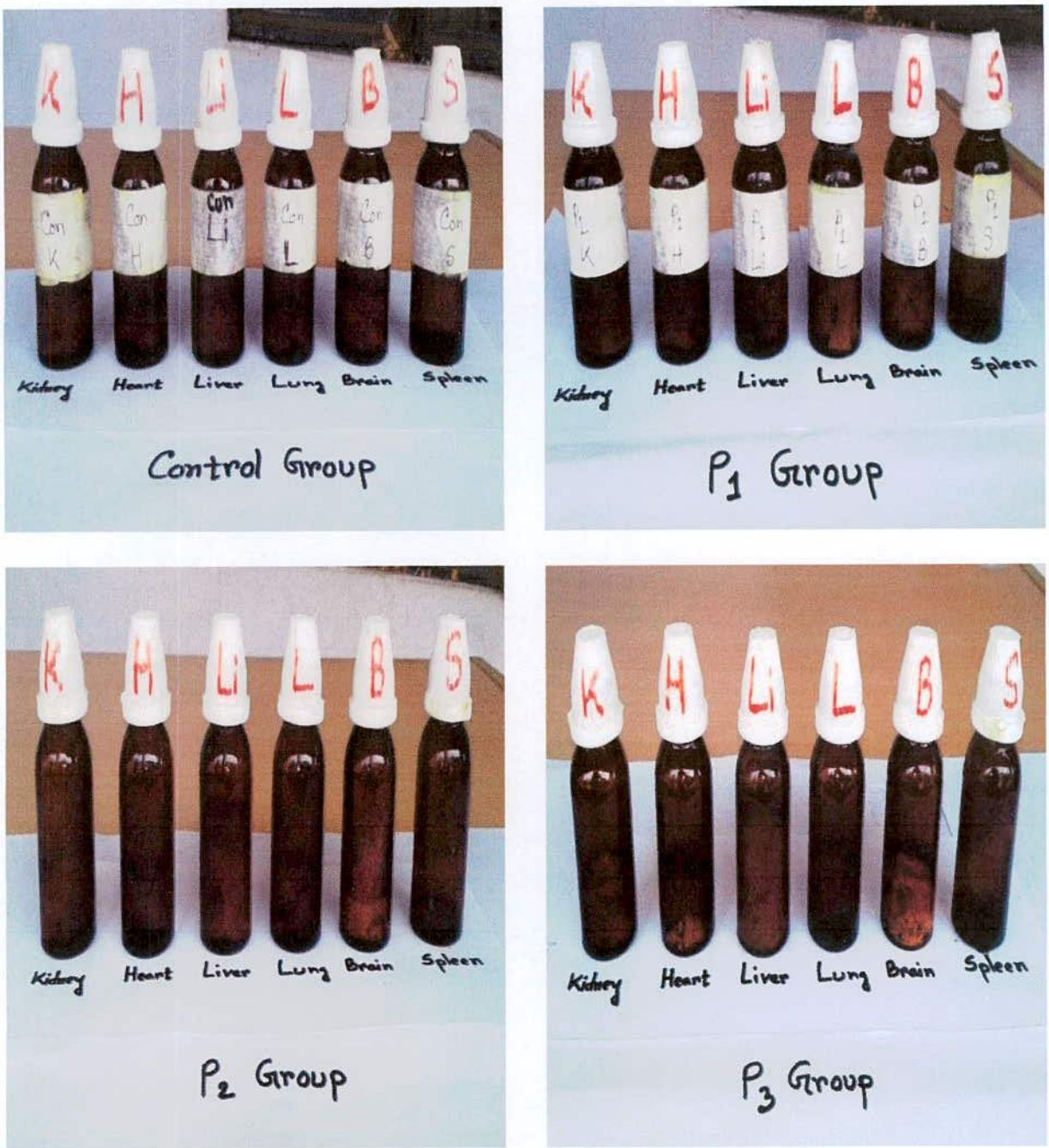


Figure 3.4: Preservation of control and heated palm oils treated rabbit organs

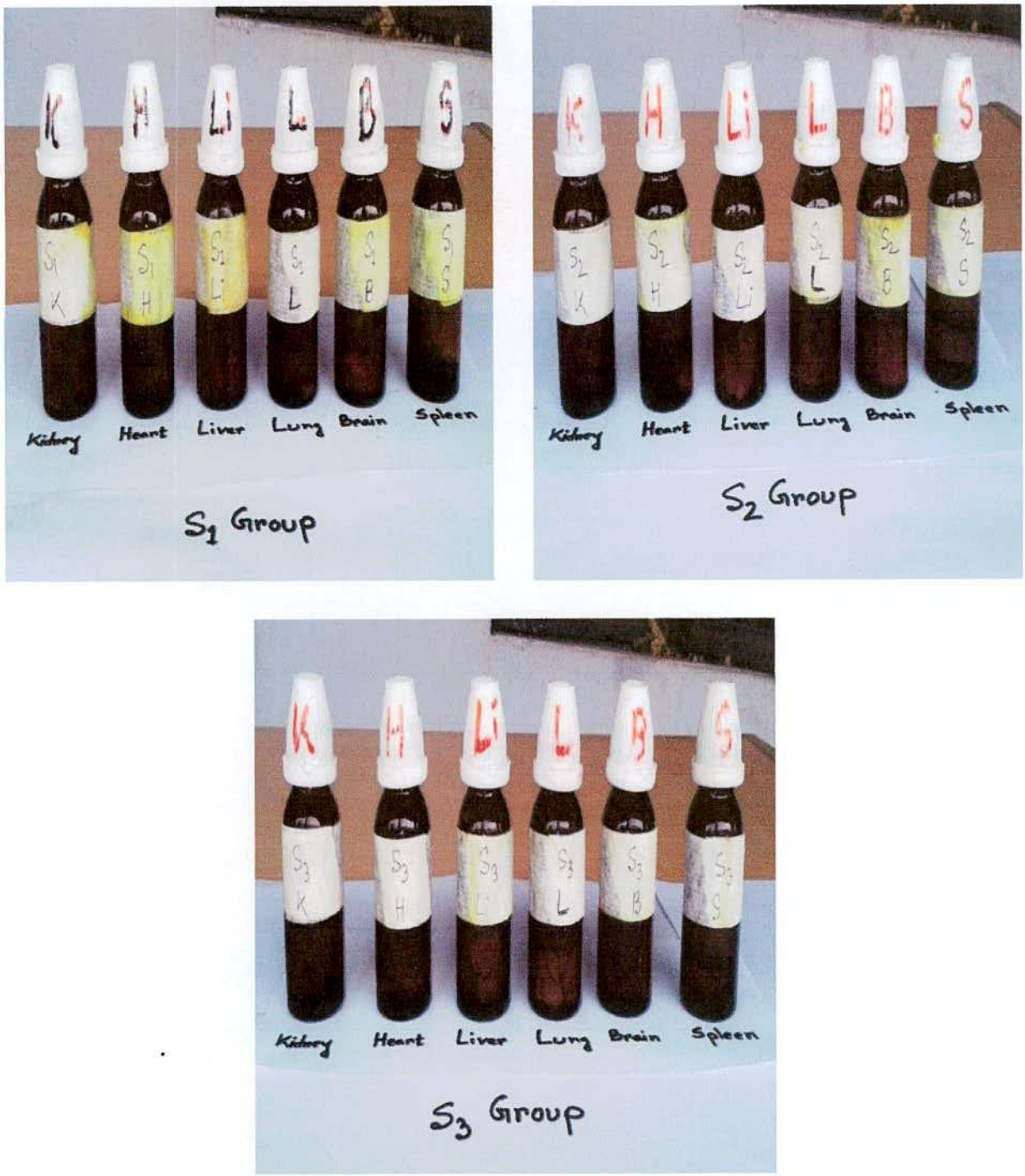
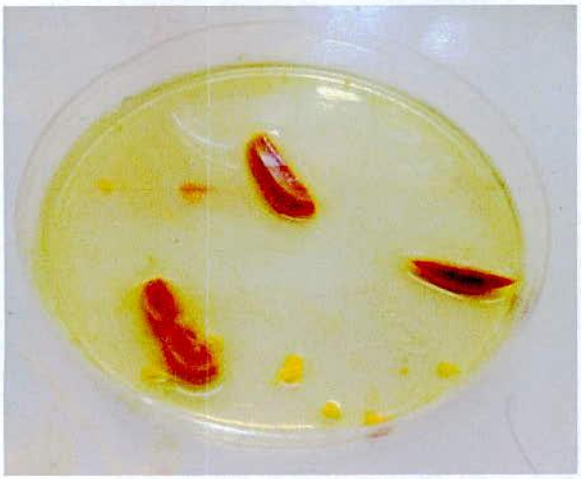


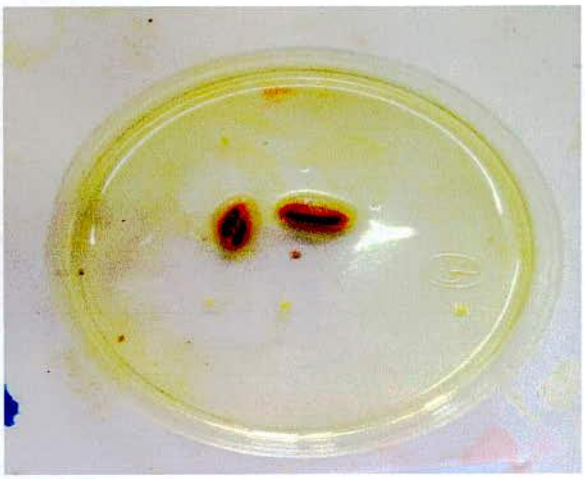
Figure 3.5: Preservation of heated soybean oils treated rabbit organs



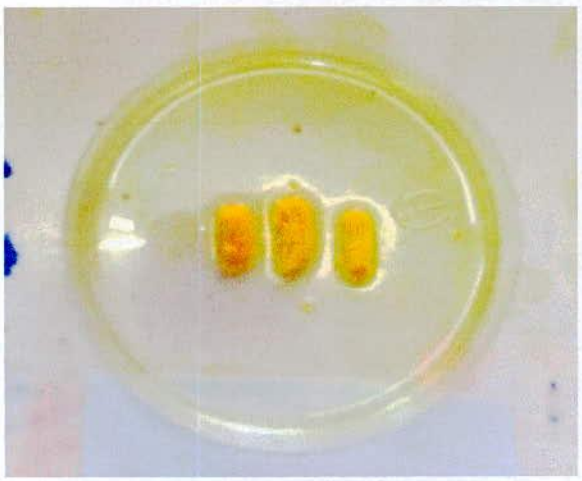
Kidney

Liver

Heart



Lung



Brain

Spleen

Figure 3.6: Sliced tissues

Figure 3.7: Block preparation

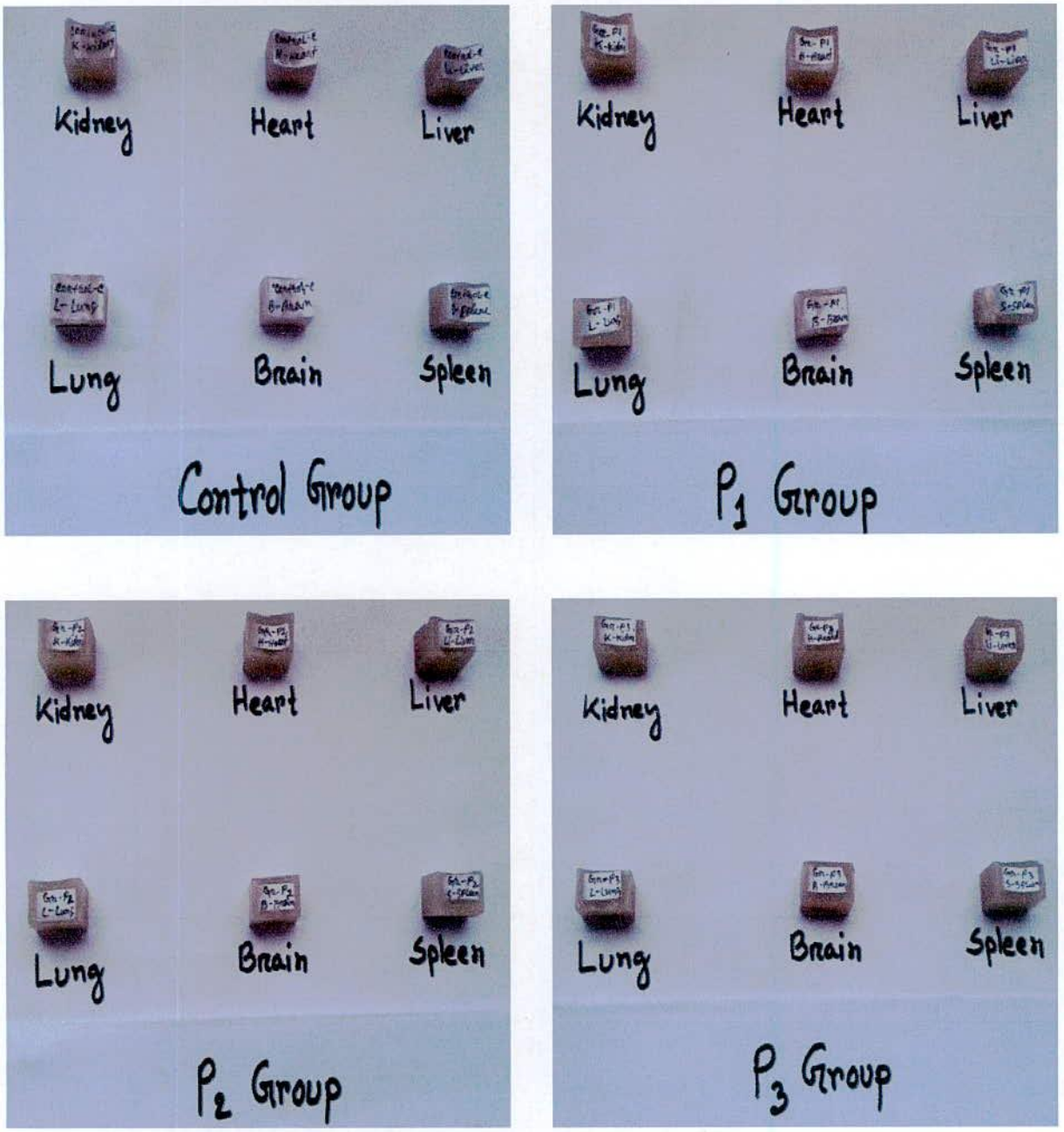


Figure 3.8: Paraffin block of control and heated palm oil treated rabbit organs

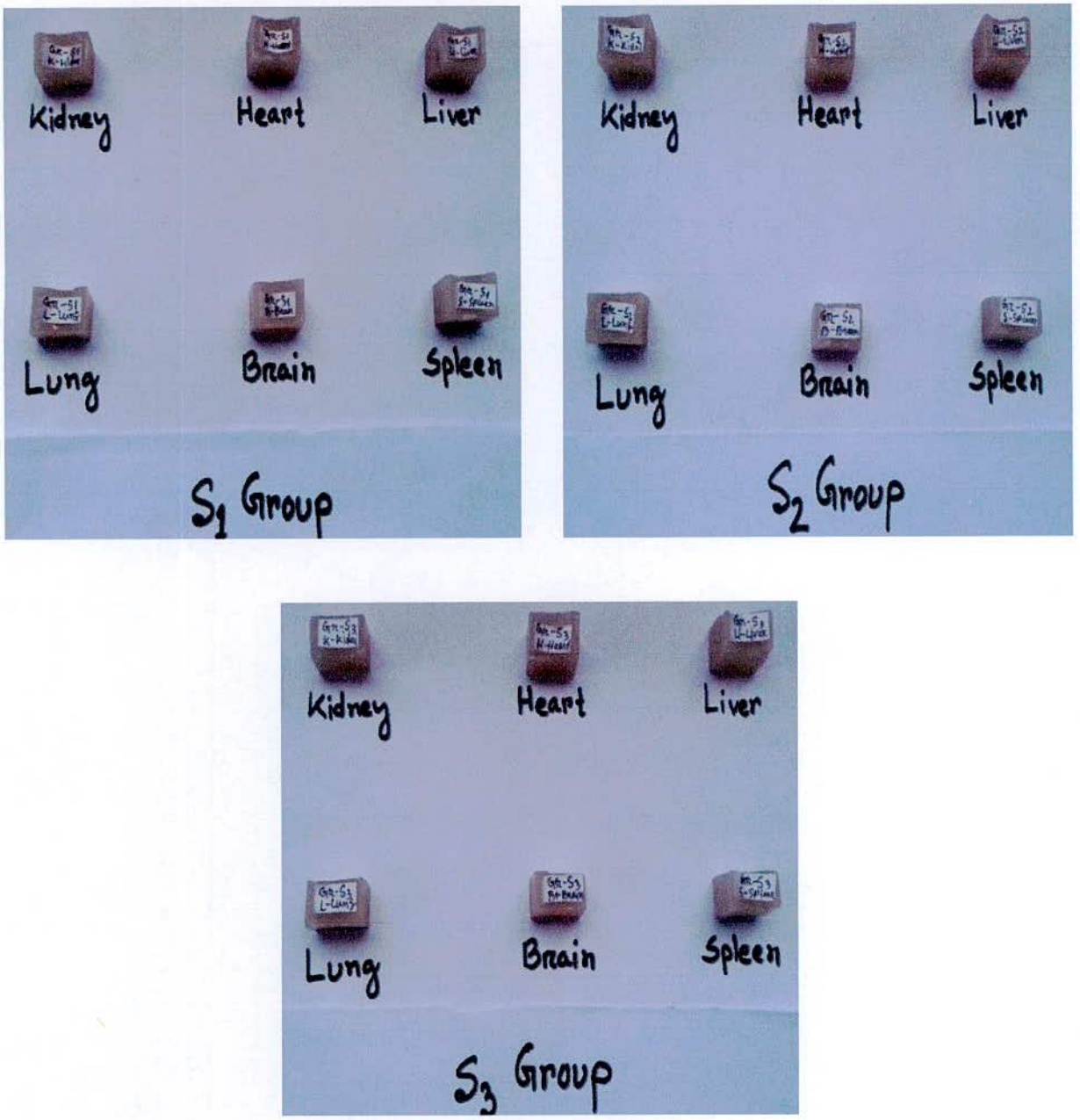
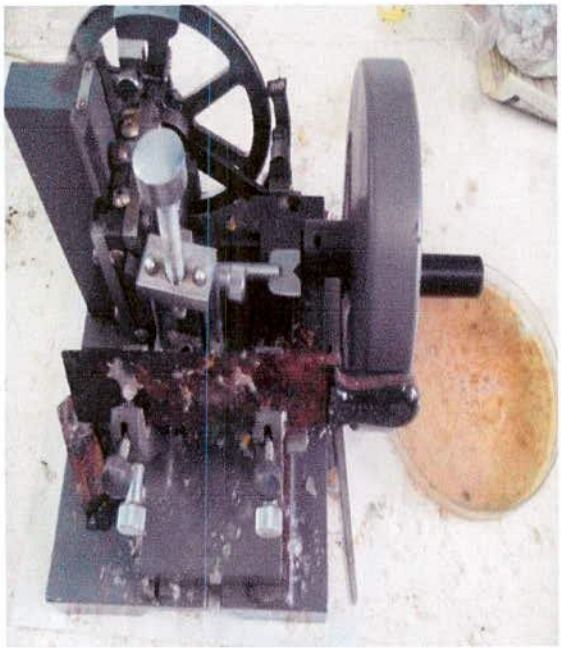
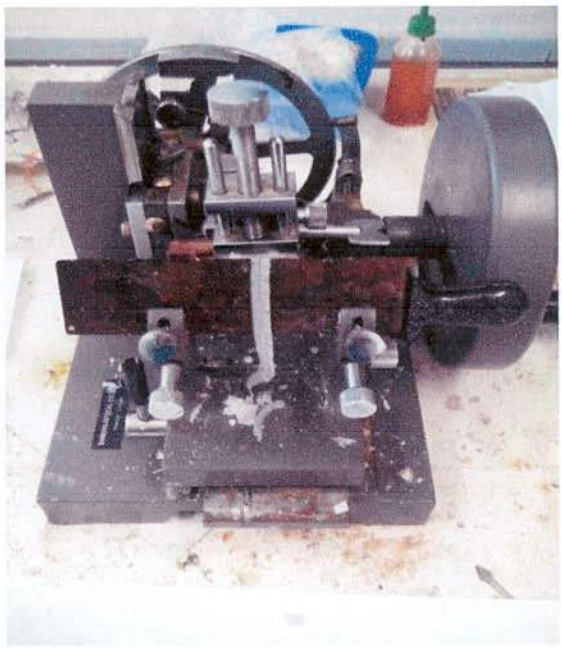


