PHYSICO CHEMICAL, NUTRITIONAL AND TOXICOLOGICAL EVALUATION OF THERMALLY OXIDISED EDIBLE OILS

BY

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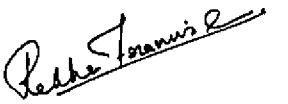
Dedicated to

The Eternal Power guiding me

DECLARATION

I hereby declare that this thesis entitled "**Physico chemical nutritional and toxicological evaluation of thermally oxidized edible oils**" is a bonafide record of the research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.



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INTRODUCTION

INTRODUCTION

Kerala, the land of coconut is famous for intellectual power, mental stability and long life of inhabitants for centuries. Coconut and coconut oil are introduced into the day to day life of Keralites some 500 years ago. This is a daily edible item to almost 99.9 per cent of the population of Kerala (Vidya Prakash, 1994).

For ages people of Kerala have been using coconut oil for cooking. They enjoy food cooked in coconut oil better than any other oil. It is used among Keralites more than any other part of India. Coconut oil is used traditionally as an additive to curries to improve appetite. Since people are acquainted with its use traditionally the allegation that it creates heart disease seems illogical. For the last fifty years the whole world had been (Keralites in particular) made to believe that coconut and coconut oil are most dangerous food to man and that no one who is really interested in not getting a heart attack should touch them even with a barge pole. In Kerala cooking oil used per day per head is only 15-30 grams. But when both coconut is being

eaten along with oil consumption comes to about 25-40 grams per head (George, 1994).

Coming to nutritional significance of fats and oils they are important components of human diet. They represent highly concentrated source of energy and they delay hunger by slow digestion. These oils and fats also have some important roles in maintaining our health and nutrition.

Among the cooking trends of today, deep fat frying is one of the common methods of preparing food in the Indian culinary system. There is a practice of using edible oils for repeated deep fat frying. In oils and fats various physical and chemical changes occur during repeated heating at high temperature. Effect of intake of thermally damaged oils is a important aspect from point of view of the consumer, regarding health especially in todays changing trend in food habits where most of the people resort to deep fat fried snacks and fast foods.

The deteriorative changes occuring in reheated oils have received much attention from oil chemists, biochemists and nutritionists. The possible toxicity and changes in

fatty acid composition of coconut oil which is the most widely used frying oil in our state has not been studied indepth so far. Hence the present investigation is undertaken with the following objectives.

1. To study the Progressive changes in physical and chemical qualities during reheating of oils.

2. To study the Changes in essential and free fatty acid composition during repeated heating

3. To study the Proximate composition of oil heated to extreme conditions to see whether it becomes carcinogenic or not.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Lipid oxidation is an extremely complex process involving numerous reactions that give rise to a variety of physical and chemical changes. Since oxidative decomposition is a major significance in regard to both the acceptability and nutritional quality of food products (Fennema, 1979).

One of the fastest growing industries is that of food service which requires enormous quantities of cooking oils. In fast food services frying fats are subjected to severe conditions ie high temperature for 10-12 hrs/day for 10-14 day periods, generally in uncovered deep fryers. According to Thomas and John (1985) the combination of heat, air, light, moisture and fragments of food being fried along with type of frying operations contributes to oxidation of fats.

Fennema (1979) noted the deteriorative changes in lipids ie lipolytic, oxidative, polymeric or other degradative mechanisms which affect both physical as well as biological properties of lipids. Other changes mentioned are lower energy values, alteration of enzyme systems, toxic and

carcinogenic effects, lower weight gains and growth rates, nutrient deficiency symptoms and in some cases death.

According to Nawar (1985) lipids especially when unsaturated, undergo many kinds of chemical changes during processing and some of these changes can affect their nutritional value and wholesomeness.

2.1. Physico Chemical Changes

Fats and oils are heated during commercial processing, but care is to be taken to exclude oxygen. Under normal condition no oxidation or degradation takes place. High temperature results in autoxidation process. In addition several other changes take place in frying fats.

Stevenson et al. (1984) reviewed the chemical and physical changes that occur in frying fats affecting fat life and product quality. According to Deman (1976) free fatty acids are formed at level of about 1 per cent during severe frying conditions. Iodine value may decrease considerably and colour of oil darkens.

According to Fire stone et al. and Friedman et al. (1961) regarding the oxidation of oils at high temperatures (200^{o} C) for extremely long heating times have shown presence of cyclic compounds classified as non urea aduct forming compounds. Michael (1966) from his studies isolated both aromatic and alicyclic non urea aduct forming compounds from thermally abused fats. Johnson (1987) also reported that several changes are taking place in edible oil during thermal oxidation.

Dupy et al. (1973) studied as quality of oil decreased the concentration of volatiles increased. According to Fennema (1985) the reactions during frying are responsible for a variety of physical and chemical change that can be observed at that time. According to him such changes include increase in viscosity, free fatty acid content, development of dark colour, a decrease in iodine value, changes in refractive index, a decrease in surface tension and an increased tendancy of oil to foam. According to Deman (1976) the most important changes are formation of polymers which results in increased viscosity which in turn may greatly increase foaming of the frying fat.

Chang et al. (1978) from their studies concluded that oxidative reactions involving the formation and decomposition of hydroperoxides of such compounds as unsaturated aldehydes, Ketones, hydrocarbon lactones, alcohols, acids and esters are produced during frying. According to Chiba and co-workers (1979) considerable deterioration occured in quality of ground nut oil during the frying operations due to increase in content of oxirane oxygen, conjugated double bonds, oxidised fatty acids and decrease in linoleic acid content.

Deep fat fried foods present another problem which may have biological significance to the consumer. According to Fred (1979) if foods are fried in fats and oils containing poly unsaturated fatty acids the frying medium gradually accumulates hydroxy acids, aldehydes, ketones, epoxides, alkoxy substituted unsaturated esters and cyclic acids more rapidly than mono unsaturated fatty acids. According to Fred (1979) more than twenty one volatile oxidation products are formed in heated oil which remains in oil and are most concern to biological significance of fats.