Figure 3.9: Paraffin block of heated soybean oils treated rabbit organs



Microtome



Microtome

Block cutting

Figure 3.10: Block cutting and slide preparation

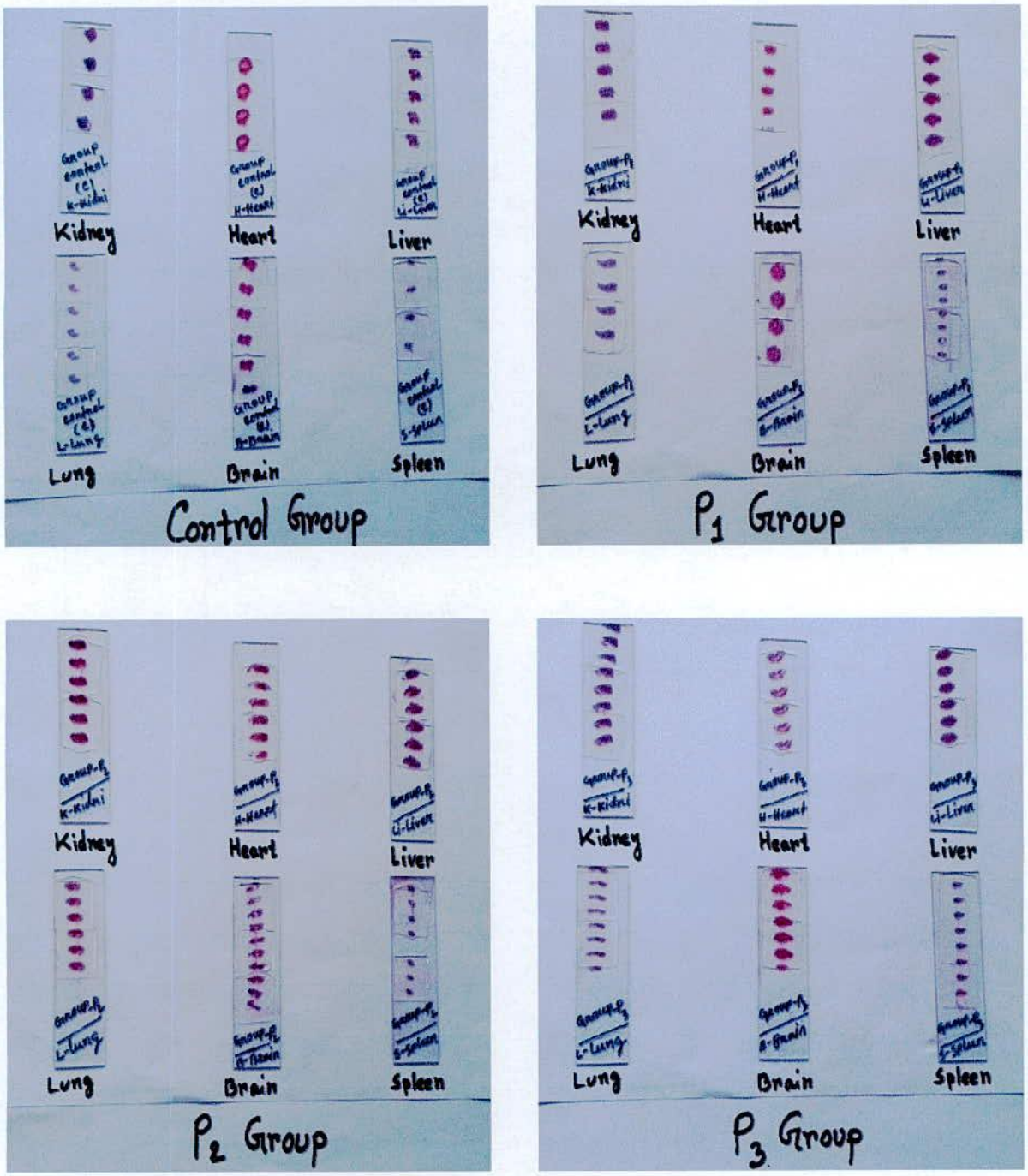


Figure 3.11: Slide of control and heated palm oils treated rabbit organs

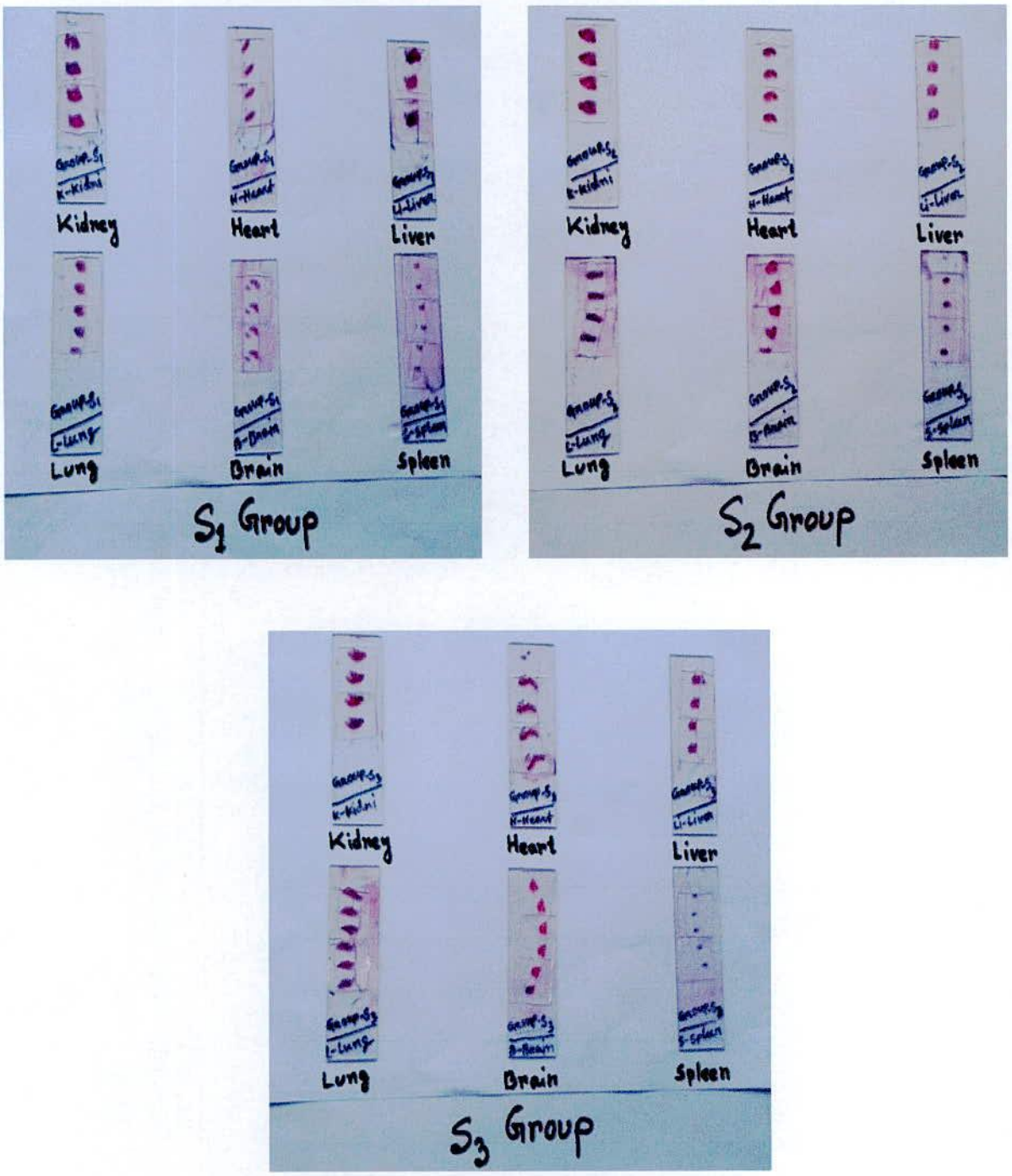


Figure 3.12: Slide of heated soybean oils treated rabbit organs

CHAPTER IV

Results and Discussion

4.1 Measurement of iodine value of fresh oil and heated oils

The iodine value of heated palm and soybean oils are reported in Table 4.1.

Table 4.1 Iodine value of heated palm and soybean oils

Heating Time	Palm Oil n = 6 M±SD	Soybean Oil n = 6 M±SD
Fresh oil	50.70±0.05	131.96±0.05
2 hours	46.86±0.09	121.95±0.08
4 hours	43.69±0.12	115.98±0.05
6 hours	41.08±0.08	110.18±0.05
8 hours	40.36±0.08	108.25±0.22
10 hours	38.08±0.09	106.01±0.11
12 hours	35.01±0.09	102.83±0.10
14 hours	31.02±0.08	101.20±0.17
16 hours	28.96±0.07	96.99±0.07
18 hours	23.06±0.05	90.03±0.11
20 hours	18.02±0.05	83.06±0.08

n= Number of titrations M= Mean value SD= Standard deviation

Iodine value of heated palm and soybean oil were decreased gradually shown in table 4.1. The highest decrease of iodine value was observed in 20 hours heated oils. Iodine value is a measure of degree of unsaturation and is used to characterize fats and oils [64]. It determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined

qualitatively [65]. As it is shown in results, the iodine value of mentioned oils gradually decreased. These low iodine values may have contributed to its greater oxidative storage stability [1]. The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid contents and a decrease in the total unsaturation of oils [66]. This decrease can be attributed to the destruction of double bonds by oxidation, scission and polymerization [67-68]. It is well established that saturated fatty acid possesses detrimental effects to the human health [18].

4.2 Measurement of acid value of fresh oil and heated oils

The acid value of heated palm and soybean oils are reported in Table 4.2.

Table 4.2 Acid value of more time heated palm and soybean oil

Heating Time	Palm Oil n = 6 M±SD	Soybean Oil n = 6 M±SD
Fresh oil	2.65±0.06	1.17±0.02
2 hours	2.76±0.05	1.34±0.04
4 hours	2.92±0.03	1.88±0.07
6 hours	3.31±0.06	2.32±0.05
8 hours	3.54±0.04	2.80±0.06
10 hours	4.01±0.06	3.22±0.04
12 hours	4.60±0.06	3.75±0.05
14 hours	5.22±0.09	4.31±0.05
16 hours	6.06±0.07	5.08±0.12
18 hours	6.68±0.08	5.29±0.08
20 hours	7.01±0.03	5.68±0.05

n= Number of titrations M= Mean value SD= Standard deviation

The acid value of heated palm and soybean oil were increased gradually shown in table 4.2. The highest increase of acid value was observed in 20 hours heated palm and soybean oil. The increase in acidity is undoubtedly due to the splitting of ester linkages of triglyceride molecules as a result of heating [69]. Acid value measures the degree of unsaturation of oil. It corresponds to the amount of potassium hydroxide needed to neutralize free fatty acids. The lower the acid value of oil, the fewer free fatty acids it contains which makes it less exposed to the phenomenon of rancidification [70].

4.3 Spectral analysis

FT-IR spectroscopy is an excellent tool for analysis as the intensities of the bands in the spectrum are proportional to concentration. Mid IR spectra have been used to characterize edible oils and fats because they differ in the intensity and the exact frequency at which the max absorbance or transmittance of the band appears, according to the nature and composition of the sample [71].

4.3.1 Spectral analysis of palm oil

Figure 4.1 and 4.2 are showing the spectra of fresh and 2 hours heated palm oil respectively. Figure 4.3 and 4.4 are showing the spectra of 10 hours heated and 20 hours heated palm oil respectively. The oil composition affects the exact positions of the band and yields a shift when the proportion of fatty acids changed. Fig. 4.1 to 4.4 the band around 3647.62–3649.32 cm^{-1} assigned to phenolic hydroxyl O–H stretching vibration, while the fig. 4.2, 4.3 and 4.4 showed an additional peak at 3711.04–3722.61 cm^{-1}. 3062.96–3099.61 cm^{-1} as C–H stretching vibration of the cis-double bond (=C–H) and 2854.65 – 2927.94 cm^{-1} shows C–H symmetric and asymmetric stretching vibrations of the aliphatic CH_2. At different times of heating, the percentage transmittance of almost all the peaks increased indicating a decrease in absorbance which may be due to the hydrolysis of oil during heating and the formation of free fatty acids and mono and diglycerides [1].

Figure 4.1 to 4.4 the band at 1737.86 cm^{-1} represents C=O ester carbonyl of triglycerides. Fresh, 2 hours heated, 10 hours heated palm oil (fig. 4.1, 4.2, 4.3 respectively) at 1618.28–1622.13 cm^{-1} indicates C=C stretching vibration of the cis-olefins. In addition, unheated and 20 hours heated palm oil (fig. 4.1 and 4.4) at 1591.27 cm^{-1} band shows an aromatic C=C stretching vibration. As the spectral regions undergoes several changes during oxidation processes at the long times heating oils showed some regions of other deformations and bending at 1456.26–1458.18 cm^{-1} of –C–H bending vibrations of the CH_2 and CH_3 aliphatic groups. The result which shows the bands at 1284.59–1288.45, 1242.16–1244.09 and 1166.93 cm^{-1}, some of them could be assigned to the stretching vibrations of the (C-O) esters group.

The unheated palm oil and 2, 10, 20 hours heated palm oils showed very similar FT-IR spectra in this study (figure 4.1, 4.2, 4.3 and 4.4).

4.3.2 Spectral analysis of soybean oil

All the edible oils are constituted basically of fatty triglyceride esters with different substitution patterns, lengths and degree of saturation of the chains and of other minor components. The importance of FTIR spectroscopy in the identification of molecular structures originates from the much information content obtained and the possibility to assign certain absorption bands related to its functional groups [72]. Fig. 4.5 and 4.6 are showing the spectra of unheated and 2 hours heated soybean oil respectively. Fig. 4.7 and 4.8 are showing the spectra of 10 hours heated and 20 hours heated soybean oil respectively. Band at 3007.02 cm^{-1} and 3062.86–3086.11 cm^{-1}, resulting from the stretching vibration of the cis olefinic double bond (=C–H). But fig. 4.8 shows the spectrum of 20 hours heated soybean oil has week band at 3722.61 and 3647.39 cm^{-1} associated with O–H stretching vibration of hydrogen bond free absorption.

The bands at 2854.65–2858.51 cm^{-1} and 2908.65–2954.95 cm^{-1}, assigned to (C-H) symmetrical and asymmetrical stretching of the aliphatic CH_2. The C=O ester carbonyl group of triglycerides resulting a stretching vibration at 1737.86–1741.72 cm^{-1}. A small band at 1618.28–1647.21 cm^{-1} represents C=C stretching vibration of the cis-olefins (fig. 4.6, 4.7,

4.8). A spectrum at 1377.71 cm^{-1} and 1456.26–1463.97cm^{-1} are due to bending vibrations of CH_2 and CH_3 aliphatic groups. On the other hand, fig. 4.5 shows band 1417.68 cm^{-1} resulting from the rocking vibration of CH bond. The result which shows the bands at 1284.59–1286.52, 1253.73, 1240.23–1242.16 and 1166.93 cm^{-1}, some of them could be assigned to the stretching vibrations of the (C-O) esters group.

The FTIR spectra of unheated and 2, 10, 20 hours heated soybean oils have a great similarity showing almost unappreciable variations either in the frequency or in the absorbance of the bands (figure 4.5, 4.6, 4.7 and 4.8).