According to Crnjar et al. (1981) however when saturated fatty acids, their esters and their

triacylglycerols heated in air at temperature higher than 158^{o} C produced a variety of oxidation products like hydrocarbons, aldehydes, ketones and lactones. Thompson (1993) from his study on lipid changes in 100 hours heated oil concluded that poly unsaturated fatty acids and trans fatty acids were 48 and 87 per cent respectively.

Iodine value of 3 edible oils was studied by NIN (1984) after repeated heating for 6,12,18 and 24 hours found that a decrease in iodine value in all the edible oils after heat treatment.

According to Nagaraj (1987) when the free fatty acid content of fat is above 2 per cent their quality is considered to be poor and a fishy odour develops in oils due to oxidation. Ahmed (1988) reported that an increase in refractive index and triacylglycerols in thermally oxidised oils. Perez (1990) did a study about the precision of some methods for evaluating oxidative degradation of different vegetable oils concluded that there is significant difference (P 0.01) among the different methods when they are applied simultaneously. The type of studied oil does not exert a significant effect (P 0.05).

According to Nagaraj (1987) repeated deep frying and storing increase the fat acidity which in turn increase the smoking point. He also concluded that such a deterioration in quality is more in the case of poly unsaturated fatty acid oil. Subbulakshmi et al. (1990) studied the effect of re heating and storage on physico chemical constituents of palmolein oil and organoleptic acceptability of food products which resulted in slight increase in free fatty acids, refractive index and specific gravity by no change in colour units. According to her study she concluded that the products fried in heated-stored-reheated oil were highly acceptable even after third frying.

Tamura et al. (1991) studied the changes in essential fatty acid resulting in formation of certain aldehydes upon oxidation shows the presence of formaldehyde (a Suspected Carcinogen) as the major product along with unsaturated aldehydes like acrolein and malonaldehyde. According to his assumption hexanal was the main aldehyde from arachidonic acid and linoleic acid and propanal from linolenic acid and its ethyl esters.

According to Hassan (1991) from his study on quality of used frying oils from restaurants concluded that discarded oils found to be heat damaged to a varying extend according to degree of quality control applied by restaurants. According to his assumption peroxide value followed by per cent free fatty acid did not significantly correlate with total polar compounds.

Prior et al. (1991) from his studies concluded that oxidative stability was found to be associated with phospholipid content. Neelima and Sarojini (1991) from their studies to compare the free fatty acid, peroxide value, iodine value of oil used for frying were analysed and compared with those at laboratory conditions ended in conclusion that the oil used at laboratory conditions were found to be relatively superior in quality as this oil contained much lower free fatty acid and peroxide value than those from market. According to Lane and Smathers (1992) acrolein and other low molecular weight aldehydes are formed by degradation of frying medium without any difference in foods fried whether protein or carbohydrate foods. They concluded that freshness of frying medium, frying time and batch size did not seem to influence low molecular weight aldehyde production in certain foods.

According to Billek (1992) there is changes in fats and oils at high temperature during steam deodourisation resulting in formation of triglyceride dimers, isomerisation of unsaturated fatty acids, changes in frying fats and in concentration of polar constituents.

According to Gumuskenson (1992) higher temperature and longer time lead to increase in free fatty acid, peroxide value, unsaponifiable matter and decrease in oxidation period. Chang and Shu (1992) studied about the volatile compounds mainly aldehydes in oils after deep frying or stir frying and during subsequent storage. During heating and storing total volatiles increased 260-1100 fold, volatiles increased upto 1-6 per cent.

Smith et al. (1986) found that frying times correlate with increase in dielectric constant, polar compounds and essential fatty acid. According to Thompson (1983) lipid changes were influenced by the quantity of potatoes fried than frying time.

During use at deep frying temperature, frying oils are prone to oxidative and thermal degradation with the

formation of volatile and nonvolatile de composition products some of which in excessive amounts may be harmful to human health. Products include volatiles and polymers (Chang et al. 1978) dimers (Veazey, 1980), oxysterols (Bascoul et al., 1986) cyclic fatty acids (Frankel et al. 1984) and alkaline compounds, soaps (Blue menthol and stockler, 1986).

2.2 Nutritional Changes

Any significant level of autooxidation or catalytic oxidation in a lipid system will reduce the essential fatty acid content, linoleic acid and linolenic acids because of unsaturated nature. Destruction of unsaturated lipids such as carotene, vitamin A and tocopherols will directly affect nutritional level resulting in reduced efficiency and nutritive value (Nawar, 1985).

According to Raju et al. (1965) heated oils for deep fat frying were poorly absorbed by the body, it tended to produce cancerous tumours and symptoms resembling vitamin E deficiency. Raju et al. (1965) also reported destruction of certain vitamins especially vitamin A resulted in lower nutritive value and oxidation products of fats produce an

inhibitory effect. Nawar (1977) reported that extensive decomposition resulting from lack of adequate control of frying operations can be a potential source of damage not only to sensory quality for fried products but also to its nutritive value. According to Sommerfield (1983) trans EFAS are devoid of physiological functionality and are much more susceptible to polymerisation during heating than all counter parts. When present in the diet of animals at high concentration for long periods trans fatty acids may have effects that are unhealthful.

Fatty acid profile and iodine value of 3 edibile oils was studied by NIN (1984) after repeated heating for 6, 12, 18 and 24 hours and found that there was increase in free fatty acid content in all edibile oils after heat treatment. Sukhdev Singh (1986) reported from his studies conducted using groundnut oil, heated at different levels of temperature and time intervals that levels of linoleic acid and linolenic acids were decreased with a corresponding increase in levels of short chain fatty acids.

Leith (1987) reported that the content of both saturated and unsaturated fatty acids as well as the content

of total fatty acids are decreased by frying. Gomathi Shivaji (1987) also observed that linoleic acid was completely destroyed from 20 per cent in fresh oil to nil in the same oil heated for 24 hours and an increase in level of short chain fatty acids.

According to Fleischman (1963) the PUFA (Poly Unsaturated Fatty Acid) are chemically vulnerable to oxidation and considerable losses occur when they are used for cooking. These changes are usually observed in heating at 257^{o} C for 30 minutes followed by cooling period of 30 minutes. According to Leith (1987) the decrease in linoleic acid and decrease in total fatty acid correlate well with alpha polar compounds as fat quality evaluation criteria.