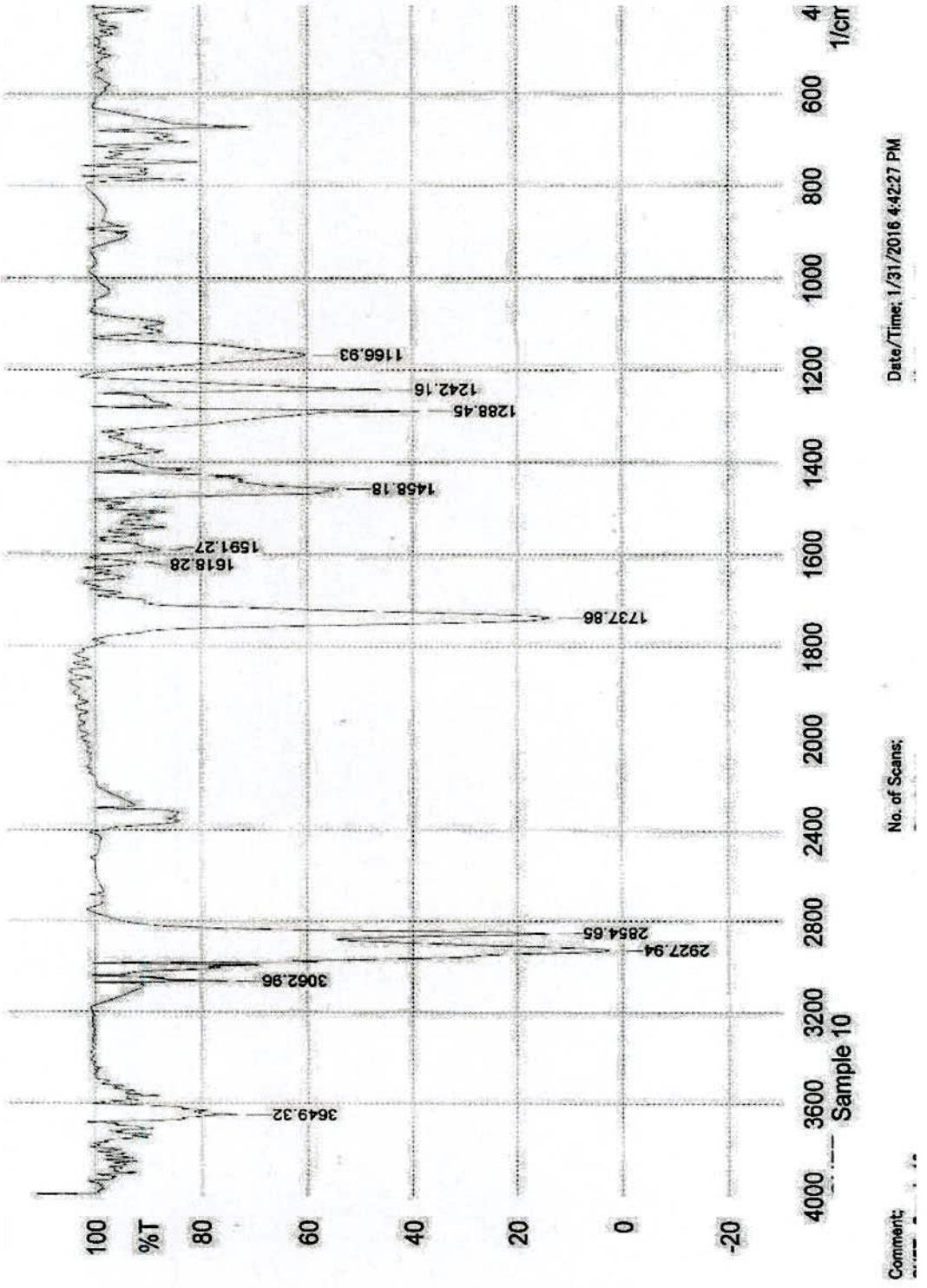


Figure 4.1: FT-IR spectrum of fresh palm oil

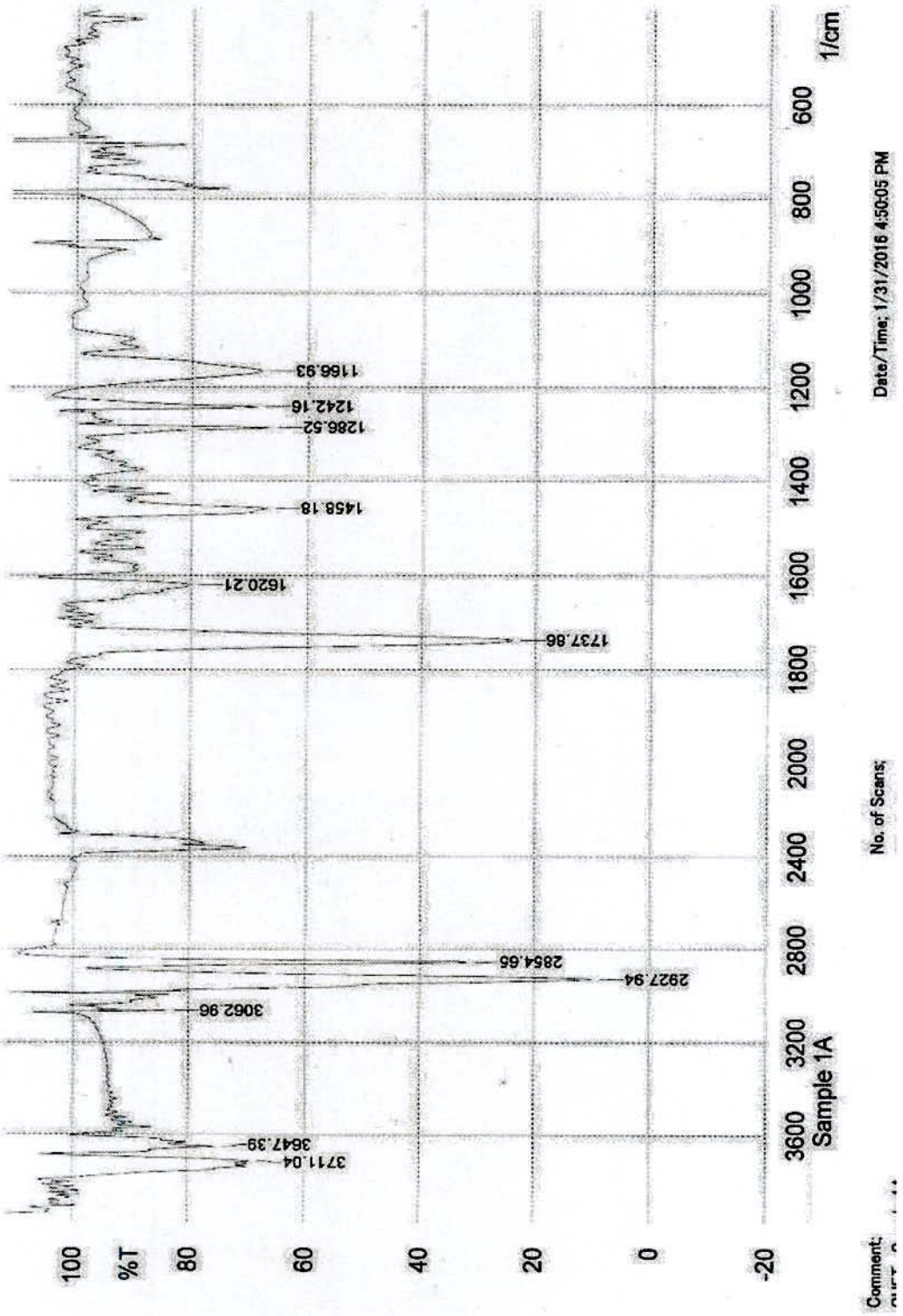


Figure 4.2: FT-IR spectrum of 2 hours heated palm oil

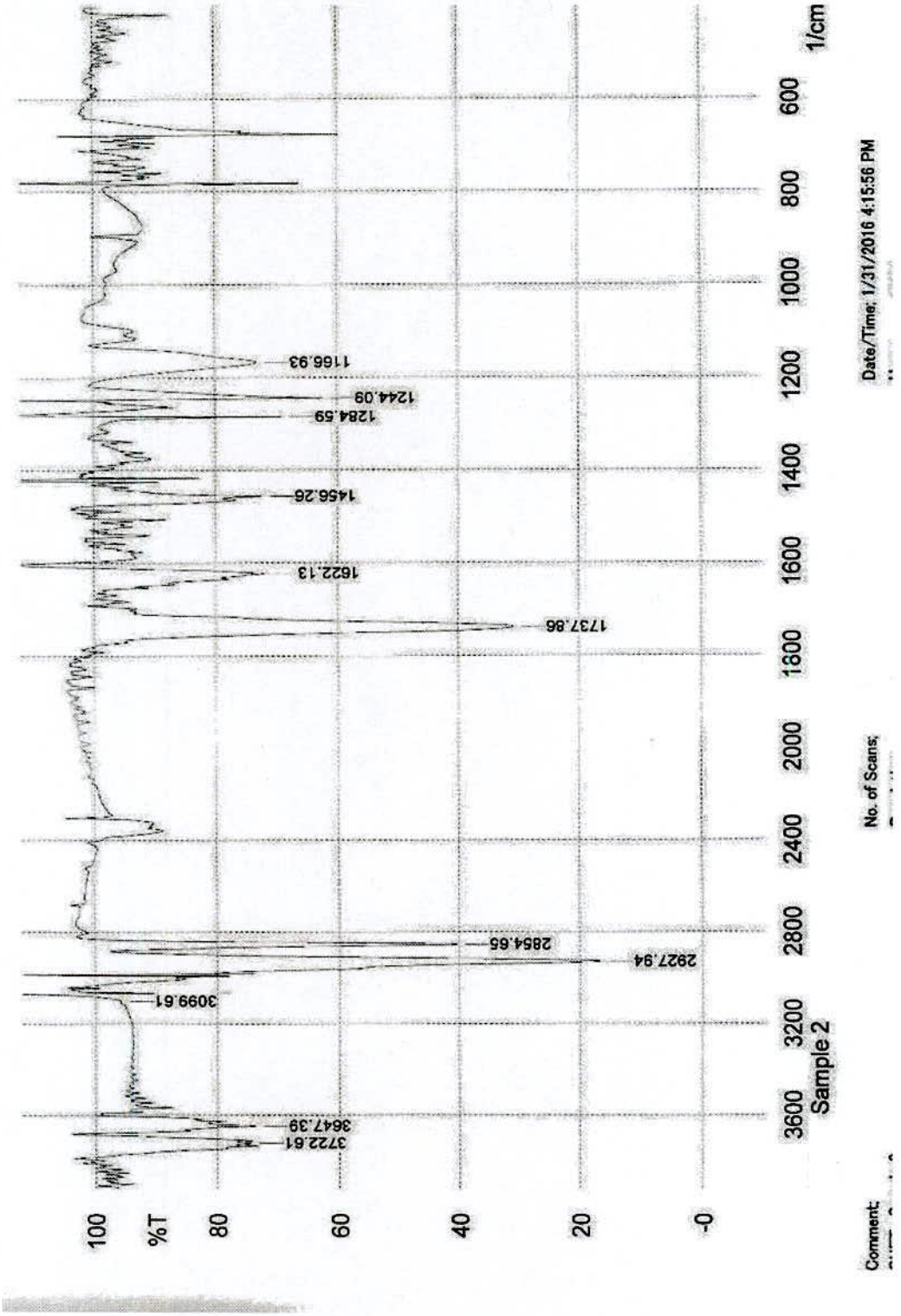


Figure 4.3: FT-IR spectrum of 10 hours heated palm oil

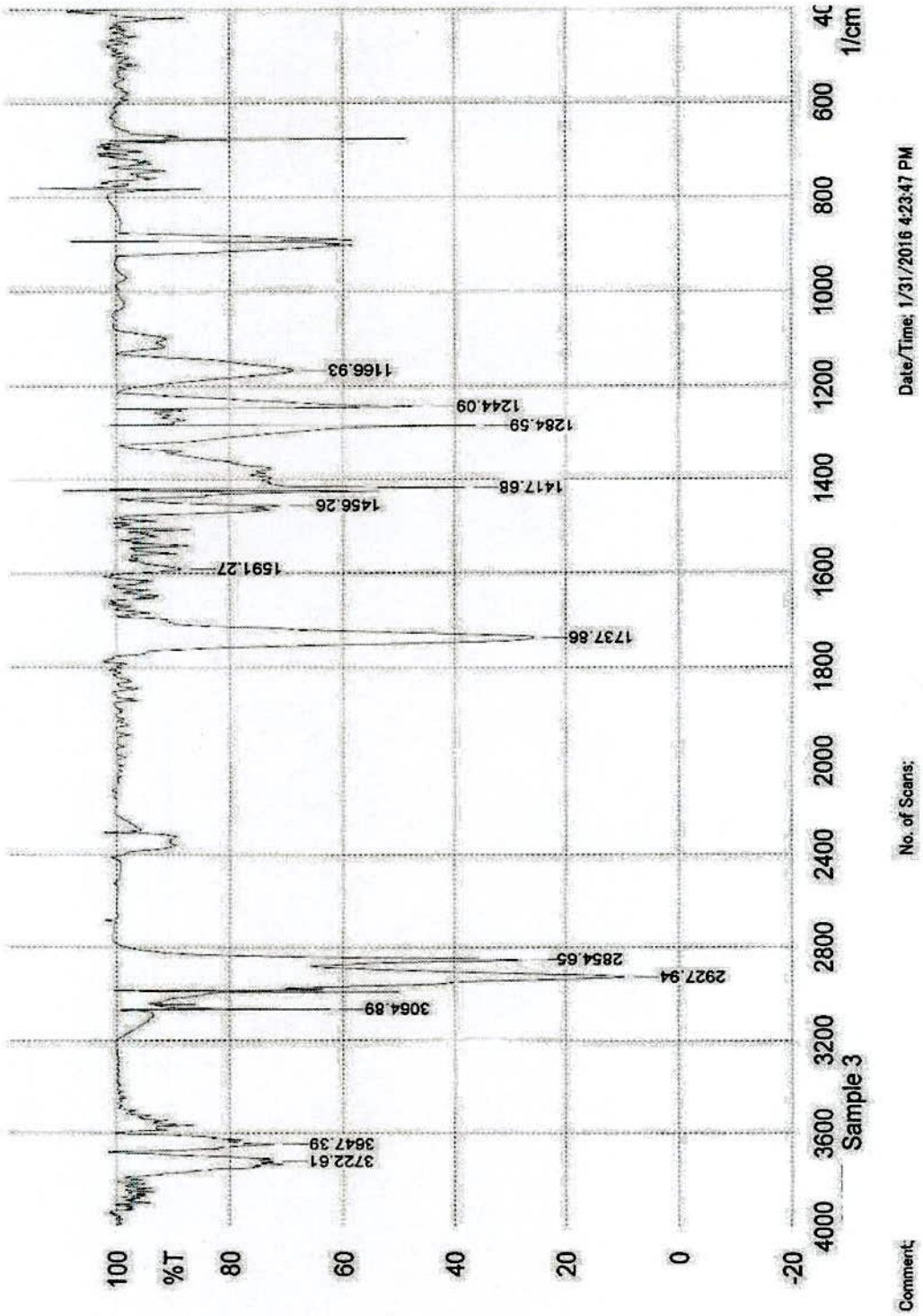


Figure 4.4: FT-IR spectrum of 20 hours heated palm oil

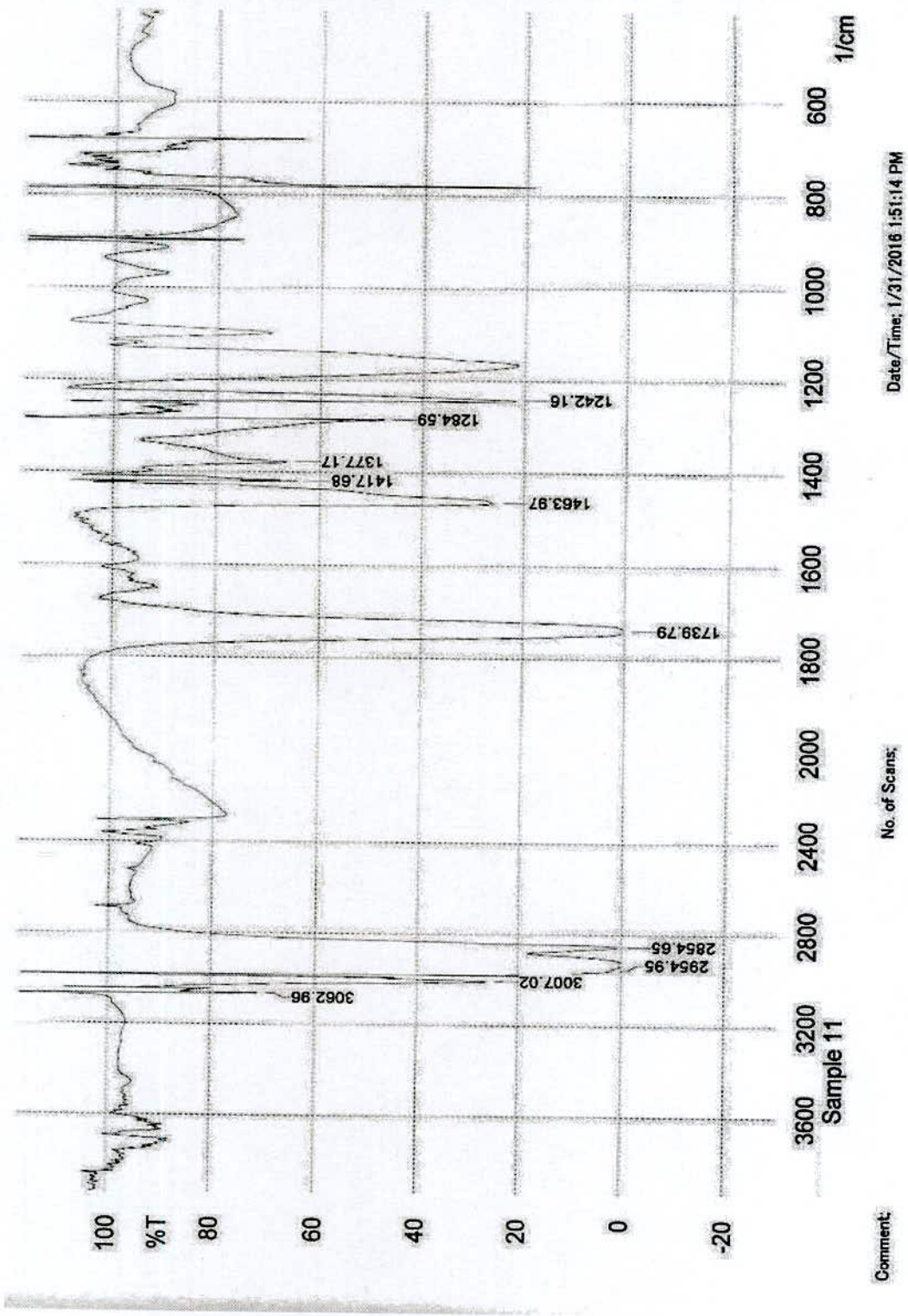


Figure 4.5: FT-IR spectrum of fresh soybean oil

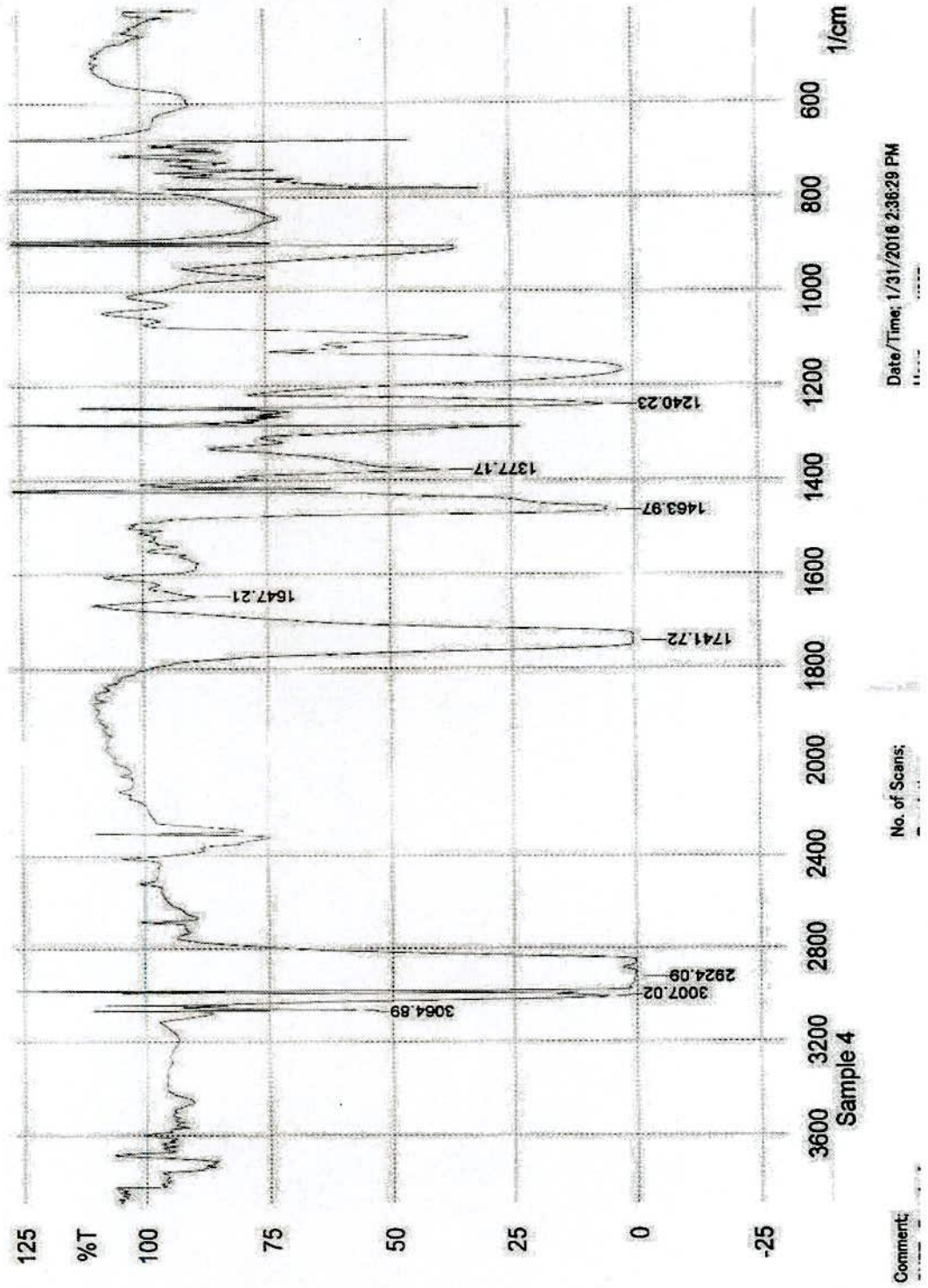


Figure 4.6: FT-IR spectrum of 2 hours heated soybean oil

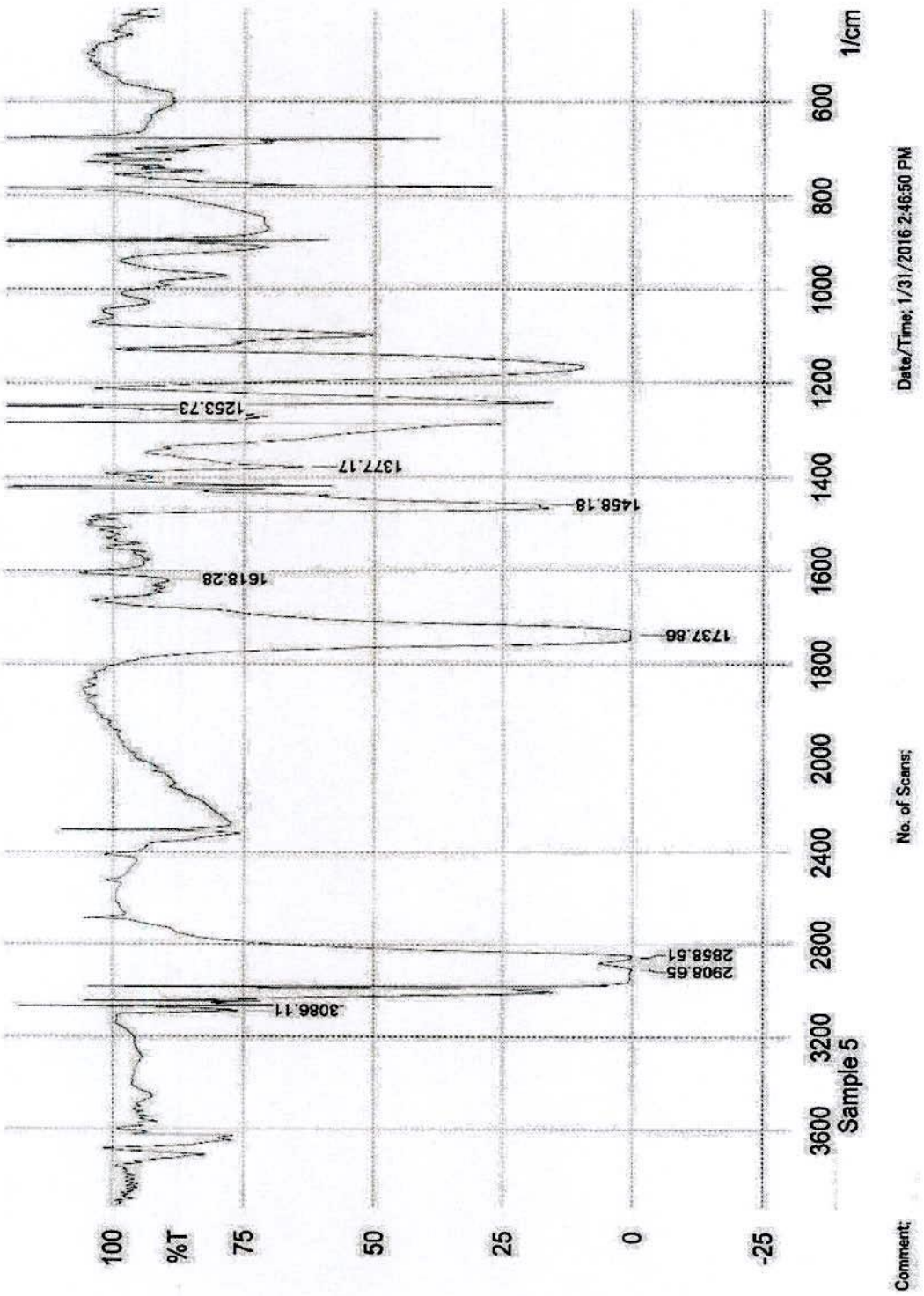


Figure 4.7: FT-IR spectrum of 10 hours heated soybean oil

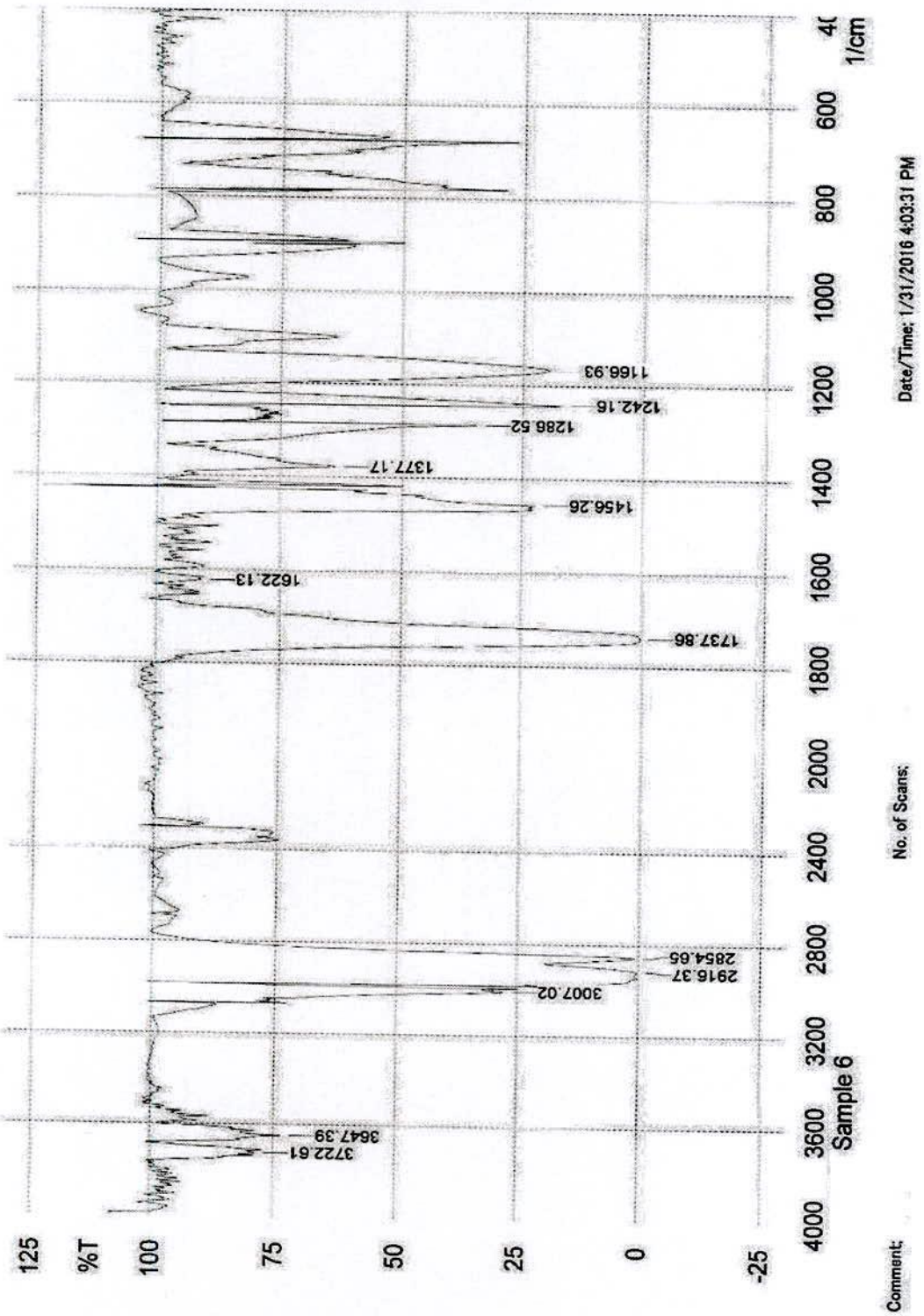


Figure 4.8: FT-IR spectrum of 20 hours heated soybean oil

4.4 Body weights were changed

The body weight of each rabbit of following groups: control group (C), 2 hours heated palm oil (P1), 10 hours heated palm oil (P2), 20 hours heated palm oil (P3), 2 hours heated soybean oil (S1), 10 hours heated soybean oil (S2), 20 hours heated soybean oil (S3) were measured before and after administration the treatment shown in table 4.3 and 4.4. Subsequently, all the rabbits were given mixed diet and heated oil were given mentioned oil group. During the study their weight gain was calculated day by day. The body weight of all group were increased significantly. The highest body weight increase was observed in P3 and S3 group. The food intake per day was also found normal. It is remarkable to highlight that the body weight gain in the 20 hours heated group showed significant changes as compared with the other group. Why the body weight increased that is not well understood, but it may be that the increase in weight due to fat tissue.

Leong et al. [17] investigated that five times heated palm oil treated rats did not gain body weight. But in this present study, the result showed that long time heated palm and soybean oil treated rabbits body weights were increased. So the present study agreed with the result obtained by Siti Khadijah Adam et al. [37] and Hussein S. Gumaih [56] who showed that the body weight of rats was significantly increased by feeding thermally oxidized palm oil.

Thermally oxidized palm oil which generates free radicals, enhances the oxidative stress secondary to estrogen deficiency and high cholesterol diet [37]. These parameters have been attributed to atherosclerosis [73].

Table 4.3 Body weight of rabbit after heated palm oil treatment

Day	Body weight(g) C n = 3 $M_1 \pm SD_1$	Body weight (g) P1 n = 3 $M_2 \pm SD_2$	Body weight (g) P2 n = 3 $M_3 \pm SD_3$	Body weight (g) P3 n = 3 $M_4 \pm SD_4$
Observation	731.43±1.14	672.13±2.05	693.45±1.03	772.37±1.94
1st	731.74±1.23	672.56±2.17	694.12±1.10	772.65±1.89
2nd	731.95±1.28	673.10±1.96	694.91±1.20	774.23±2.05
3rd	732.43±1.26	674.01±2.08	695.75±1.32	775.26±2.06
4th	732.67±1.24	674.33±1.89	696.91±1.86	776.74±2.16
5th	732.92±1.14	675.24±1.72	698.11±1.81	778.68±1.83
6th	733.14±1.18	676.12±1.86	699.44±2.36	780.75±1.74
7th	733.49±1.13	677.28±1.95	701.02±2.17	783.13±2.32
8th	733.67±1.16	678.27±1.67	701.78±2.49	784.72±2.20
9th	733.82±1.11	679.53±1.86	703.13±2.24	787.03±2.04
10th	734.29±1.04	680.33±2.13	704.08±2.09	788.67±2.51
11th	734.67±1.15	681.98±1.75	704.98±2.51	790.48±2.56
12th	734.90±1.04	682.33±2.08	705.98±2.71	792.63±1.87
13th	735.08±1.10	683.44±1.88	706.84±2.39	794.22±1.60
14th	735.42±1.11	684.48±2.62	707.85±2.21	796.01±1.05
15th	735.71±1.12	686.19±2.60	709.13±2.26	797.99±1.41
16th	736.01±1.06	687.87±2.39	710.29±2.31	799.41±1.23
17th	736.55±1.17	689.17±2.35	711.17±2.44	801.03±1.60
18th	736.97±1.17	690.24±2.82	712.49±2.32	802.24±1.75
19th	737.44±1.08	691.62±3.06	713.70±2.77	803.95±1.72
20th	737.76±1.13	692.87±2.83	715.00±2.76	806.21±2014

21th	738.13±1.01	694.06±2.67	716.87±3.02	807.96±2.38
22th	738.73±1.13	695.36±2.40	718.35±2.55	810.13±2.72
23th	739.33±1.02	697.25±2.04	719.74±2.66	811.98±2.56
24th	739.83±1.03	698.91±2.08	721.12±2.25	814.46±3.65
25th	740.71±1.27	701.50±2.42	722.59±2.58	815.43±2.82
26th	741.17±1.31	703.72±1.90	723.81±2.78	817.18±2.81
27th	741.75±1.19	705.52±2.17	725.28±5.51	819.21±2.56
28th	742.30±1.17	707.29±2.29	726.91±2.33	821.57±3.29

n= Number of rabbit, M_1, M_2, M_3, M_4= Mean value of control (C) group, 2 hours heated palm oil (P1) group, 10 hours heated palm oil (P2) group, 20 hours heated palm oil (P3) group respectively , SD_1, SD_2, SD_3, SD_4= Standard deviations of control (C) group, 2 hours heated palm oil (P1) group, 10 hours heated palm oil (P2) group, 20 hours heated palm oil (P3) group respectively.

Table 4.4 Body weight of rabbit after heated soybean oil treatment

Day	Body weight(g) C n = 3 $M_1 \pm SD_1$	Body weight (g) S1 n = 3 $M_5 \pm SD_5$	Body weight (g) S2 n = 3 $M_6 \pm SD_6$	Body weight (g) S3 n = 3 $M_7 \pm SD_7$
Observation	731.43±1.14	641.11±0.59	681.78±1.14	701.25±1.08
1st	731.74±1.23	641.47±0.95	682.16±1.64	702.51±2.06
2nd	731.95±1.28	642.14±0.89	682.99±1.63	703.73±1.92
3rd	732.43±1.26	642.68±1.12	683.72±1.48	704.93±1.74
4th	732.67±1.24	643.38±0.88	684.64±1.52	705.66±1.94
5th	732.92±1.14	644.03±0.79	685.48±1.72	706.71±1.93
6th	733.14±1.18	644.86±0.97	686.28±1.30	707.66±1.70
7th	733.49±1.13	645.52±0.99	686.92±1.33	708.26±1.43
8th	733.67±1.16	646.42±1.08	687.43±1.14	709.02±1.39
9th	733.82±1.11	647.22±1.17	687.94±1.10	709.74±1.07
10th	734.29±1.04	647.85±1.29	688.76±0.88	710.39±1.16
11th	734.67±1.15	648.58±1.07	689.52±0.53	711.28±0.80
12th	734.90±1.04	649.08±0.88	690.33±0.47	712.35±0.81
13th	735.08±1.10	649.88±0.75	691.06±0.57	713.31±0.67
14th	735.42±1.11	650.44±0.74	691.94±0.38	714.26±0.66
15th	735.71±1.12	651.20±0.86	692.75±0.49	714.92±0.77
16th	736.01±1.06	651.83±0.68	693.51±0.44	715.95±0.56
17th	736.55±1.17	652.74±0.66	694.69±0.50	717.23±0.26
18th	736.97±1.17	653.38±0.72	695.92±0.66	718.49±0.70
19th	737.44±1.08	653.92±0.58	696.69±0.40	719.58±0.74
20th	737.76±1.13	654.87±0.40	697.49±0.45	720.62±1.04
21th	738.13±1.01	656.02±0.38	698.50±0.66	722.01±1.21

22th	738.73±1.13	656.75±0.40	699.27±0.62	723.02±1.38
23th	739.33±1.02	657.47±0.37	699.96±0.76	724.22±1.46
24th	739.83±1.03	658.17±0.61	701.12±0.82	725.25±1.68
25th	740.71±1.27	659.11±0.75	702.18±0.92	726.61±2.01
26th	741.17±1.31	659.92±0.57	703.16±0.85	727.89±1.88
27th	741.75±1.19	660.97±0.58	704.18±0.94	729.11±2.25
28th	742.30±1.17	662.38±0.55	705.27±0.95	730.50±2.20

n= Number of rabbit, M_1, M_5, M_6, M_7= Mean value of control (C) group, 2 hours heated soybean oil (S1) group, 10 hours heated soybean oil (S2) group, 20 hours heated soybean oil (S3) group respectively , SD_1, SD_5, SD_6, SD_7= Standard deviations of control (C) group, 2 hours heated soybean oil (S1) group, 10 hours heated soybean oil (S2) group, 20 hours heated soybean oil (S3) group respectively.

4.5 Effect of heated oil diets on haematological profiles

The present study, long term administration of heated palm oil and soybean oil treated rabbits have been observed to cause alterations in count white blood cell (WBC), count red blood cell (RBC) and hemoglobin (Hb) (table 4.5 and 4.6).

The results showed that the total white blood cell count (WBC) of heated palm oil and soybean oil fed all groups were increased. WBC count was increased in palm oil and soybean oil treated rabbits with respect to control. Total WBC of P1group ($9.5 \times 10^3 \pm 0.16 \times 10^3$ blood cell/µl) and S2 group ($6.5 \times 10^3 \pm 0.16 \times 10^3$ blood cell/µl) were significantly higher than that of untreated rabbits.

White blood cells (WBCs), also called leukocytes, are the cells of the immune system that are involved in protecting the body against infectious disease. The present study showed that the WBC of all heated oil treated group were increased compared with control group. A high number of WBC is called leukocytosis. Leukocytosis may be occur hepatic damage reported by Finlayson et al. [74]. This study agree with E.J. Ani et al. [75] who reported that it may be an indication of inflammation, infection, stress to major organs or certain diseases.

The red blood cell count (RBC) was increased in all heated palm oil fed groups with respect to control shown in table 4.5. The RBC of P3 group (6.58±0.03 m/ µl) was higher than that of untreated, P1 and P3 group. On the other hand, RBC of all heated soybean oil diet group were decreased compared to control shown in table 4.6. The RBC of S2 group (3.69±0.03 m/ µl) was lower than that of control (5.48±0.01 m/ µl), S1 and S3 group.

In previous study O. E. Mesembe et al. [55] and Hussein S. Gumaih [56] investigated that the RBC count was decreased in the rats fed thermoxidized palm oil compared with control group. This study showed that the RBC count of heated palm oils treated rabbits were increased with respect to untreated rabbits. But the RBC count was decreased all heated soybean oil diet rabbits group compared with control group. This decrease may be due to the suppressive

effect of the hazardous constituents of thermally oxidized palm oil on the bone marrow [54]. In addition, red blood cells (RBC) are also called erythrocytes. Erythropoietin is a part of RBC. The decrease of RBC count in the heated soybean oils treated rabbits may have also caused the failure of erythropoietin production supported by O. E. Mesembe et al [55].