Yurkov (1991) reviewed from his studies that deep frying of different batches of potatoes in the same frying oil every 8 hour for 48 hours resulted in pronounced hydrolytic and oxidative changes, leading to quality deterioration in both the oil and fried product. In oil the marked change being decrease in contents of linoleic acid with repeated deep frying.

2.3. Toxicological evaluation of oxidized oils

2.3.a. Effect of reheated oil on animal feeding experiments

According to Alexander (1978) the possibility that consumption of heated and oxidised fats may produce adverse effects and has been a major concern. Several reviews on this subject are available. Perkins (1979) conducted animal feeding experiments involving heated fat or used frying oils oxidised under controlled conditions resulted in nutritional toxicity.

Andrew et al. (1960) fed oxidized oils to Weanling rats to see the effect of peroxide on growth. He concluded that oils with peroxide value between 800-1200 there was no weight gain but ultimately ended in loss of weight and death within 3 weeks. Studies by Rasheed et al. (1963) Holman and Greenberg (1954 and 1958) Kaunitz et al. (1960 and 1965) have shown that lipids oxidised at normal temperature exhibited no toxicity or growth effects when peroxide value did not greatly exceed 100.

According to Andrew et al. (1960) in general the feeding of highly oxidised fats (at levels of of 10-20 per

cent in the diet) results in loss of appetite and growth retardation. Enlargement of livers, kidneys and accumulation of peroxides in adipose tissue were also reported. Raju et al. (1965) conducted experiments in coconut oil heated to 270° F (132° C) for 8 hours in an open pan and fed to albino rats at 15 per cent level resulted in depressed growth of rats, reduced liver weights, showed fatty liver, elevated blood glucose level and cholesterol value in rats.

According to Poling et al. (1969) in most cases feeding of heated fats that have become severly oxidised produces various detrimental effects in animals. He also concluded that oils heated in absence of air was more toxic than oil heated in air. Artman (1969) observed negative effect upon feeding used oils in biological system, when oil heated upto 180° C for repeated frying incorporated at a 10 per cent level in diet of rats.

Decreased feed intake, lower fat absorbability decreased growth and liver enlargement are among various adverse effects reported in several animal feeding studies by Alexander (1978) involving highly abused frying oils. According to Fred (1979) when rats were kept on diets

marginal in protein and vitamin fed with used frying fat developed diarrhoea and enlarged livers. According to Iwaoka and perkins (1978) a level of only 0.15 per cent cyclic fatty acid in diets containing 8 per cent protein produced fatty livers and also decreased rates of lipogenesis.

According to Billeck (1979) animal group fed polar fraction of discarded sunflower oil used for industrial production of fish fingers showed growth retardation, a certain amount of liver damages, increased liver and kidney weights where as in control group fed unheated oil greatest increase in body weight were observed. Yonnai (1980) from his study concluded that fatty acid dimers and polymers when fed to rats have no nutritive value and cause growth suppression and poor reproduction.

Shalini Rao (1985) conducted animal studies with thermally oxidised oils and found an increase in requirement of all vitamins, poor adaptation to reduced calorie intake, enlargement and lesions of liver and increased susceptibility to certain diseases. Kaunitz (1987) found that when sesame, peanut and coconut oil heated at 270^{0} C were incorporated in

experimental diets at 15 per cent level growth rate was markedly depressed.

Yoshida et al. (1989) from their studies concluded that rats fed on autooxidised soyabean oil showed increased haemolysis, peroxide value in tissue lipids and a decrease in linoleic acid and arachidonic acids except in liver. Animal studies conducted by Huang et al. (1989) showed high mortality rate in rats when they were fed a low protein diet containing 15 per cent deteriorated used frying oil. According to Artman (1989) cyclic compounds developed during thermal oxidation of fats have been reported to be present in the lymph of rats which had been fed with thermally oxidised fats.

Blond et al. (1990) studied the effect of geometrical isomers of heated linseed oil on feeding rats at 10 per cent level concluded that geometrical isomers of linoleic acid (18:3n-3) were high in oils heated at 240^{o} C for 10 hours and affected rats slightly.

Hageman (1991) worked on the effect of saturated fatty acid rich coconut oil and PUFA rich (60 per cent PUFA)

vegetable oil by feeding animals at 10 per cent level. He reported that short term consumption of such deep frying oils which contain many oxidation products can cause increased cell proliferation.

Alexander (1992) conducted an animal feeding experiment at 15 per cent level concluded that oxidised oils are no longer effective in supressing glutathione peroxidase activity but more effective in enhancing catalase activity. According to Franca (1992) rectal proliferation data were negatively correlated with concentration and percentage of butyric acid and positively correlated with percentage of acetic acid, acetic acid and butyric acid ratio. Behniwal (1991) reported that feeding of peroxidized oil at 10 per cent level has been found to supress the growth and protein efficiency ratio. It reduces the haemoglobin content and altered the activities of membrane bound enzymes.

2.3.a.(1) Toxicological Evaluation- Mutagenicity causes

According to Abelson (1983) reported that our diet contains several chemicals that are mutagenic and carcinogenic some formed during cooking via reactions

involving fats or proteins. Black (1983) have been well documented the influence of diet on rate of formation of certain chemically induced and spontaneous tumours.

According to Alexander (1978), Artman (1969) and Perkins (1976) the possibility that consumption of heated and oxidised fats may produce adverse effects has been a major concern and has stimulated extensive research.

Grasas (1985) reported that the toxicity of certain natural fatty acids in edible oils and fats are contributed by acids like erucic acid, marine oils, cyclopropenic acids, C_{18} poly unsaturated fatty acids, peroxides, cyclic monomers, dimers and polymers of fatty acids and fatty acid isomers.

2.3.b. Mutagenic effect of reheated oil

Taylor (1983) found that abusive frying conditions like abnormally long frying times, high frying temperature, repeated reuse of frying oil were necessary to produce appreciable levels of mutagenic activity in french fried potatoes. Various other studies have failed to show evidence of carcinogenic effects from normally used frying fats.

In a study by Rice et al. (1960) fat at 182° C was used for frying potatoes, onions and fish until the point of severe foaming occured and then this was fed to rats at 15 per cent of diet for 2 years resulted in growth retardation. There were no incidence of tumour or any bio chemical or histological irregularities.

In a study by Scheutwinkel Reich (1980) for testing mutagenicity of used frying fat negative result were obtained when a polar fraction isolated by column chromatography and so called fatty acid fraction obtained by petroleum ether fractionation were subjected to salmonella/microsome mutagenicity test. At higher concentrations however limited test reliability resulted from direct toxic effects on bacterial growth.

According to Taylor (1983) repetitive frying of 45 batches of french fried potatoes in the same batch of shortening elicited no increase in mutagen formation. But repetitive frying of fish fillets resulted in generating appreciable levels of mutagen even in the 7th batch itself as it contained low levels of mutagenic activity.

In another study by Saleh et al. (1986) for mutagenic activity using Salmonella typhimurium assay with and without activation 59.8 of heated samples showed significant mutagenic activity, None of fresh unheated oils were mutagenic. S. typhimurium TA 102 gave a higher response than either TA 100 or TA 98.

In spite of disparity of results and contradictory conclusions existing in literature it is evident that toxic compounds can be generated in fat by severe heating or by oxidation. However it also appears reasonable to conclude that no significant hazard to health is to be expected from moderate ingestion of fried foods, provided high quality oils are used and recommended practices are followed.

MATERIALS AND METHODS

MATERIALS AND METHODS

The study on "Physico chemical, nutritional and toxicological evaluation of thermally oxidized edible oils" is an assessment of changes in physico chemical properties, fatty acid composition and toxicological safety of reheated edible oils. The influence of long time high temperature deep frying of foods taken into consideration to ascertain the changes.

Method of study

1. Commonly used edible oils viz. coconut oil and ground nut oil were studied for their changes in physico chemical properties and fatty acid composition. Toxicological evaluation of fresh and reheated coconut oil were conducted through animal feeding experiments since it is more widely used frying oil in Kerala.

2. The coconut oil and ground nut oil were heated seperately to smoking temperature and small quantity of fresh oil kept aside for fresh oil analysis. A known quantity of banana was fried continuously for one hour (plate 1). The

Plate 1. Frying banana chips in heated oil continuously for one hour by the investigator.

heated oil was cooled to room temperature and a small quantity of heated oil sample (200 ml) was drawn and kept aside for lab analysis. The cooled oil sample was heated to smoke temperature in the next day and banana was fried for 1 hour. This procedure of reheating the oil sample for one hour every day and cooling to room temperature was continued for 12 days. At the end of 12th day, total number of 12 oil samples reheated for various periods were ready for physico chemical analysis.

3. Assessing the changes in physico chemical properties by estimating

   a. Smoke point (Aocs, 1978)
   b. Boiling point (Aocs, 1978)
   c. Iodine number (Wij's method, 1987 (IUPAC))
   d. Saponification number (AOCS & AOAC, 1979 & IUPAC, 1987)
   e. Acid value (IUPAC 2.201, 1987)

3a. Smoke point

A thermometer designated upto 500^{o}C was deeply immersed in hot oil while heating. The readings were taken

at the time when dense white fumes came out of the hot oil. This is noticed as smoke point or smoke temperature (Plate 2). The smoke point was recorded daily during heating.

3b. Boiling point

Boiling point was recorded with a thermometer designated upto 500^{o}C immersed with bulb deeply in hot oil. The reading was taken at that time when oil starts slightly boiling and was recorded everyday before frying banana (Plate 3).

3c. Iodine value

The iodine value is often the most useful and easily determined figure for identifying an oil. The iodine value is usually determined by Wij's method (CF Bs 684 section 2.13 (ISO 3961); IUPAC; 1991.

3d. Saponification value

As many oils have some what similar values, the saponification value is not in general as useful for

Plate 2. Dense white fumes coming out of the oil denotes smoking temperature.

Plate 3. Noting the boiling temperature of the oil when bubbles starts coming out.

identification purpose as the iodine value. But it is useful in some oils like coconut oil to see the presence of lower fatty acids. It is determined by AOCS & AOAC, 1979 & IUPAC, 1987.

3e. Acid value

As rancidity is usually accompanied by free fatty acid formation. The acid value determination is used as general indicator of the condition and edibility of oils. It is determined by Bs 684 section 2.10 method 3; ISO 660, IUPAC 2.201, 1991.

4. Fatty acid profile

The commercial introduction of gas liquid chromatography in the late 1950s and early 1960s made feasible a rapid and accurate measurement of the fatty acid composition in oils and fats following conversion of triglyceride esters into the more volatile methyl esters. The finger print fatty acid profile so obtained in many cases made virtually absolute most of the earlier semispecific colour and turbidity tests for individual oils.

4a. Packed Column Gas Chromatography

Methyl esters of coconut oil were determined using a sealed vial method and transesterification with sodium methoxide was done. The method outlined by Timms (1978) and Chalvardjean et al. (1964). The metheyl esters were analysed in a Hewelett packed Gas chromatography using a 2m x 2m glass column packed with SP 2330 packing (Supal Co) run for 30 minutes 0.1 micro ml. of the fatty acid methyl ester was injected. This experiment was conducted with oils of two hours intervals.

5. Toxicological evaluation of fresh and reheated coconut oil by

a. Screening for mutagenicity in bacterial system..

b. Conducting animal feeding experiment for observing histopathology of tissues

Toxicological evaluation of reheated oil was done using coconut oil heated for 12 hours.

Long feeding experiments in animals have shown that processed deep frying fats may have deleterious effects of special toxicological interest at certain fat fractions which arise in considerable amounts during deep frying (Scheutwinkel, 1980).

5a. Mutagenicity testing

Assays were conducted using the bacterial mutagenesis test of Ames et al. as modified by Pariza et al. (1983) (Bacteria strain used :- The test organism was salmonella typhimurium TA 1535 strains plated at Ca 6.6 x 10.7 bacteria per plate). Two replicate plates were done with duplicate oil samples by dividing it into equal proportions. Results are reported as the ratio of induced revertants per plate to spontaneous revertants per plate.