The hemoglobin concentration of heated soybean oil diets all groups, heated palm oil treated P1 and P2 group were decreased compared to control rabbits. But the hemoglobin concentration of heated palm oil diet P3 group was increased. The hemoglobin concentration of P1 group (10.7±0.21 g/dl) and S2 group (8.0±00 g/dl) were lower than that of control (11.5±0.08 g/dl) shown in table 4.5 and 4.6. In addition, the hemoglobin concentration of P3 group (12.3±0.21) was higher than that of untreated rabbits shown in table 4.5.

The hemoglobin concentration was decreased in heated palm and soybean oil fed group except P3 group compared with control. In previous study O. E. Mesembe et al. [55] reported that this decrease in hemoglobin concentration may be a consequence of reduced uptake of iron by the damaged intestinal mucosa of rats resulting in a reduced bioavailability of iron in the system. The liver stores iron as ferritin and hemosiderin [74]. The decrease in hemoglobin concentration may also be attributed to the decreased storage of iron in the liver as a result of damage to the liver [75].

Heated palm oil and soybean oil diets were significantly affected Erythrocyte Sedimentation Rate (ESR) in this study. ESR was higher in S2 fed group (15±0.82 g/dl) but ESR was stabled in S1 and S3 fed group compared with control group shown in table 4.6. On the other hand, ESR of all heated palm oil fed group were decreased, compared to untreated shown in table 4.5. ESR of P1 group (1.00±00 g/dl) was lower than that of control group (10±00 g/dl).

The erythrocyte sedimentation rate (ESR) is the rate at which red blood cells sediment in a period of one hour. It is a common hematology test and is a non-specific measure of inflammation. The present study showed that the ESRs of heated palm oil treated groups were

decreased with respect to control rabbits. It may be an indication of sickle cell anemia, leukemia.

The Platelet Count (PC) was increased in P1, P2 heated palm oil fed groups and in S1, S2 heated soybean oil fed groups compared with control shown in table 4.5 and 4.6. PC of P2 group ($593x10^3 \pm 2.44x10^3$ blood cell/µl) was higher than that of untreated and all heated oil fed groups. PC of P3 and S3 group were decreased compared with control. PC of P3 group ($217 x10^3 \pm 0.81x10^3$ blood cell/µl) and S3 group ($221x10^3 \pm 1.41x10^3$ blood cell/µl) were lower than that of control ($241x10^3 \pm 0.81x10^3$ blood cell/µl) and other heated oil fed groups.

Abnormalities in platelet number are an indication of a defect in primary hemostasis. An increase in platelet number above normal serves as a marker of vascular disease [76]. This study agrees with Mohammad Anwar et al. [77] who reported that the platelet count of high fat diet fed rabbits group were increased.

Table 4.5 Effect of heated palm oil diets on Hematological profiles

Parameters		Units	C n = 3 $M_8 \pm SD_8$	P1 n = 3 $M_9 \pm SD_9$	P2 n = 3 $M_{10} \pm SD_{10}$	P3 n = 3 $M_{11} \pm SD_{11}$
Total WBC		thous and/ µl	2.7, 2.9,3.1	9.3, 9.7, 9.5	6.2, 5.9, 6.2	5.1, 5.3, 5.5
			2.8±0.16	9.5±0.16	6.1±0.14	5.3±0.16
Differential count of WBC in %	Neutrophils	%	49, 50, 51	53, 54, 55	38, 36, 37	48, 45, 48
			50±0.82	54±0.82	37±0.82	47±1.14
	Lymphocytes	%	50, 49, 48	46, 45, 44	57, 60, 60	50, 51, 49
			49±0.82	45±0.58	59±1.41	50±0.82
	Monocytes	%	01, 01,01	01, 01, 01	05, 04, 03	01, 03, 02
			01±00	01±00	04±0.82	02±0.82
	Eosinophils	%	00, 00, 00	00, 00, 00	00, 00, 00	01, 01, 01
			00±00	00±00	00±00	01±00
Total RBC		m/ µl	5.46, 5.48, 5.50	5.71, 5.73, 5.78	5.90, 5.95, 6.12	6.54, 6.59, 6.61
			5.48±0.01	5.74±0.03	5.99±0.09	6.58±0.03
Hemoglobin		g/dl	11.4, 11.5, 11.6	10.4, 10.8, 10.9	11.0, 11.3, 11.0	12.1, 12.2, 12.6
			11.5±0.08	10.7±0.21	11.1±0.14	12.3±0.21

ESR	g/dl	10, 10, 10	01, 01, 01	02, 02, 02	02, 02, 02
		10±00	1±00	2±00	2±00
PC	thous and/ µl	240, 242, 241	410,415, 411	590, 596, 593	216, 217, 218
		241±0.81	412±2.16	593±2.44	217±0.81

n= Number of rabbit, M_8, M_9, M_{10}, M_{11}= Mean value of control (C) group, 2 hours heated palm oil (P1) group, 10 hours heated palm oil (P2) group, 20 hours heated palm oil (P3) group respectively , SD_8, SD_9, SD_{10}, SD_{11}= Standard deviations of control (C) group, 2 hours heated palm oil (P1) group, 10 hours heated palm oil (P2) group, 20 hours heated palm oil (P3) group respectively.

WBC= White Blood Cell, RBC= Red Blood Cell, ESR= Erythrocyte Sedimentation Rate, PC= Platelet Count.

Table 4.6 Effect of heated soybean oil diets on Hematological profiles

Parameters		Units	C n = 3 $M_8 \pm SD_8$	S1 n = 3 $M_{12} \pm SD_{12}$	S2 n = 3 $M_{13} \pm SD_{13}$	S3 n =3 $M_{14} \pm SD_{14}$
Total WBC		thousand/ µl	2.7, 2.9,3.1	4.2, 4.4, 4.3	6.3, 6.5, 6.7	5.2, 5.5, 5.5
			2.8±0.16	4.3±0.08	6.5±0.16	5.4±0.14
Differential count of WBC in %	Neutrophils	%	49, 50, 51	53, 56, 56	65, 68, 68	43, 44, 45
			50±0.82	55±1.41	67±1.41	44±0.82
	Lymphocytes	%	50, 49, 48	45, 42, 42	33, 30, 30	54, 53,52
			49±0.82	43±1.41	31±1.41	53±0.82
	Monocytes	%	01, 01, 01	02, 02, 02	02, 02, 02	02, 02, 02
			01±00	02±00	02±00	02±00
	Eosinophils	%	00, 00, 00	00, 00, 00	00, 00, 00	01, 01, 01
			00±00	00±00	00±00	01±00
Total RBC		m/ µl	5.46, 5.48, 5.50	5.34, 5.37, 5.40	3.65, 3.71, 3.71	4.86, 4.91, 4.93
			5.48±0.01	5.37±0.03	3.69±0.03	4.90±0.03
Hemoglobin		g/dl	11.4, 11.5, 11.6	9.4, 9.6, 9.8	8.0, 8.0, 8.0	10.6, 10.7, 10.8
			11.5±0.08	9.6±0.16	8.0±00	10.7±0.08

ESR	g/dl	10, 10, 10	10, 10, 10	14, 15, 16	10, 10, 10
		10±00	10±00	15±0.82	10±00
PC	thousand/ µl	240, 242, 241	571, 576, 578	424, 428, 429	219, 222, 222
		241±0.82	575±2.94	427±2.16	221±1.41

n= Number of rabbit, M_8, M_{12}, M_{13}, M_{14}= Mean value of control (C) group, 2 hours heated soybean oil (S1) group, 10 hours heated soybean oil (S2) group, 20 hours heated soybean oil (S3) group respectively, SD_8, SD_{12}, SD_{13}, SD_{14}= Standard deviations of control (C) group, 2 hours heated soybean oil (S1) group, 10 hours heated soybean oil (S2) group, 20 hours heated soybean oil (S3) group respectively.

WBC= White Blood Cell, RBC= Red Blood Cell, ESR= Erythrocyte Sedimentation Rate, PC= Platelet Count

4.6 Effect of heated oil diets on biochemical profiles

Biochemical profiles as Creatinine, SGPT, SGOT, ALP and Uric acid of heated palm oil and soybean oil diets by rabbits have been observed (table 4.7 and 4.8).

Creatinine was increased in heated palm oil treated groups except P2 group compared to untreated shown in table 4.7. Creatinine of P3 group (1.0±0.14 mg/dl) was higher than that of control, P1 and P2 groups. On the other hand, creatinine of heated soybean oil diet groups were decreased except S3 group with respect to control shown in table 4.8. Creatinine of S2 group (0.6±0.08 mg/dl) was lower than that of untreated, S1 and S3 groups. In addition, creatinine of S3 group (1.1±0.08 mg/dl) was higher than that of control, all heated palm oil treated and heated soybean oil diet groups.

The present study showed that serum creatinine in the heated palm oil fed rabbits group was significantly increased compared with control. Creatinine levels in plasma are usually measured to determine acute or chronic renal insufficiency [78]. They are usually raised in renal disease. So it may be suggestive of possible renal system damage agree with Elemi J. Ani et al. [79].

The serum glutamic pyruvic transaminase (SGPT) of heated palm oil and soybean oil treated all groups were increased. The SGPT was increased in palm oil and soybean oil fed groups compared with control. The SGPT of P3 group (81±2.16 U/L) and S3 group (79±2.16 U/L) were higher than that of untreated rabbits (33±0.82 U/L).

Serum glutamic pyruvic transaminase (SGPT), an enzyme that is normally present in liver and heart cells. SGPT is released into blood when the liver or heart is damaged. The blood SGPT levels are thus elevated with liver damage or with an insult to the heart [80]. The present study showed that the SGPT of all heated palm oil and soybean oil diet groups were significantly increased compared with control group. So, it indicates that the liver or heart of heated palm oil and soybean oil diet rabbit groups may be damaged. Rashid et al. [81] who studied the

effects of dietary cooked fats and oils on blood lipids reported that a relatively high SGPT level in palm oil fed group indicates the possible hepatic damage.

The serum glutamic oxaloacetic transaminase (SGOT) was increased in all heated palm oil and soybean oil treated groups with respect to control shown in table 4.7 and 4.8. The SGOT of P3 group (147±0.82 U/L) was higher than that of untreated and all heated oil diet groups.

SGOT is a liver enzymes which made by liver cells. When liver cells are damaged, SGOT leaks out into the bloodstream and the level of SGOT in the blood becomes higher than normal. SGOT is found in parts of the body other than the liver including the heart, kidneys, muscles and brain. When cells in any of those parts of the body are damaged, SGOT can be elevated [82]. In previous study, Nageswari et al. [83] observed a maximum increase in SGOT in rats indicative of myocardial damage in the coconut oil fed group. In addition, this study showed that the SGOT of all heated palm oil and soybean oil fed groups were significantly increased compared with control group. So it indicates that the liver, heart, kidneys, muscles and brain of heated palm oil and soybean oil diet rabbit groups may be damaged.

Alkaline phosphatase (ALP) of heated palm oil and soybean oil fed of all groups were decreased, compared with control group. ALP of P1 group (174±1.63 U/L) and S2 group (141±1.41 U/L) were lower than that of control (260±2.16 U/L) shown in table 4.7 and 4.8.

In previous study, Ayodeji Osmund Falade et al. [51] showed that ALP of heated palm oil diet group was increased. But in present study, the ALP all heated palm oil and soybean oil fed groups were significantly decreased.

Uric acid was increased in all heated palm oil fed groups compared with control shown in table 4.7. Uric acid of P2 group (1.9±0.08 mg/dl) was significantly higher than that of control group (1.4±00 mg/dl). Uric acid of S3 group was decreased compared with control.

Table 4.7 Effect of heated palm oil diets on biochemicall profiles

Parameters	Unit	C n = 3 M_{15}±SD_{15}	P1 n = 3 M_{16}±SD_{16}	P2 n = 3 M_{17}±SD_{17}	P3 n = 3 M_{18}±SD_{18}
Creatinine	mg/dl	0.8, 0.8, 0.8	0.8, 0.9, 1.0	0.7, 0.8, 0.9	0.9, 0.9, 1.2
		0.8±00	0.9±0.08	0.8±0.08	1.0±0.14
SGPT	U/L	32, 33, 34	61, 64, 64	50, 54, 55	78, 82, 83
		33±0.82	63±1.41	53±2.16	81±2.16
SGOT	U/L	35, 38, 38	44, 45, 46	48, 51, 51	146, 147, 148
		37±1.41	45±0.82	50±1.41	147±0.82
ALP	U/L	257, 261, 262	172, 174, 176	233, 233, 236	255, 258, 258
		260±2.16	174±1.63	234±1.41	257±1.41
Uric acid	mg/dl	1.4, 1.4, 1.4	1.6, 1.9, 1.9	1.8, 1.9, 2.0	1.4, 1.6, 1.8
		1.4±00	1.8±0.14	1.9±0.08	1.6±0.16

n= Number of rabbit, M_{15}, M_{16}, M_{17}, M_{18}= Mean value of control (C) group, 2 hours heated palm oil (P1) group, 10 hours heated palm oil (P2) group, 20 hours heated palm oil (P3) group respectively , SD_{15}, SD_{16}, SD_{17}, SD_{18}= Standard deviations of control (C) group, 2 hours heated palm oil (P1) group, 10 hours heated palm oil (P2) group, 20 hours heated palm oil (P3) group respectively.

SGPT= Serum glutamic pyruvic transaminase, SGOT= Serum glutamic oxaloacetic transaminase, ALP= Alkaline phosphatase.

Table 4.8 Effect of heated soybean oil diets on biochemical profiles

Parameters	Unit	C n = 3 M_{15}±SD_{15}	S1 n = 3 M_{19}±SD_{19}	S2 n = 3 M_{20}±SD_{20}	S3 n = 3 M_{21}±SD_{21}
Creatinine	mg/dl	0.8, 0.8, 0.8	0.6, 0.7, 0.8	0.5, 0.6, 07	1.0, 1.1, 1.2
		0.8±00	0.7±0.08	0.6±0.08	1.1±0.08
SGPT	U/L	32, 33, 34	62, 65, 65	59, 61, 63	77, 78, 82
		33±0.82	64±1.41	61±1.63	79±2.16
SGOT	U/L	35, 38, 38	45, 46, 47	51, 54, 54	126, 127, 128
		37±1.41	46±0.82	53±1.41	127±0.82
ALP	U/L	257, 261, 262	175, 177, 179	139, 142, 142	182, 184, 186
		260±2.16	177±1.63	141±1.41	184±1.63
Uric acid	mg/dl	1.4, 1.4, 1.4	1.3, 1.4, 1.5	1.3, 1.3, 1.6	1.3, 1.3, 1.3
		1.4±00	1.4±0.08	1.4±0.14	1.3±00

n= Number of rabbit, M_{15}, M_{19}, M_{20}, M_{21}= Mean value of control (C) group, 2 hours heated soybean oil (S1) group, 10 hours heated soybean oil (S2) group, 20 hours heated soybean oil (S3) group respectively, SD_{15}, SD_{19}, SD_{20}, SD_{21}= Standard deviations of control (C) group, 2 hours heated soybean oil (S1) group, 10 hours heated soybean oil (S2) group, 20 hours heated soybean oil (S3) group respectively.

SGPT= Serum glutamic pyruvic transaminase, SGOT= Serum glutamic oxaloacetic transaminase, ALP= Alkaline phosphatase.

4.7 Effect of heated oil diets on serum lipid profiles

Biochemical profiles of serum lipid as total cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Triglyceride (TG) in blood of heated palm oil and soybean oil diets by rabbits has been observed (table 4.9 and 4.10).

The total cholesterol (TC) of heated palm oil fed all group were increased compared with control group. But the total cholesterol of heated soybean oil fed all group were decreased compared with untreated group. The rabbits of group P3 showed the maximum level of total blood cholesterol (158±2.16 mg/dl) which differ from all other groups shown in table 4.9 and 4.10. In addition, the TC of S2 group (38±1.63 mg/dl) was lower than that of control (96±00 mg/dl) shown in table 4.10.

In previous study Siti Khadijah Adam et al. [37] who reported that the TC level of 5 hours heated palm oil fed group was increased but 1 hours heated palm oil fed group was not significant changed compared with control group. But the present study, the TC of 2 hours, 10 hours, 20 hours heated palm oil diet groups were significantly increased. This study, the TC of heated soybean oil treated all group of rabbits were decreased. So, this study agreed with the study of Hur et al. [84] who reported that heated oil reduced plasma cholesterol in rabbits.

The blood High Density Lipoprotein (HDL) level after supplementation of heated palm oil and soybean oil in different groups of rabbits are presented in the table 4.9 and 4.10. The HDL level of heated palm oil diet all groups of rabbits had significant differences from that of control group. The HDL level of rabbits of group P2 (57±1.63 mg/dl) which differ significantly than control group (26±0.82 mg/dl). The significant increase in HDL has been recorded in rabbits fed with heated palm oil group P1 (43±1.4 mg/dl) and P3 (42±0.82 mg/dl) that differs significantly from the value of control group. On the other hand, the HDL of all heated soybean oil fed group were decreased compared with control group. The HDL of S2 group (12±1.41 mg/dl) was significantly lower than that of control (26±0.82 mg/dl) shown in

table 4.10. So the present study support Chinu Chacko et al. [85] and Siti Khadijah Adam et al. [38].

The blood Low Density Lipoprotein (LDL) of heated palm oil and soybean oil fed groups are presented in table 4.9 and 4.10. The LDL of palm oil fed P1 and P2 groups were decreased but P3 group was increased compared with control group. But the LDL of heated soybean oil fed all group were decreased compared with control group. The rabbits of group P3 showed the maximum level of LDL (81±1.41 mg/dl) which differ significantly from all other groups shown in table 4.9 and 4.10. In addition, the LDL of S1 group (10±00 mg/dl) was significantly lower than that of control (61±2.45 mg/dl) shown in table 4.10.

This study showed that the LDL of all heated palm and soybean oil fed groups of rabbits except P3 group were decreased compared with control group. So, this study partially agrees with Kamsiah Jaarin et al. [62] who showed that the LDL of 5 time heated soybean and palm oil fed group were increased.

The effects of heated palm oil and soybean oil on triglyceride (TG) level of different groups of rabbits are presented in table 4.9 and 4.10. The TG level of heated palm and soybean oil fed all groups of rabbits were increased compared with control group. TG of P3 group (176±0.82 mg/dl) and S1 group (81±0.82 mg/dl) were significantly higher than that of control group.

The present study showed increase in TG concentration of heated palm and soybean oil fed all groups of rabbits. This study was in agreement with the results of Chinu Chacko et al. [85], Shastry et al. [86] and Islam Uddin et al. [87].

Table 4.9 Effect of heated palm oil diets on Lipid profiles

Parameters	Unit	C n = 3 $M_{22}\pm SD_{22}$	P1 n = 3 $M_{23}\pm SD_{23}$	P2 n = 3 $M_{24}\pm SD_{24}$	P3 n = 3 $M_{25}\pm SD_{25}$
TC	mg/dl	96, 96, 96	127, 128, 129	125, 126, 127	155, 159, 160
		96±00	128±0.82	126±0.82	158±2.16
HDL	mg/dl	25, 26, 27	41, 44, 44	55, 57, 59	41, 42, 43
		26±0.82	43±1.41	57±1.63	42±0.82
LDL	mg/dl	58, 61, 64	51, 53, 55	42, 43, 44	79, 82, 82
		61±2.45	53±1.63	43±0.82	81±1.41
TG	mg/dl	44, 44, 44	156, 157, 161	128, 128, 131	175, 176, 177
		44±00	158±2.16	129±1.41	176±0.82

n= Number of rabbit, M_{22}, M_{23}, M_{24}, M_{25}= Mean value of control (C) group, 2 hours heated palm oil (P1) group, 10 hours heated palm oil (P2) group, 20 hours heated palm oil (P3) group respectively , SD_{22}, SD_{23}, SD_{24}, SD_{25}= Standard deviations of control (C) group, 2 hours heated palm oil (P1) group, 10 hours heated palm oil (P2) group, 20 hours heated palm oil (P3) group respectively.