The Ames test is a short term mutagenicity test which was various histidine dependant S. typhimurium strains as indicator organisms. Under the influence of mutagenic substances some bacteria revert back to the histidine non dependant type. These revertant are able to grown on a histidine free minimal agar. The number of colonies which

grow is counted and compared to control showing the spontaneous mutation rate. The number of revertants per plate increase as the amount of mutagenic substance present increases. Such a response relationship is of great significance when assessing the mutagenic activity of compound.

5b. Animal feeding experiment

Animal experiment was conducted in male albino rats (Sprague - Dawley Strains weighing 60-80g). Experiment started with 24 male rats which were divided into three groups. Animals of more or less identical weights were selected and divided into 4 groups, two groups with four animals as controls and two experimental groups with 8 animals.

Diets were formulated using fresh and reheated oils (NIN, 1983). The planned diet were found to supply 9 per cent fat. The animals were kept in stock diet before starting the experiment. The dietary details is presented in Table 1 and Table 2 respectively. The formulation of mineral mixture and vitamin mixture is also presented in Table 3 and 4 respectively.

Table 1. Stock diet (NIN 1983)

Ingredients	Amounts
Groundnut oil	5g
Mineral mixture	4g
Vitamin mixture	2g
Wheat flour	15g
Roasted Bengal gram dhal	60g
Groundnut	10g
Casein	4g

Table 2. The diet used for the experiment (NIN, 1983)

Ingredients (g)	Control		Experimental	
	Group 1	Group 2	Group 3	Group 4
Skimmed milk powder	26.3	26.3	26.3	26.3
Starch	58.7	58.7	58.7	58.7
Mineral mixture	4	4	4	4
Vitamin mixture	2	2	2	2
Fresh groundnut oil	9	-	-	-
Fresh coconut oil	-	9	-	-
Reheated coconut oil	-	-	9	9

Table 3. Composition of minerals mixture

Items	Weight in g
Calcium carbonate	38.1400
Cobalt chloride	0.0023
Cupric sulphate	0.0477
Ferrous sulphate	2.7000
Magnecium sulphate	5.7300
Manganeese sulphate	0.4010
Potassium iodide	0.0790
Potassium phosphate monobasic	38.9000
Sodium chloride	13.9300
Zinc sulphate	0.0548

(NIN, 1983)

Table 4. Composition of vitamin mixture

Vitamins	Amounts
Vitamin A	2000 I.U
Vitamin D	200 I.U
Vitamin E	10 I.U
Vitamin K	0.5 mg
Thiamine	0.5 mg
Riboflavin	0.8 mg
Pyridoxine	0.5 mg
Calcium pantothenate	4.0 mg
Niacin	4.0 mg
Inositol	10.0 mg
Para amino benzoic acid	10.0 mg
Biotin	40.0 mg
Folic acid	0.2 mg
Vitamin B12	3.0 mg
Choline chloride	200.0 mg

(NIN, 1983)

The rats were housed in cages with wire mesh floors (plate 4) 10 g of diet mixed with boiled water and fed to animals. Each rat was given about 10 g of food daily. The intake of minerals and vitamins were kept the same in the four groups. Water was provided adlibitum. Experimented group was fed with a diet containing 9 per cent 12 hours reheated oil without replenishing.

The rats were maintained on the respective diets for six months. The monthly body weights of the animals were recorded for a period of 6 months in the experimental period (Plate 5). During this period the conditions were maintained as uniform as possible.

The histopathological study was conducted at two intervels during the feeding experiment.

At the end of 3 months a set of control and experimental rats (one from each group) were killed and the organs viz., liver, kidney and intestine were taken and kept in formalin solution to study the histopathological changes.

Plate 4. Rats in wire mesh cages with enough food and water

Plate 5. Monthly weight assessment using triple beam balance

2. At the end of 6 months period the animals were killed and the organs viz. liver, kidney and intestine were taken and kept in formalin solution to study the histopathological changes.

The organs taken were processed and the slides prepared were observed under microscope to note the histopathological changes.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The study entitled "Physico chemical, nutritional and toxicological evaluation of thermally oxidized edible oils" comprises an assessment of repeatedly reheated oils on

i. Physico chemical changes

ii. Changes in Fatty acid composition

iii. Toxicological evaluation

4.1 Physico chemical changes

Smoke point, Boiling point, Iodine number, saponification number and acid value were determined to assess the physico chemical changes occured during reheating through repeated frying. Data obtained for two commonly used edible oils viz. Coconut oil and ground nut oil with respect to the above parameters are presented in the Table 5 and 6 respectively.

Changes in physico-chemical properties

A close scrutiny of the data reveals a decrease in boiling point consequent to reheating of both the oils with

Table 5. Assessment of physico-chemical changes of coconut oil heated upto 12 hrs

Hours of heating	Smoking temperature	Boiling point	Iodine number	Acid value	Saponification number
Fresh oil	162	215	8.50	4.2	206.5
1st hour	163	212	7.25	3.8	210.7
2nd hour	165	210	6.03	3.5	215.9
3rd hour	166	205	3.02	3.6	210.7
4th hour	167	203	6.4	3.9	214.2
5th hour	168	201	6.4	3.8	215.9
6th hour	169	199	5.6	3.2	209.7
7th hour	169	195	5.7	3.5	208.7
8th hour	170	193	5.4	3.5	217.3
9th hour	171	190	3.08	3.7	201.3
10th hour	171	190	3.8	3.5	216.3
11th hour	172	189	3.02	3.0	215.7
12th hour	173	189	2.7	3.4	217.9

time. The lowest value for boiling point was recorded in the case of samples subjected to 12 hours of heating (cumulative). In fresh oil, low values with respect to smoking temperature and the higher value, for boiling point were obtained. On an average there was a difference of about 26°C in temperature between the fresh and last sample of coconut oil with respect to boiling point. Corresponding increase in temperature for smoke point was 11°C between the fresh sample and 12 hrs reheated sample.

Iodine number and acid value of coconut oil was found to be decreasing consequent to reheating. The change was marginal for acid value where as it was substantial for Iodine number. Saponification value has increased from 206.5 to 217.9 between the fresh and last sample. Intermediate samples had shown values ranging between the above two. However no clear conclusions could be drawn from the data.

Decrease in Iodine number was from 8.5 to 2.7 between the first sample and the 12 hrs reheated sample. In general a gradual decrease in Iodine number was noticed with increase in cumulative heating times.