TC= Total Cholesterol, HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein, TG= Triglyceride.

Table 4.10 Effect of heated soybean oil diets on Lipid profiles

Parameters	Unit	C n = 3 $M_{22}\pm SD_{22}$	S1 n = 3 $M_{26}\pm SD_{26}$	S2 n = 3 $M_{27}\pm SD_{27}$	S3 n = 3 $M_{28}\pm SD_{28}$
TC	mg/dl	96, 96, 96	42, 42, 42	36, 38, 40	45, 47, 49
		96±00	42±00	38±1.63	47±1.63
HDL	mg/dl	25, 26, 27	15, 16, 17	11, 11, 14	16, 19, 19
		26±0.82	16±0.82	12±1.41	18±1.41
LDL	mg/dl	58, 61, 64	10, 10, 10	11, 11, 11	13, 14, 15
		61±2.45	10±00	11±00	14±0.82
TG	mg/dl	44, 44, 44	80, 81, 82	77, 77, 77	75, 76, 77
		44±00	81±0.82	77±00	76±0.82

n= Number of rabbit, M_{22}, M_{26}, M_{27}, M_{28}= Mean value of control (C) group, 2 hours heated soybean oil (S1) group, 10 hours heated soybean oil (S2) group, 20 hours heated soybean oil (S3) group respectively , SD_{22}, SD_{26}, SD_{27}, SD_{28}= Standard deviations of control (C) group, 2 hours heated soybean oil (S1) group, 10 hours heated soybean oil (S2) group, 20 hours heated soybean oil (S3) group respectively.

TC= Total Cholesterol, HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein, TG= Triglyceride.

4.8 Histopathological study of heated oil diets on rabbits

In the present study, liver, kidney, heart, lung, brain and spleen of rabbits were histopathological examined for the detection of pathological lesions if any. In histopathology, some specific lesions are found in the liver, kidney, heart, lung, brain and spleen of heated palm oil and soybean oil diet groups as compared with the control rabbits.

4.8.1 Histopathological study of liver of rabbits

Histological structures of liver of rabbits are presented in figure 4.9 and 4.10. Control group showed the liver is divided into hepatic lobules formed of radially arranged strands of hepatocytes that extend from the central vein to periphery of the lobule. The hepatocytes strands are separated from each other by blood sinusoids that are lined with the endothelial cells and Kupffer cells (a) (Image CLi). 2 hours heated palm oil diet group P1 showed fatty degenerative change and the portal area showing severe dilatation (b), vacuolation of central vein and hepatocytes (c) (Image P1Li). 10 hours heated palm oil diet group P2 showed abnormal shape of central vein (d), swollen cell with chronic inflammatory cell infiltration (e), tissue lost its attachment and vacuolation (f) (Image P2Li). 20 hours heated palm oil diet group P3 showed vacuolation and abnormal shape of central vein (g) and the liver cells were swollen (h) (Image P3Li).

On the other hand, 2 hours heated soybean oil diet group S1 showed abnormal shape of central vein and hemorrhage around the central vein (i), vacuolation (j) and edema (k) (Image S1Li). 10 hours heated soybean oil diet group S2 showed enlarged central vein (l), focal inflammatory cells infiltration was abundant (m), focal Kupffer cells proliferation and the hepatocytes appeared with cytoplasmic vacuolation and pyknotic nuclei (n) (Image S2Li). 20 hours heated soybean oil diet group S3 showed cell debris inside the central vein (o), coagulation necrosis (p) and tissue lost its attachment and hemorrhage (q) (Image S3Li).

4.8.2 Histopathological study of kidney of rabbits

Figure 4.11 and 4.12 showing histological structures of kidney of rabbits. Histological examination of the kidney of control group of rabbits revealed normal histological features. Control group showed normal histological structure of glomeruli (a) and renal tubules of kidneys in rabbits (b) (Image CK). 2 hours heated palm oil diet group P1 showed cells in the medullary region vacuoles (c), focal inflammatory cells infiltration in between the tubules associated with dilatation in the blood vessels (d) and the glomerular tuft showed vacuolization in the lining endothelium (e) (Image P1K). 10 hours heated palm oil diet group P2 showed abnormal shape of glomeruli (f), vacuolation (g), atrophy of a glomerulus with degeneration in the lining epithelial cells of renal tubules and edema of tissue (h) (Image P2K). 20 hours heated palm oil diet group P3 showed blood capillaries in between the degenerated tubules were congested (i), vacuolation (j), eroded wall of bowman's capsule (k), cell debris inside the glomeruli and edema of tissue (l) (Image P3K).

2 hours heated soybean oil diet group S1 showed vacuolation (m), congested glomeruli (n) and cells in the medullary region vacuoled with occasional tubule containing esinophilic materials (o) (Image S1K). 10 hours heated soybean oil diet group S2 showed hemorrhage (p), shrinked glomeruli and focal fibrosis between renal tubules (q) (Image S2K). 20 hours heated soybean oil diet group S3 showed hemorrhage (r), congestion in blood capillaries in between the degenerated renal tubules (s) and the glomerular showing vacuolization (t) (Image S3K).

4.8.3 Histopathological study of heart of rabbits

Histological examination of the heart of control group of rabbits showed normal structure (figure 4.13 and 4.14). Control group showed normal arrangement of cardiac muscular layer (a) (Image CH). 2 hours heated palm oil diet group P1 showed congested myocardial (b), few vacuolation in papillary muscle and splitting of longitudinal muscles (c) (Image P1H). 10 hours heated palm oil diet group P2 showed hemorrhage (d), vacuolation (e), myocardial degeneration and necrosis (f) (Image P2H). 20 hours heated palm oil diet group P3 showed

hemorrhage (g), vacuolation (h), hypertrophy of cardiac muscle (i) and thickening of the ventricular septum and brown atrophy (j) (Image P3H).

2 hours heated soybean oil diet group S1 showed brown atrophy (k), fragmentation of myocardial nucleus fibres with extensive infiltration (l) and splitting of longitudinal muscles (m) (Image S1H). 10 hours heated soybean oil diet group S2 showed myocardial degeneration (n), extensive infiltration (o), vacuolation (p) and edema (q) (Image S2H). 20 hours heated soybean oil diet group S3 showed vacuolation (r), edema (s) and cellular infiltration and congested myocardial (t) and vacuolation in papillary muscle (u) (Image S3H).

4.8.4 Histopathological study of lung of rabbits

Histological structures of lung of rabbits are showing in figure 4.15 and 4.16. Untreated rabbits showed the lung pulmonary tissues compact configuration with airway, interalveolar septa, regular alveolar sacs and capillaries (Image CL). Bronchiolar (a) and alveolar (b) structures in the control group in their normal structures. 2 hours heated palm oil diet group P1 showed lung tissue containing collagen fiber accumulation along with distinctive cell proliferation (c) and mononuclear cell invasion in the alveolar septa (d) (Image P1L). 10 hours heated palm oil diet group P2 showed a bronchus lined with pseudostratified epithelium and containing lymphocytes in their lamina propria and surrounding these structures the saccus alveolaris (e), alveoli with regular walls, interalveolar septa (f) and interalveolar connections connecting the alveoli to each other were observed in the lung parenchyma (g) (Image P2L). 20 hours heated palm oil diet group P3 showed inhalation (h), revealed lymphocyte infiltration into the interalveolar septa and also into the bronchiolar lamina propria (i) and the structural organization of the alveoli seemed to be disturbed and the interalveolar septa were thickened (j) (Image P3L).

2 hours heated soybean oil diet group S1 showed increased accumulation of inflammatory cells that vacuolated the bronchioles and the alveolar sac (k), with thickness of alveolar smooth muscle and trachea (l) (Image S1L). 10 hours heated soybean oil diet group S2 showed histological appearance, increase in infiltration with inflammatory cells (m) and

relatively unclear bronchial and alveolar sacs (n) with remarkable increase in the thickness of alveolar epithelium and tracheal smooth muscles (o) (Image S2L). 20 hours heated soybean oil diet group S3 showed a terminal bronchiole with the epithelium (p) and the underlying strip of smooth muscle (q), in addition to the respiratory bronchiole, alveolar ducts, alveolar sacs (r) and numerous alveoli separated by alveolar septum (s) (Image S3L).

4.8.5 Histopathological study of Brain of rabbits

Untreated and heated oils treated rabbits histological structures of brain are presented in figure 4.17 and 4.18. Image CB showed the histological structure of brain of control rabbits. Normal histological structure of the meninges (a) and cerebral cortex (b) were showed in brain of control rabbits. 2 hours heated palm oil diet group P1 showed the medulla oblongata vacuolation (c) in the matrix (Image P1B). 10 hours heated palm oil diet group P2 showed the deep cerebrum had fat vacuoles in the matrix (d) as well as focal gliosis (e) (Image P2B). 20 hours heated palm oil diet group P3 showed edema of tissue (f), necrosis (g) and vacuolation in the matrix with focal gliosis (h) (Image P3B).

On the other hand, 2 hours heated soybean oil diet group S1 showed the vacuolation (i) in the matrix (Image S1B). 10 hours heated soybean oil diet group S2 showed the vaculation in the matrix (j), necrosis of the tissue (k) (Image S2B). 20 hours heated soybean oil diet group S3 showed blood hemorrhage (l), vaculation in the matrix (m) (Image S3B).

4.8.6 Histopathological study of spleen of rabbits

Figure 4.19 and 4.20 showing histological structure of spleen of rabbits. Image CS showed the normal splenic structure of control rabbits. The histopathological examination of spleen of the control group showed normal structure which composed of normal white and red pulp (a), normal capsule and blood vessels (b). 2 hours heated palm oil diet group P1 showed atrophy in white pulp and edematous in red pulp (c), vaculation in matrix (d) (Image P1S). 10 hours heated palm oil diet group P2 showed atrophy of lymphoid tissue of white pulp (e), while the red pulp showed foamy vacuolated macrophages (f) (Image P2S). In addition, 20 hours heated

palm oil diet group P3 showed severe atrophy of lymphoid tissue of white pulp (g) while the red pulp showed foamy vacuolated macrophages (h), congestion of sinusoids (i) (Image P3S).

2 hours heated soybean oil diet group S1 showed severe depletion and necrosis in the lymphocytes of most white pulp (j), the red pulp showed foamy vacuolated macrophages (k) (Image S1S). 10 hours heated soybean oil diet group S2 showed atrophy in white pulp (l) and edematous in red pulp (m), vacuolated macrophages (n), congestion of sinusoids (o) and hemosiderin laden macrophages (p) (Image S2S). 20 hours heated soybean oil diet group S3 showed atrophy of lymphoid tissue of white pulp (q), the red pulp showed foamy vacuolated macrophages (r), congestion around blood vessels with fibrosis figure (s) (Image S3S).

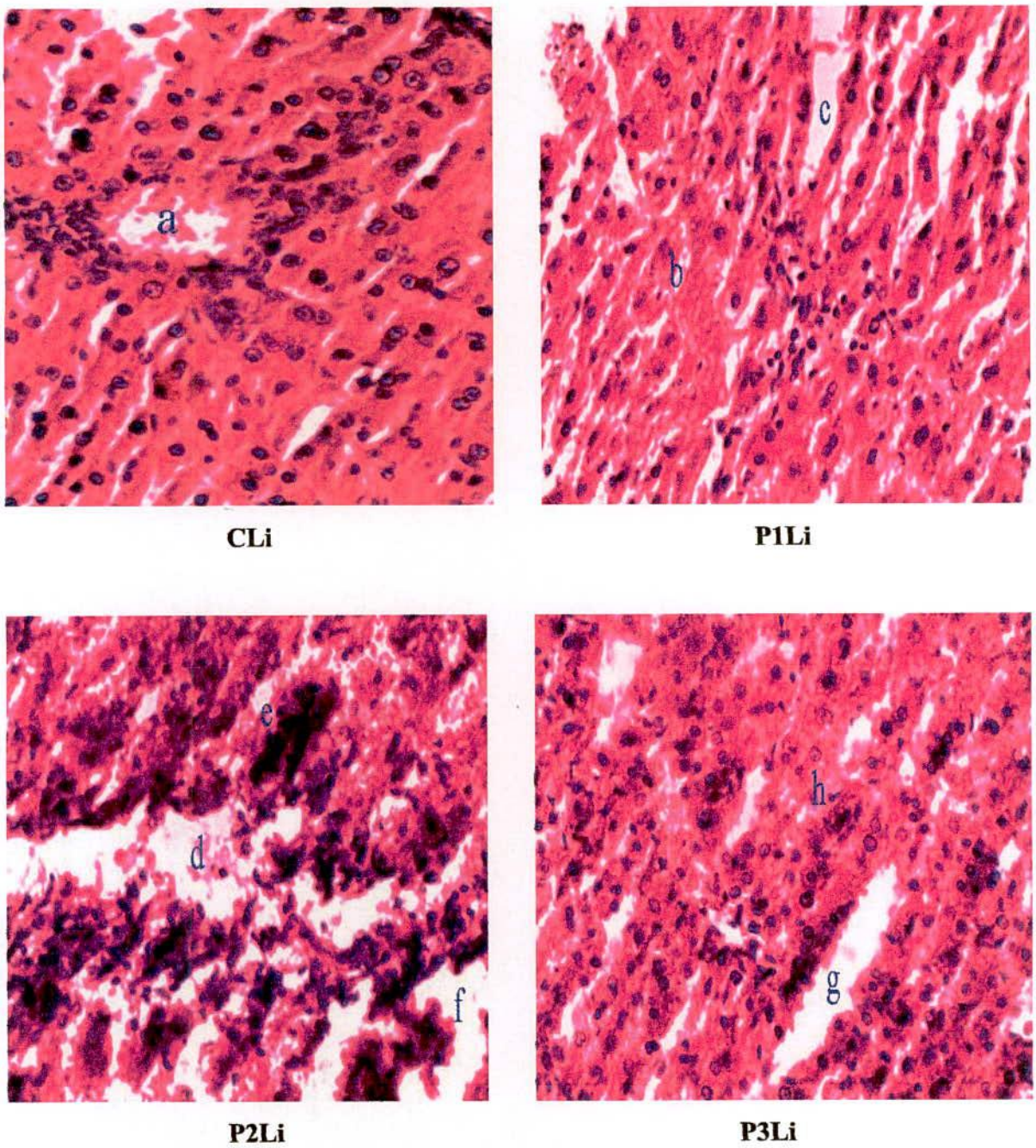
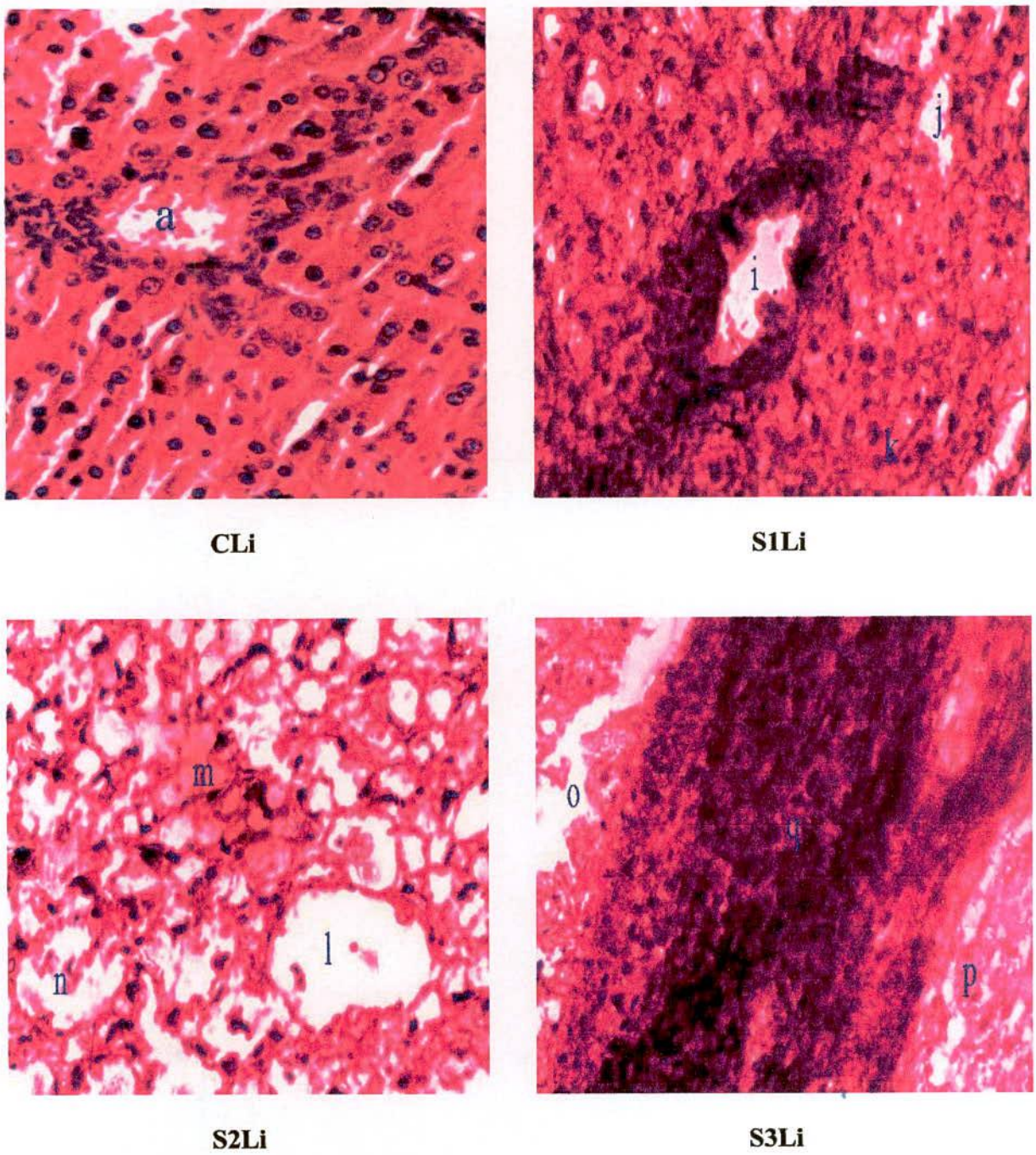


Figure 4.9: Histopathological section of liver of rabbits (Hematoxylin & Eosin x 200) (CLi) Control group liver; (P1Li) 2 hours heated palm oil diet group liver; (P2Li) 10 hours heated palm oil diet group liver; (P3Li) 20 hours heated palm oil diet group liver.



CLi **S1Li**

S2Li **S3Li**

Figure 4.10: Histopathological section of liver of rabbits (Hematoxylin & Eosin x 200) (CLi) Control group liver; (S1Li) 2 hours heated soybean oil diet group liver; (S2Li) 10 hours heated soybean oil diet group liver; (S3Li) 20 hours heated soybean oil diet group liver.