Table 6. Assessment of physico-chemical changes of ground nut oil heated upto 12 hrs using suitable laboratory techniques

Hours of heating	Smoking temperature	Boiling point	Iodine number	Acid value	Saponification number
Fresh oil	182	219	89.20	1.1	158.80
1st hour	183	218	88.40	1.2	157.30
2nd hour	184	217	89.60	1.4	156.80
3rd hour	185	217	88.01	1.0	157.10
4th hour	185	216	87.02	1.6	156.80
5th hour	186	216	86.03	1.5	155.80
6th hour	187	215	84.60	1.6	154.60
7th hour	188	215	84.40	1.7	153.50
8th hour	189	214	82.80	1.8	152.90
9th hour	190	213	80.02	1.7	151.80
10th hour	191	213	80.01	1.9	150.90
11th hour	192	212	80.03	1.8	150.02
12th hour	193	210	79.9	1.9	150.04

Groundnut oil also exhibited a similar pattern for smoke point, boiling point and iodine number. However in the case of acid value and saponification value a reverse trend was observed with increase in heating time. The increase in smoking temperature was from 182^{o}C to 193^{o}C for groundnut oil between the fresh and 12 hrs reheated sample with a difference of about 11 degrees over a period of 12 hours of cumulative heating. The boiling temperature decreased from 219^{o}C for the fresh sample to 210^{o}C for 12 hrs. reheated samples which was subjected to 12 hours of cumulative heating. The drop in boiling point was only 9^{o}C as compared to 26^{o}c in the case of coconut oil.

Saponification value of ground nut oil decreased from 158.8 to 150.04 between the fresh oil and 12 hrs. reheated sample respectively. The corresponding figures for acid value was 1.1 and 1.9 respectively for fresh sample and the 12 hrs. reheated sample.

The observed decrease in boiling point and increase in smoke point of the two oils used for the study during reheating is attributed to the possible physico-chemical changes that are likely during heating process such as the

formation and decomposition of hydroperoxides, saturated and unsaturated aldehydes, ketones, hydrocarbons, lactones, alcohols, short chain acids and esters. Further increase in free fatty acid content was also reported to occur during deep frying and reheating. Accumulation of hydroxy acids, aldehydes, ketones, epoxides, alkoxy substituted unsaturated esters and cyclic acids in addition to a variety of nonvolatile oxidation products which accumulates in the frying medium (oil), also would have changed the chemical composition of the oil. Similar results have been reported by Chang et al. (1978), Fred (1979) and Thompson (1983) to occur in reheated oils. The increase in smoke point is also attributed to an increase in the free fatty acid content and fat acidity during frying process. Similar results have been reported by Nagaraj (1987). Further increase in smoking temperature can also be due to the observed increase in viscosity of the oil during frying and changes in refractive index. Such changes in viscosity, refractive index, decrease in surface tension consequent to reheating of oils have been reported by Fennema (1979). As the above changes are already known, those parameters were not quantified. When compared to groundnut oil, coconut oil had given lower values for both surface tension and boiling point in all the observations.

This is supposedly due to the higher viscosity and the long chain nature of the fatty acids present in the glyceride esters of ground nut oil. Coconut oil on the contrary are light oils having low viscosity with more number of medium and short chain fatty acids present in the glyceride esters.

The drastic decrease in boiling point of coconut oil due to reheating (26^{o} towards 12 hrs. reheated sample) when compared to groundnut oil is also attributed to the faster oxidation and degradation of the fractions of coconut oil at higher temperatures than groundnut oil.

Decrease in Iodine value of both coconut oil and groundnut oil consequent to reheating is attributed to lipid peroxidation and saturation of double bonds. Studies by Gumuskenson (1992) and Chang and Shu (1992) also reported decrease in Iodine value consequent to reheating. Release of free fatty acid during heating process might be the reason for the observed increase in acid value of groundnut oil. However coconut oil did not show any significant change in acid value, probably due to the volatilisation loss of short and medium chain fatty acids released during reheating. The observed decrease in saponification value of groundnut oil is

also due to the escape of volatile free fatty acids and the degradation of other fatty acids which were made free during reheating process. Variation observed between the fresh and the 12 hrs. reheated sample is only of 8 units which is indicative of the lesser amounts of free fatty acid lost during the process.

Saponification value of coconut oil though had increased from 206 to 217 between the fresh and 12 hrs reheated sample has not given any definite trend for the change. The variation observed was erratic and hence no clear cut inference could be made from the data. Further experimentation with more number of samples is needed in the case of coconut oil to arrive at a conclusion with regard to changes in saponification value due to reheating.

4.2 Change in fatty acid composition

Table 7 and 8, figures 1-14 and 15&16 represents the data on changes occuring in reheated oil samples of both coconut oil and groundnut oil with respect to fatty acid composition. Fractionation of coconut oil and groundnut oil with 2 hours intervals to find out the fatty acid composition

Table 7. Coconut oil - Percentage of each fatty acid in oil samples (GLC method)

Sample No.	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{16.1}$	C_{18}	$C_{18.1}$	Saturated fatty acid	USFA
FC	7.87	5.73		49.65	20.05	7.62		5.24	3.20	96.17	3.20
2C	9.20	6.50		51.70	20.05	6.42		3.00	3.16	96.87	3.20
4C	9.20	6.68		34.40	25.70	12.45		7.50	4.20	95.53	4.20
6C	9.80	7.14		41.90	25.01	8.70		5.05	2.09	97.60	2.09
8C	10.10	7.80		55.90	18.04	1.50		3.87	2.30	97.14	2.30
10C	11.10	7.90		48.40	25.90	1.40		3.01	1.80	97.70	1.80
12C	10.0	7.34		43.03	24.09	10.07	1.75	2.79	0.95	99.47	0.95

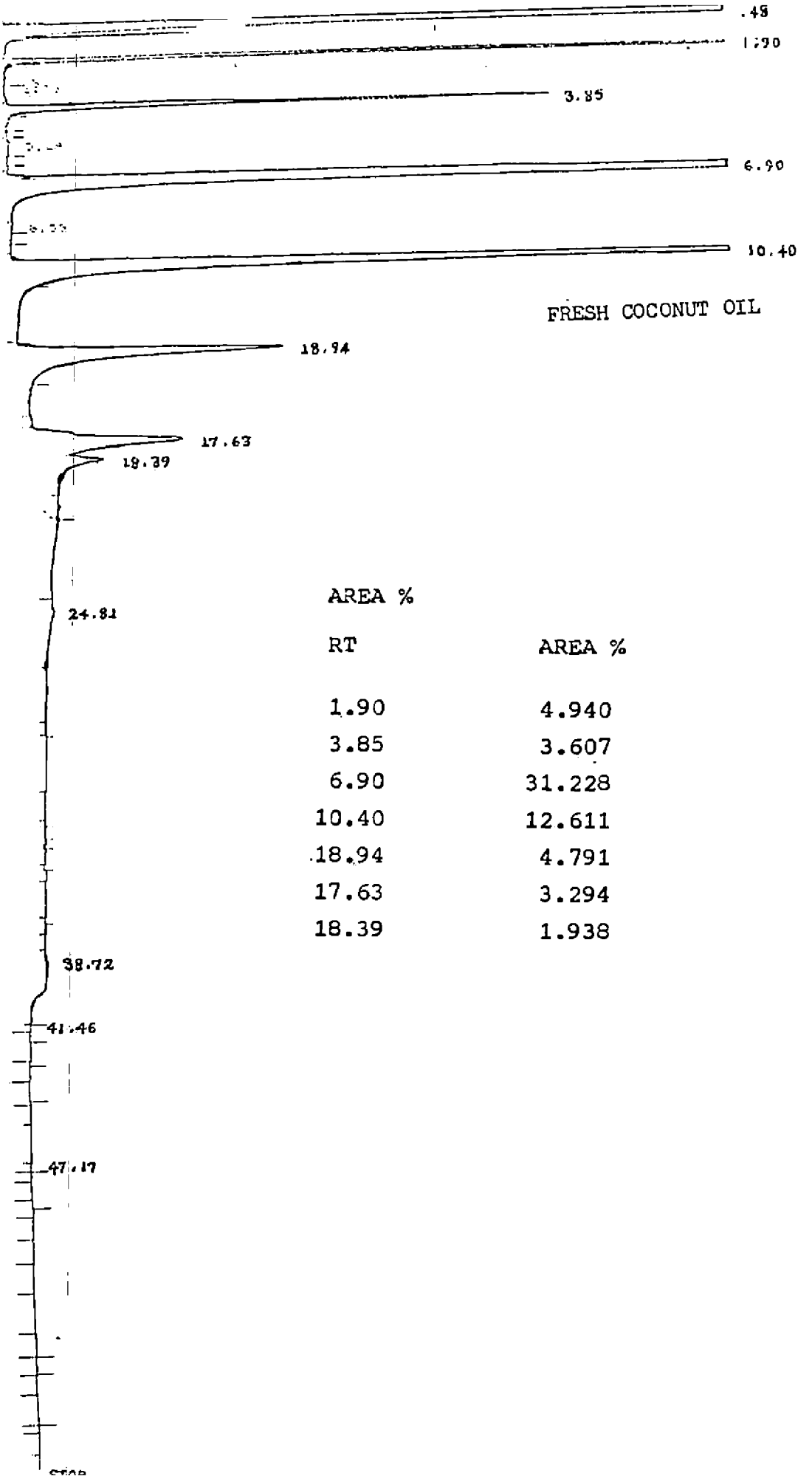
was achieved by GLC analysis (BS 684, section 2.34:1980 or by IUPAC 2.302 and AOCS cd 1-62)

Table 7 and 8 represent data on fatty acid composition of fresh and reheated oils used in the present study.

Changes in fatty acid composition

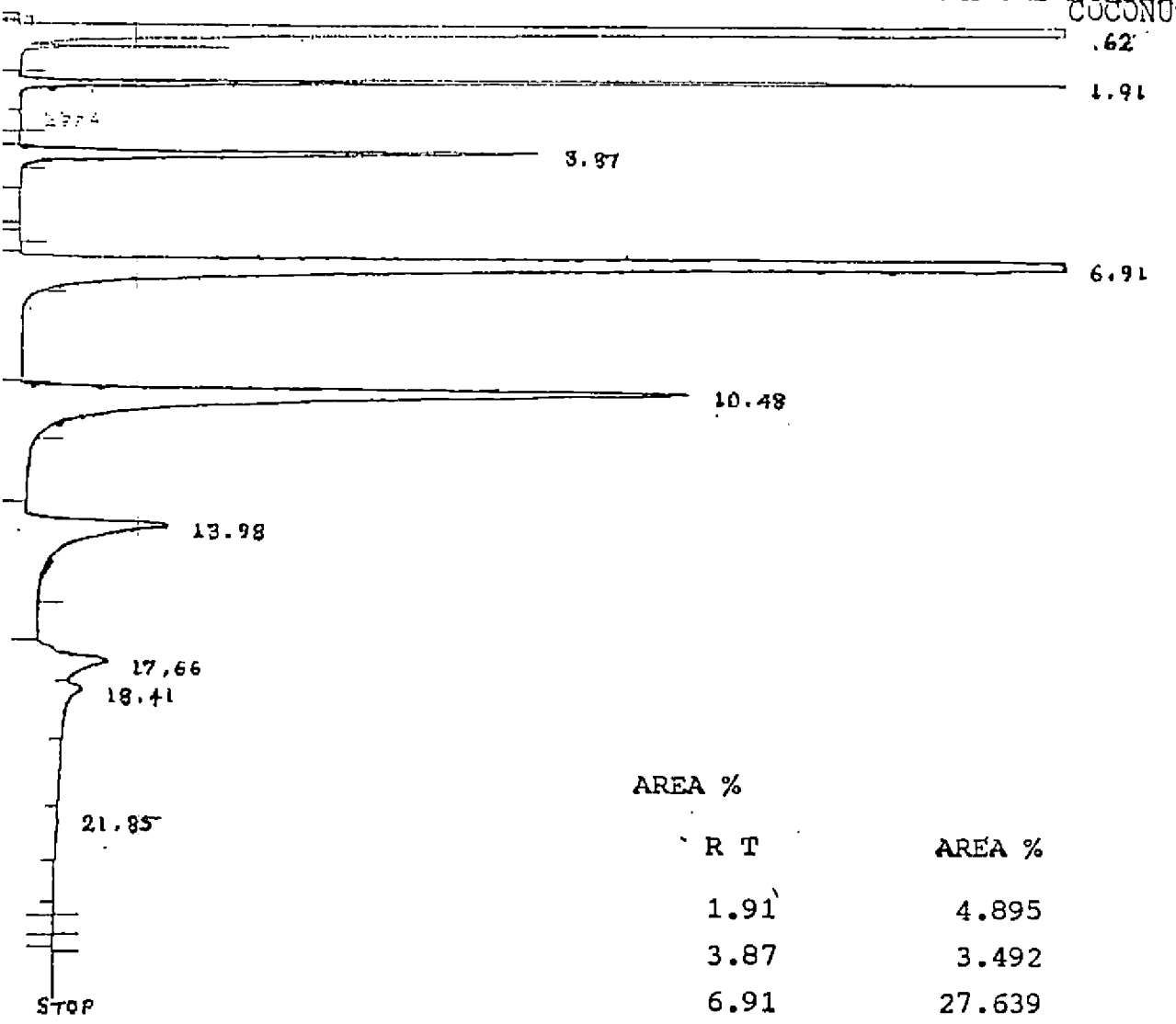
Table 7 and 8 represents data on chemical changes occuring in reheated oil samples of both coconut oil and groundnut oil, with respect to their fatty acid composition. Fractionation of oil samples for this purpose was achieved through GLC analysis.