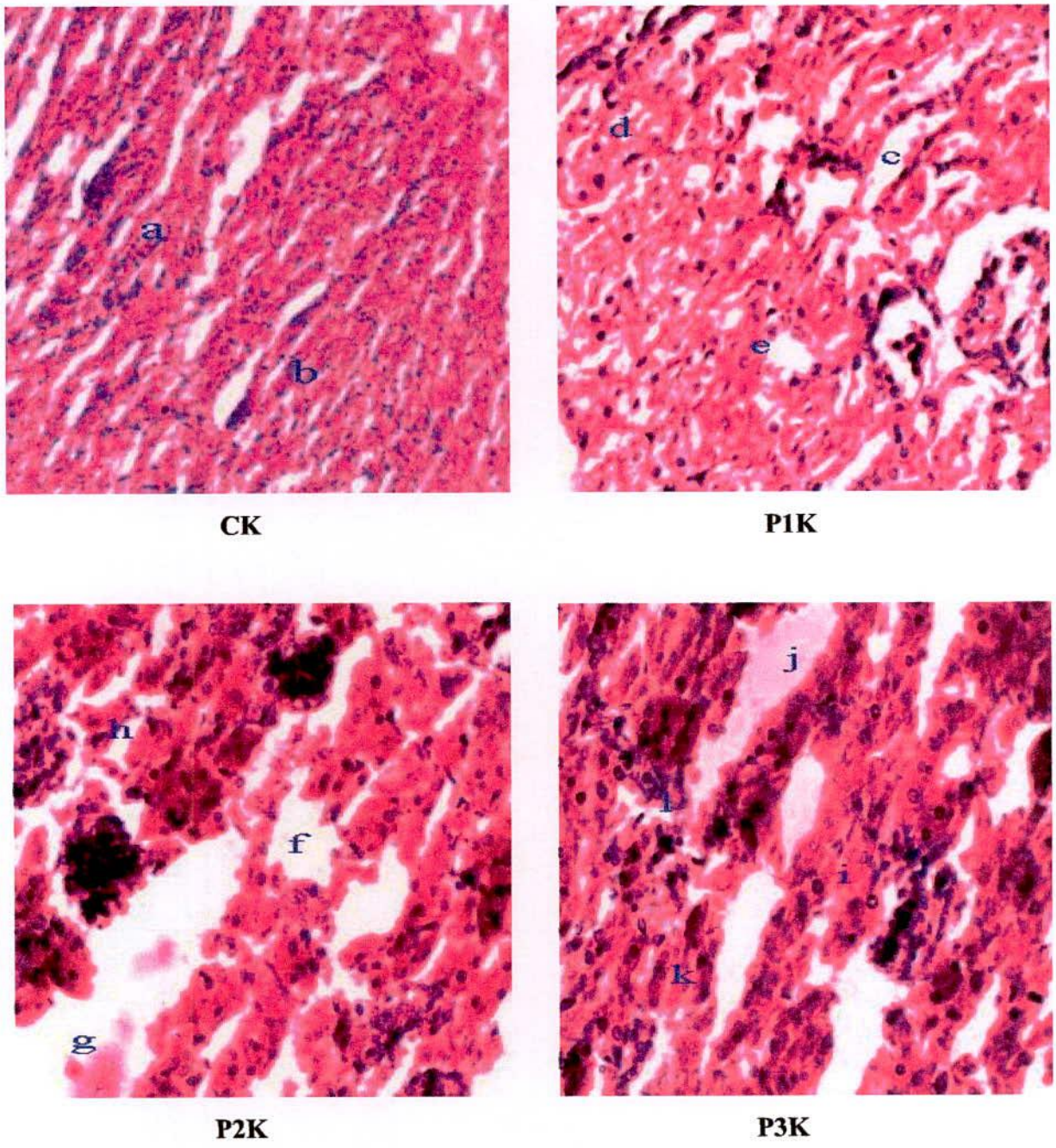
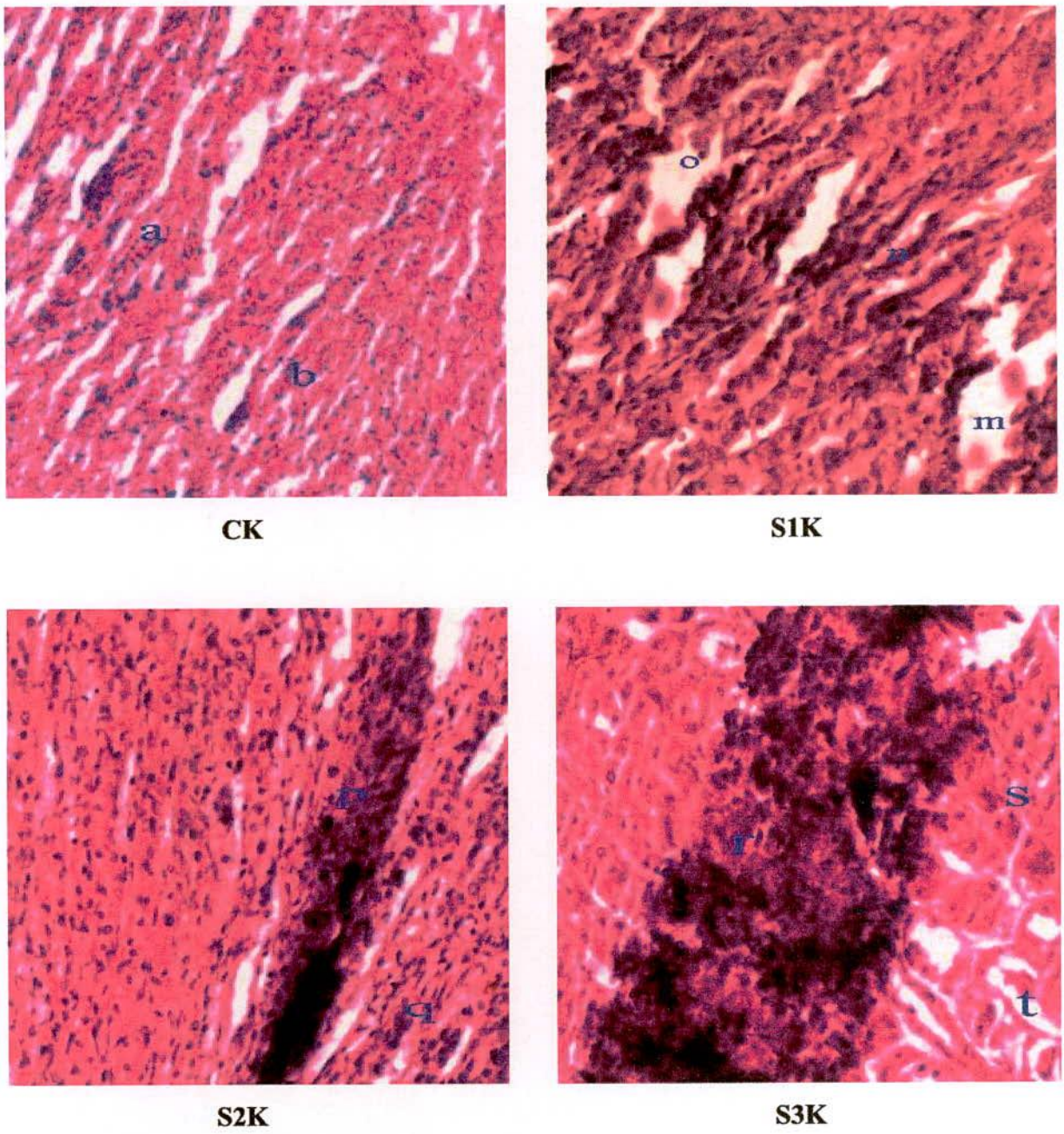


Figure 4.11: Histopathological section of kidney of rabbits (Hematoxylin & Eosin x 200) (CK) Control group kidney; (P1K) 2 hours heated palm oil diet group kidney; (P2K) 10 hours heated palm oil diet group kidney; (P3K) 20 hours heated palm oil diet group kidney.



CK **S1K**

S2K **S3K**

Figure 4.12: Histopathological section of kidney of rabbits (Hematoxylin & Eosin x 200) (CK) Control group kidney; (S1K) 2 hours heated soybean oil diet group kidney; (S2K) 10 hours heated soybean oil diet group kidney; (S3K) 20 hours heated soybean oil diet group kidney.

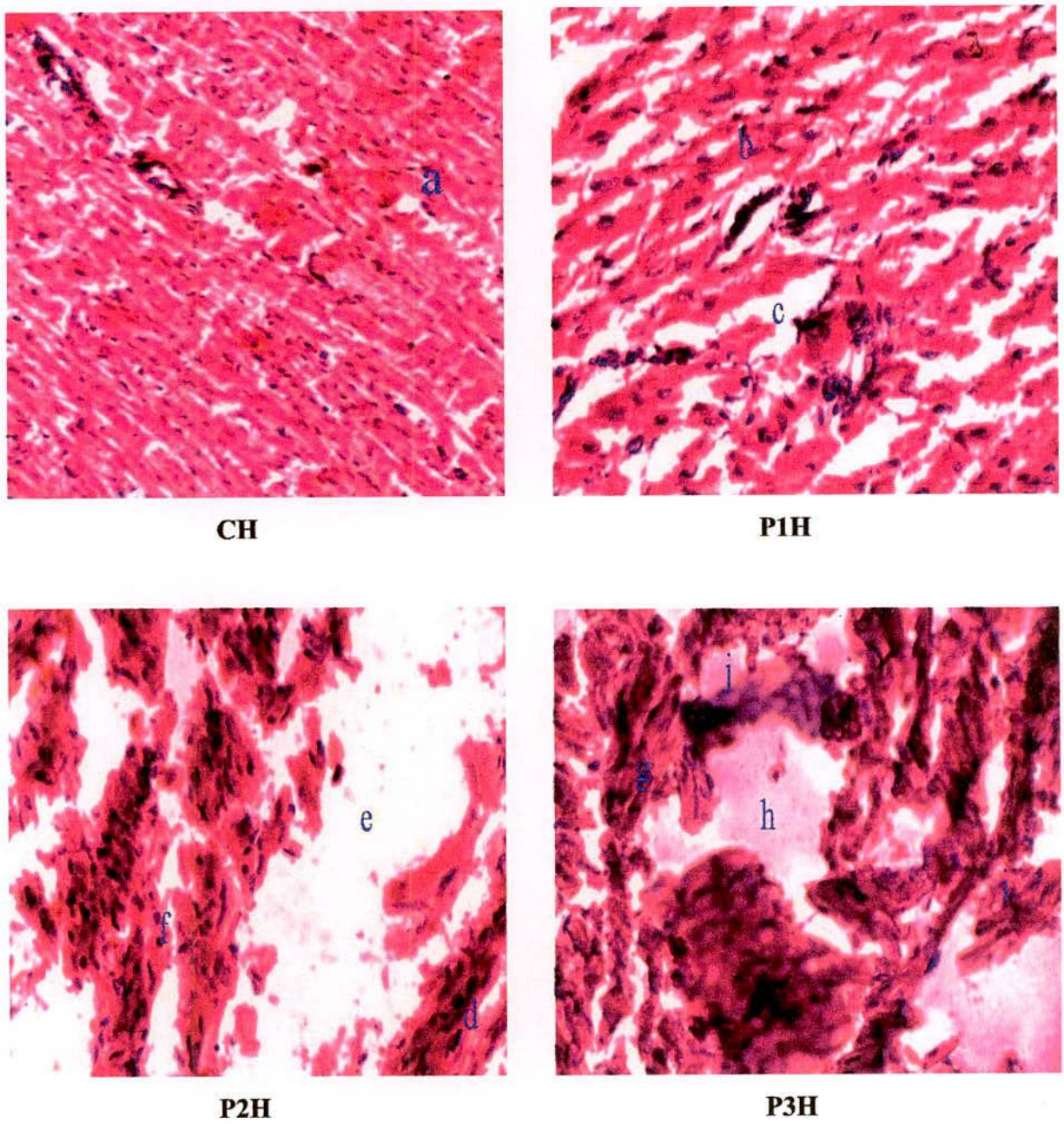


Figure 4.13: Histopathological section of heart of rabbits (Hematoxylin & Eosin x 200) (CH) Control group heart; (P1H) 2 hours heated palm oil diet group heart; (P2H) 10 hours heated palm oil diet group heart; (P3H) 20 hours heated palm oil diet group heart.

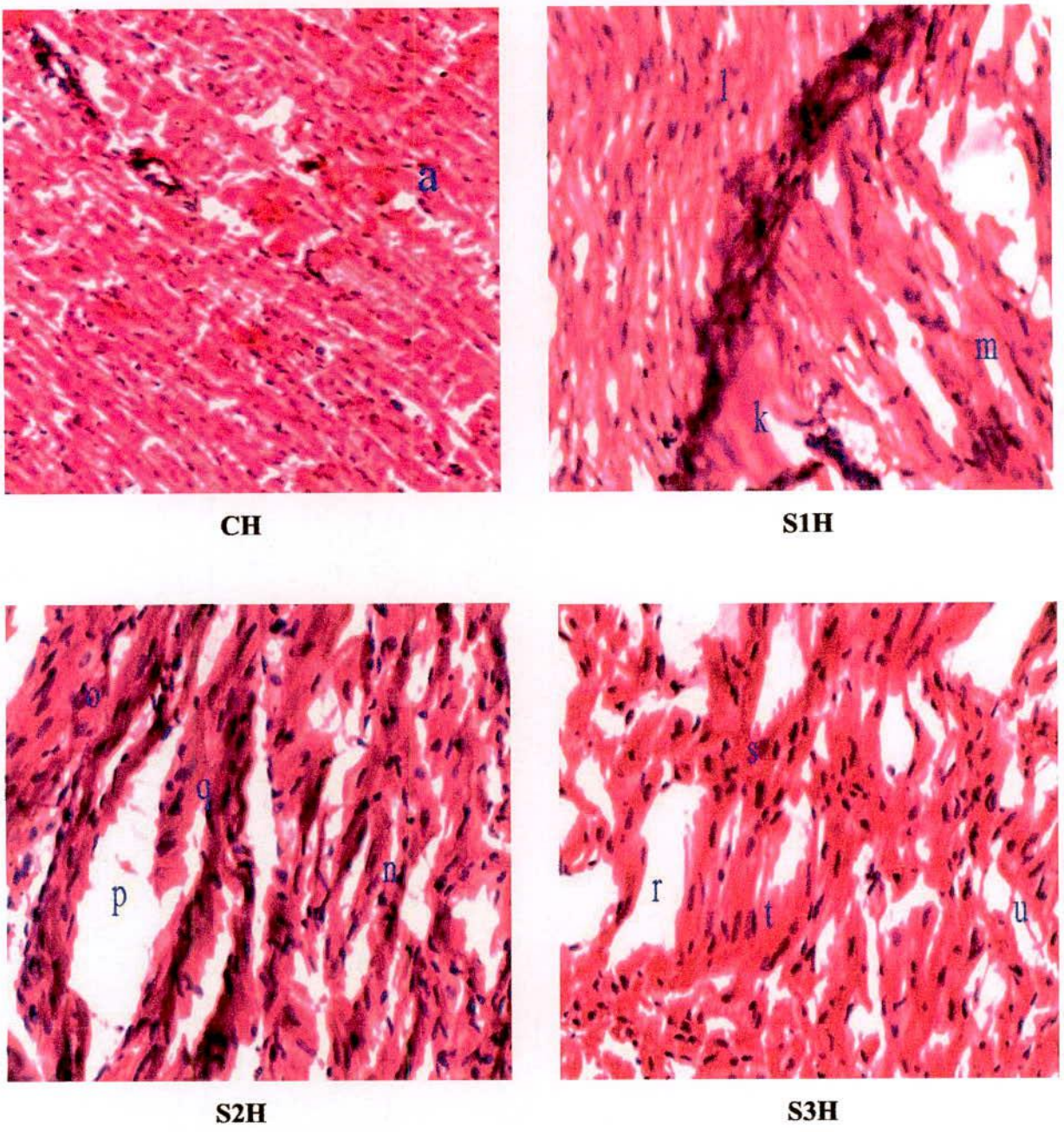


Figure 4.14: Histopathological section of heart of rabbits (Hematoxylin & Eosin x 200) (CH) Control group heart; (S1H) 2 hours heated soybean oil diet group heart; (S2H) 10 hours heated soybean oil diet group heart; (S3H) 20 hours heated soybean oil diet group heart.

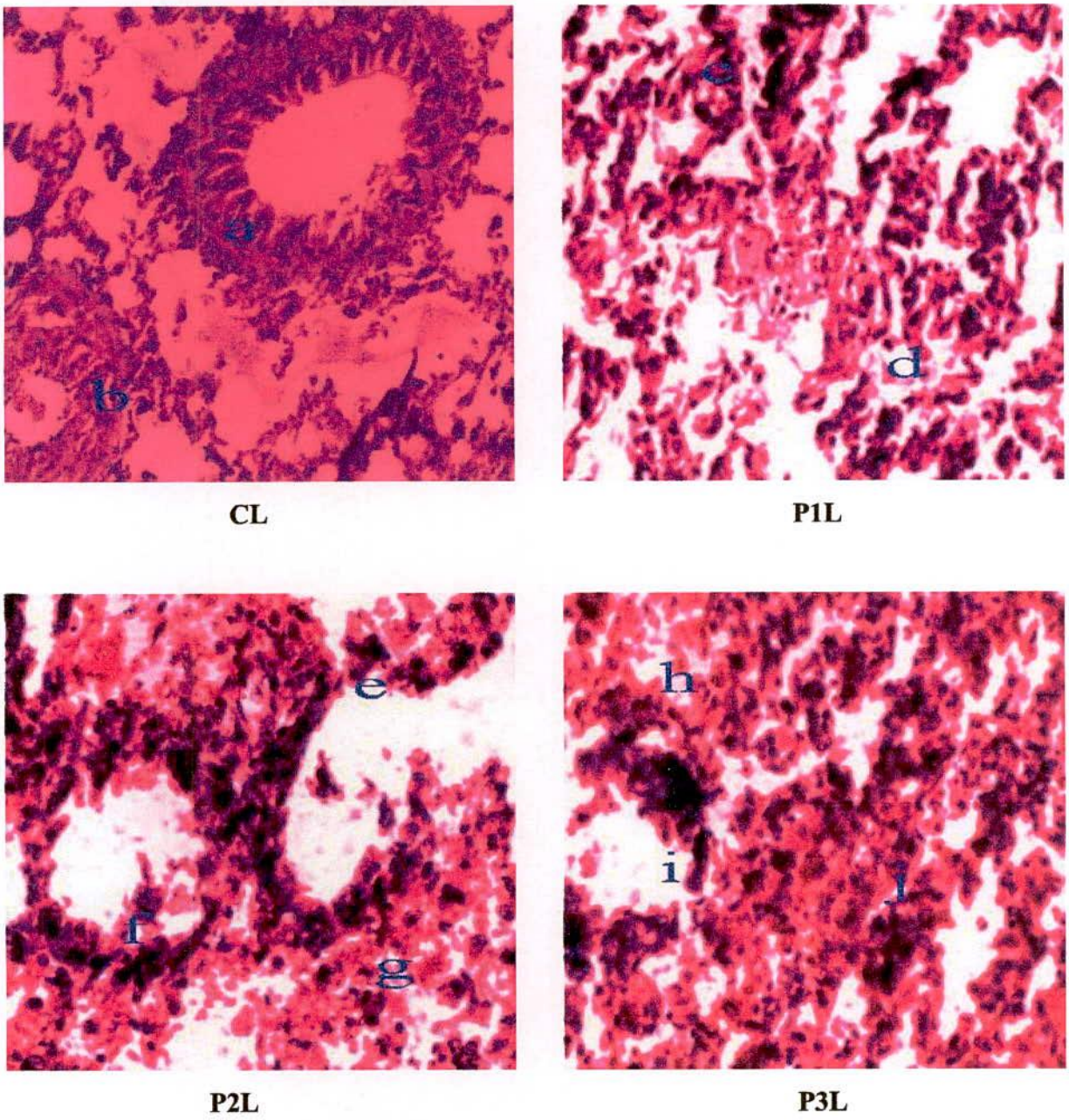
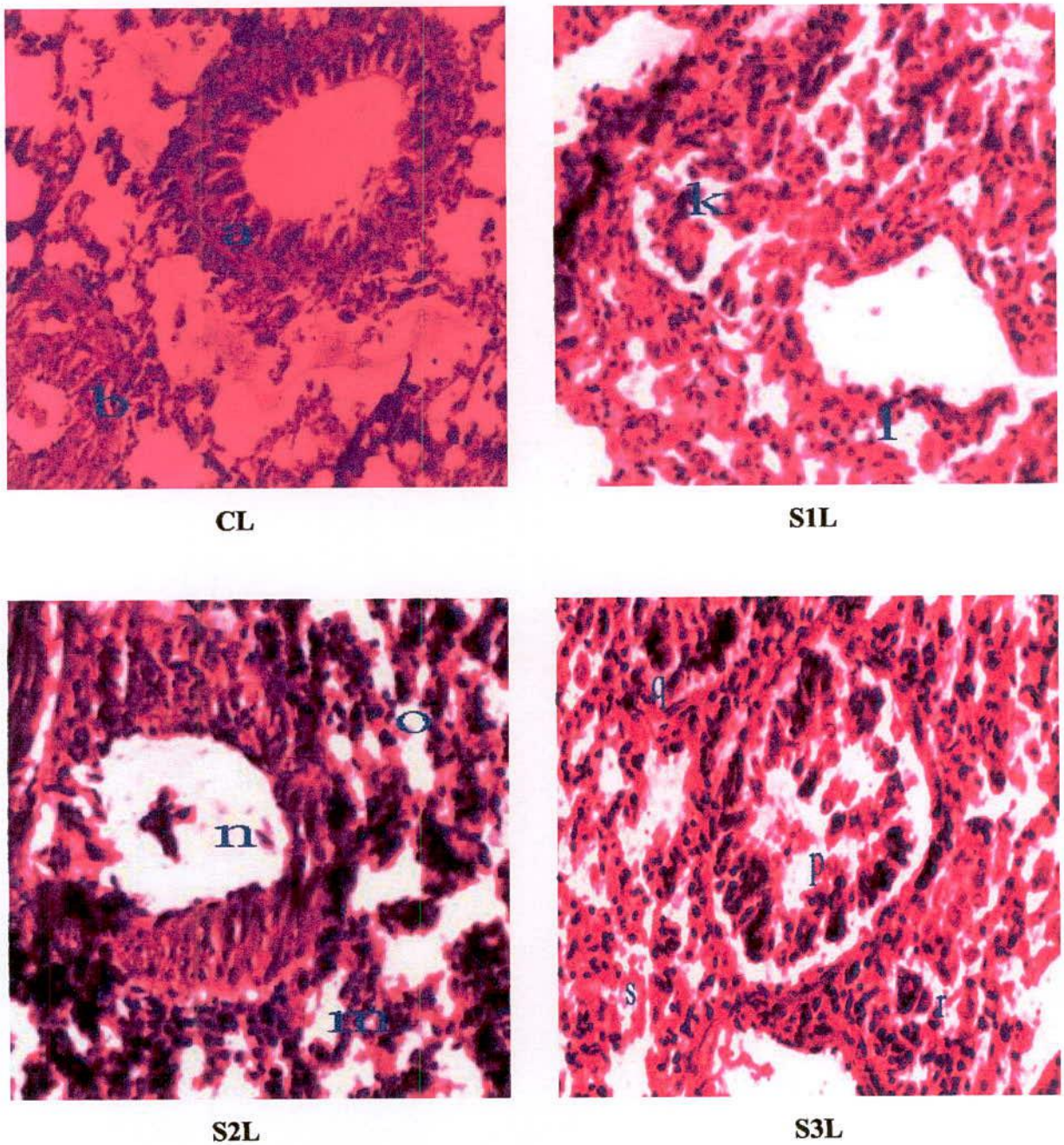


Figure 4.15: Histopathological section of Lung of rabbits (Hematoxylin & Eosin x 200) (CL) Control group lung; (P1L) 2 hours heated palm oil diet group lung; (P2L) 10 hours heated palm oil diet group lung; (P3L) 20 hours heated palm oil diet group lung.



CL **S1L**

S2L **S3L**

Figure 4.16: Histopathological section of Lung of rabbits (Hematoxylin & Eosin x 200) (CL) Control group lung; (S1L) 2 hours heated soybean oil diet group lung; (S2L) 10 hours heated soybean oil diet group lung; (S3L) 20 hours heated soybean oil diet group lung.

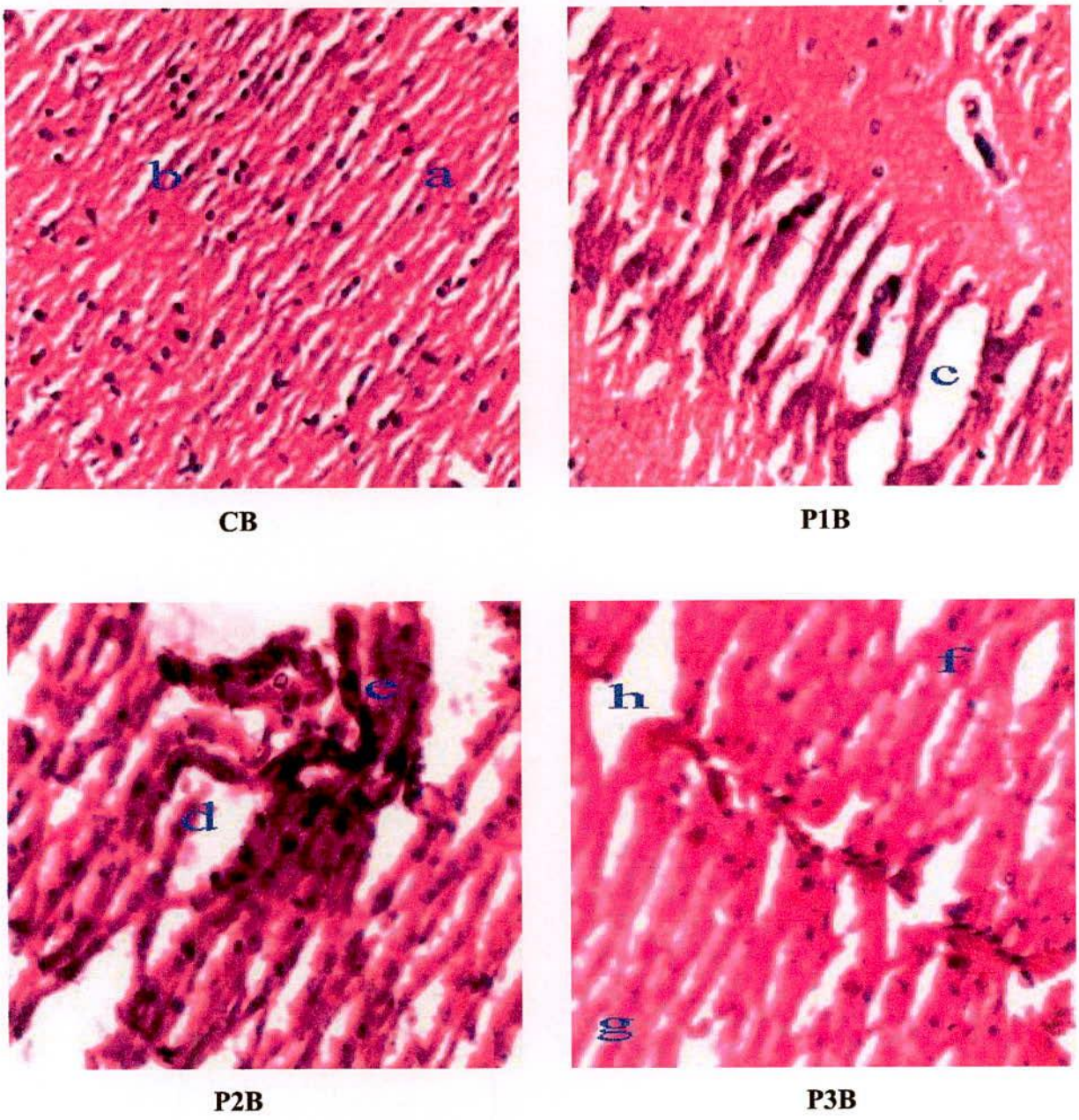


Figure 4.17: Histopathological section of Brain of rabbits (Hematoxylin & Eosin x 200) (CB) Control group brain; (P1B) 2 hours heated palm oil diet group brain; (P2B) 10 hours heated palm oil diet group brain; (P3B) 20 hours heated palm oil diet group brain.

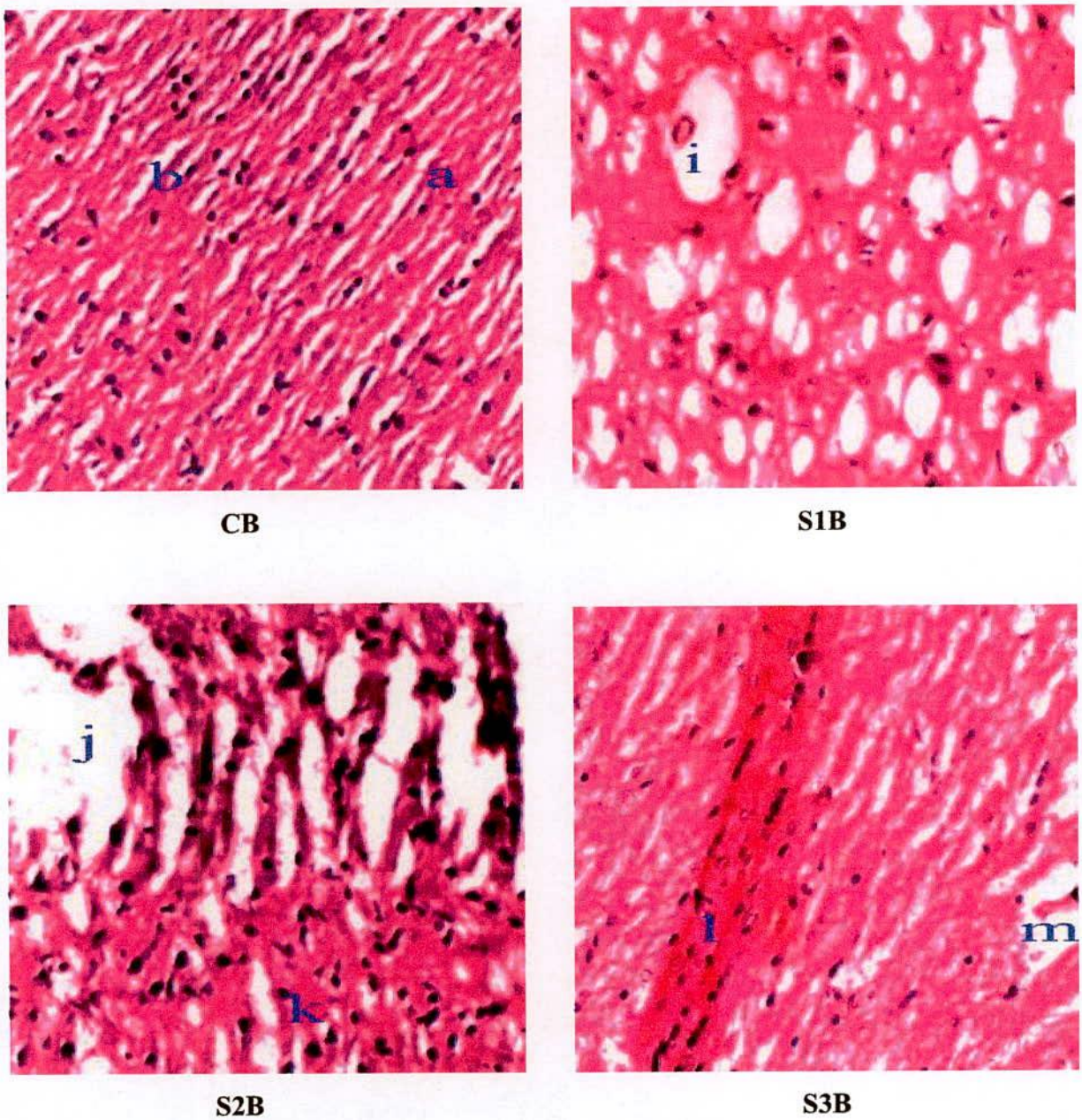


Figure 4.18: Histopathological section of Brain of rabbits (Hematoxylin & Eosin x 200) (CB) Control group brain; (S1B) 2 hours heated soybean oil diet group brain; (S2B) 10 hours heated soybean oil diet group brain; (S3B) 20 hours heated soybean oil diet group brain.

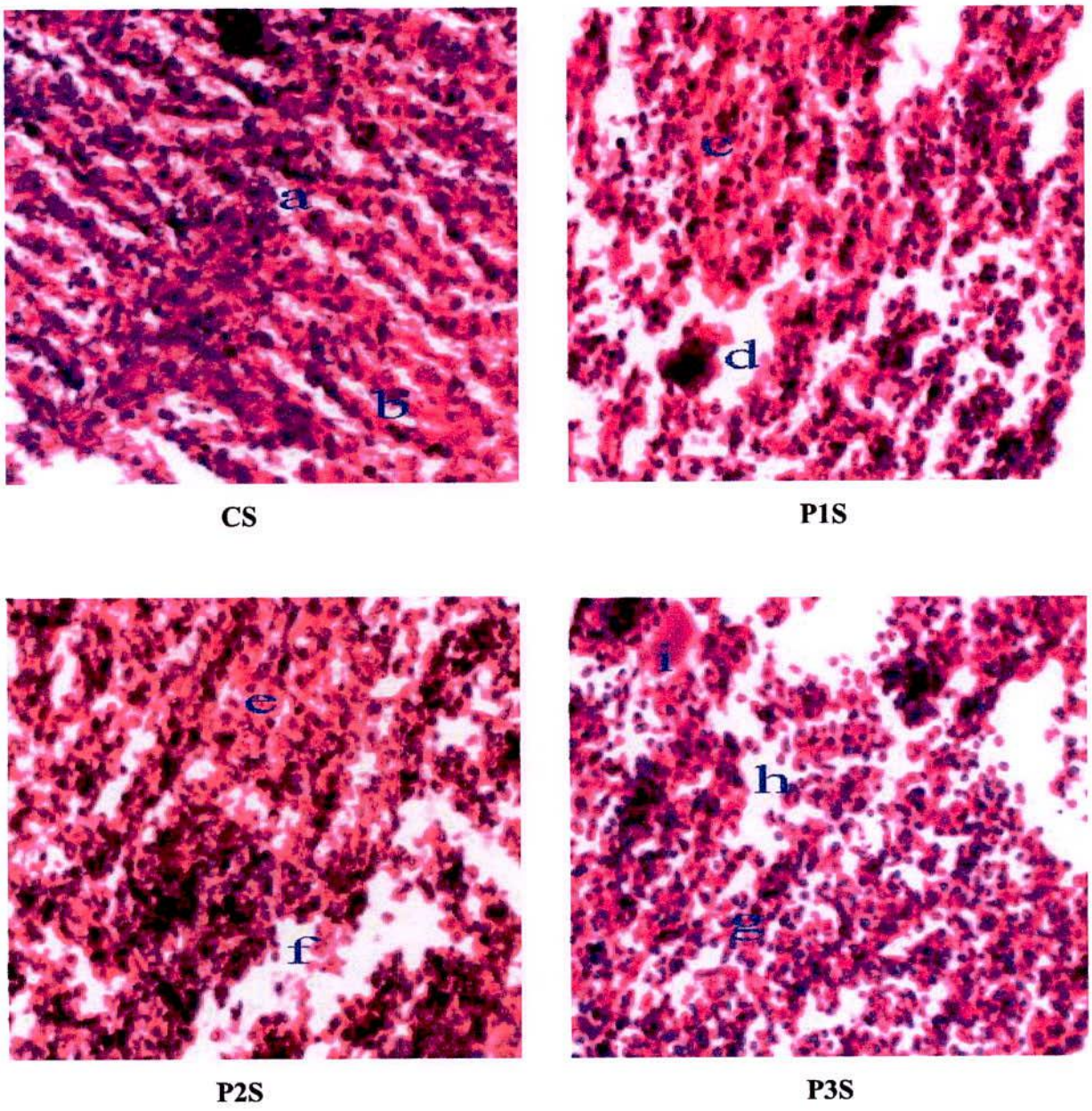


Figure 4.19: Histopathological section of Spleen of rabbits (Hematoxylin & Eosin x 200) (CS) Control group spleen; (P1S) 2 hours heated palm oil diet group spleen; (P2S) 10 hours heated palm oil diet group spleen; (P3S) 20 hours heated palm oil diet group spleen.

CHAPTER V

Conclusions and Recommendations

Edible oils are vegetable oils that are used for cooking. Upon heating, the physico-chemical, nutritional properties of the oil can be changed. Consumption of heated oils diets had deleterious effects on body weights, hematological parameters, biochemical profiles, lipid profiles and organs of rabbits. From this study, the obtained results can be presented as:

i. The iodine values of heated palm and soybean oil were decreased whereas the acid values were increased. The spectra of unheated and heated edible oils showed very similar FT-IR spectra.

ii. The body weights of all treated groups were increased with respect to control rabbits. The highest increase in body weight was observed in 20 hours heated oils diet groups.

iii. The WBC of all heated palm and soybean oil treated groups were higher than that of untreated rabbits but the RBC and hemoglobin of treated groups were decreased.

iv. The creatinine, SGPT, SGOT and uric acid of all treated rabbits were increased by chronic consumption of heated oils with respect to control rabbits.

v. The cholesterol, LDL, HDL and triglyceride of heated palm oil treated groups were increased whereas the values were decreased for heated soybean oil treated rabbits compared to untreated one.

vi. Histological studies of treated rabbit's organs showed strong abnormalities in comparison to control group.

The results of this study indicate that heated oils may be hazardous to the consumer's health. Long time heated oils consumption may occur certain diseases such as infection, stress, inflammation, allergy, anaemia, liver cirrhosis, hepatocellular disease, renal failure, pulmonary infarction, atherosclerosis (coronary artery disease) that means myocardial

infarction of the consumers. Liver, heart, kidney, lung, brain, spleen and muscles of users may be damaged by chronic consumption of heated edible oils. So this study recommends that long time heated palm and soybean oil consumption may be harmful for animal body.

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