Samples of coconut oil from selected treatments at 2 hours heating interval which were subjected to GLC analysis showed considerable variation in fatty acid composition (Fig. 1-7). Distribution pattern of individual fatty acids from different treatments indicated a total saturated fatty acid percentage of 96.17 in the fresh sample and 99.47 in reheated samples with cumulative heating time of 12 hours. Intermediate treatments with cumulative heating time of 2



AREA %

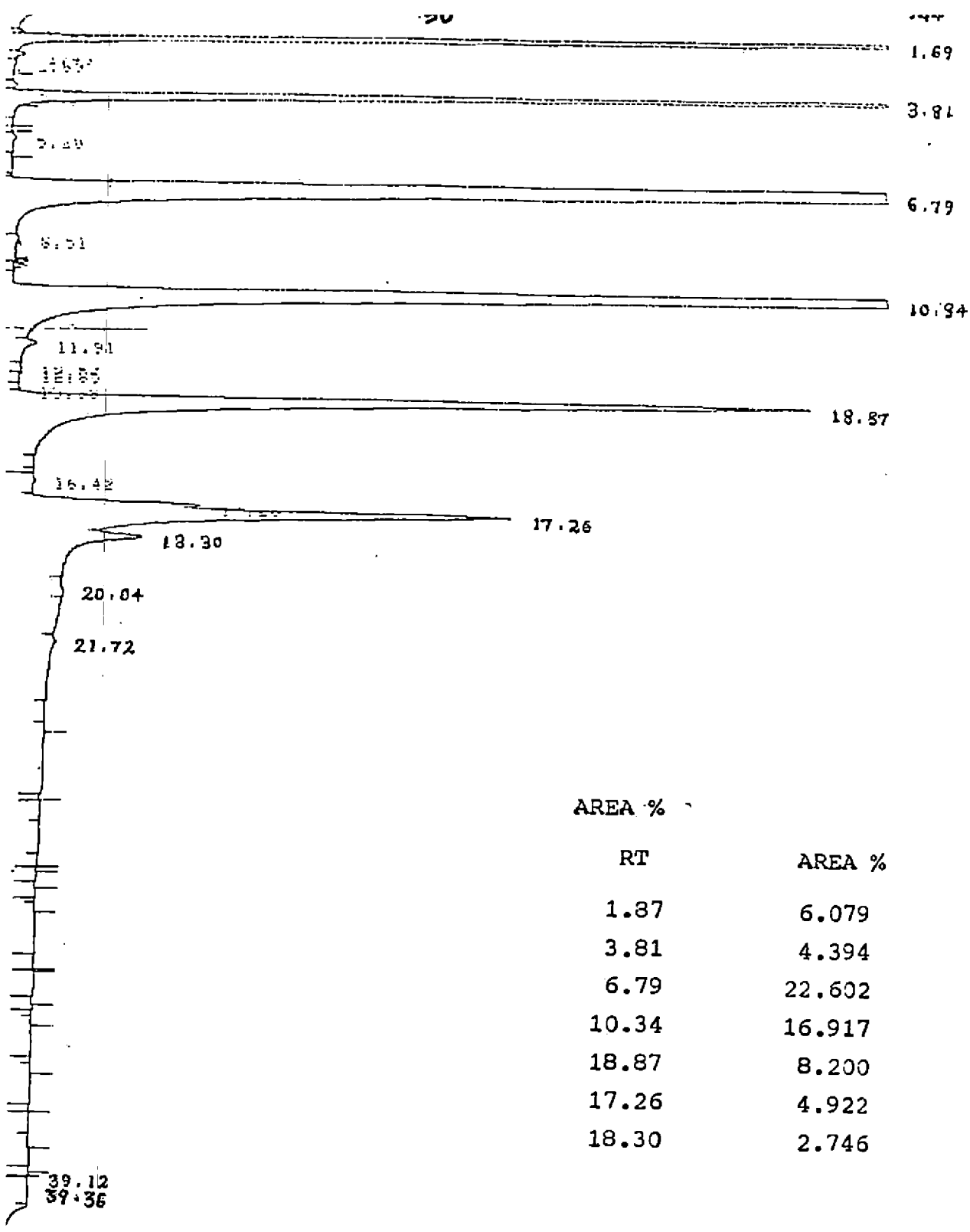
RT	AREA %
1.90	4.940
3.85	3.607
6.90	31.228
10.40	12.611
18.94	4.791
17.63	3.294
18.39	1.938



AREA %

R T	AREA %
1.91	4.895
3.87	3.492
6.91	27.639
10.48	10.722
13.98	3.433
17.66	1.606
18.41	1.690

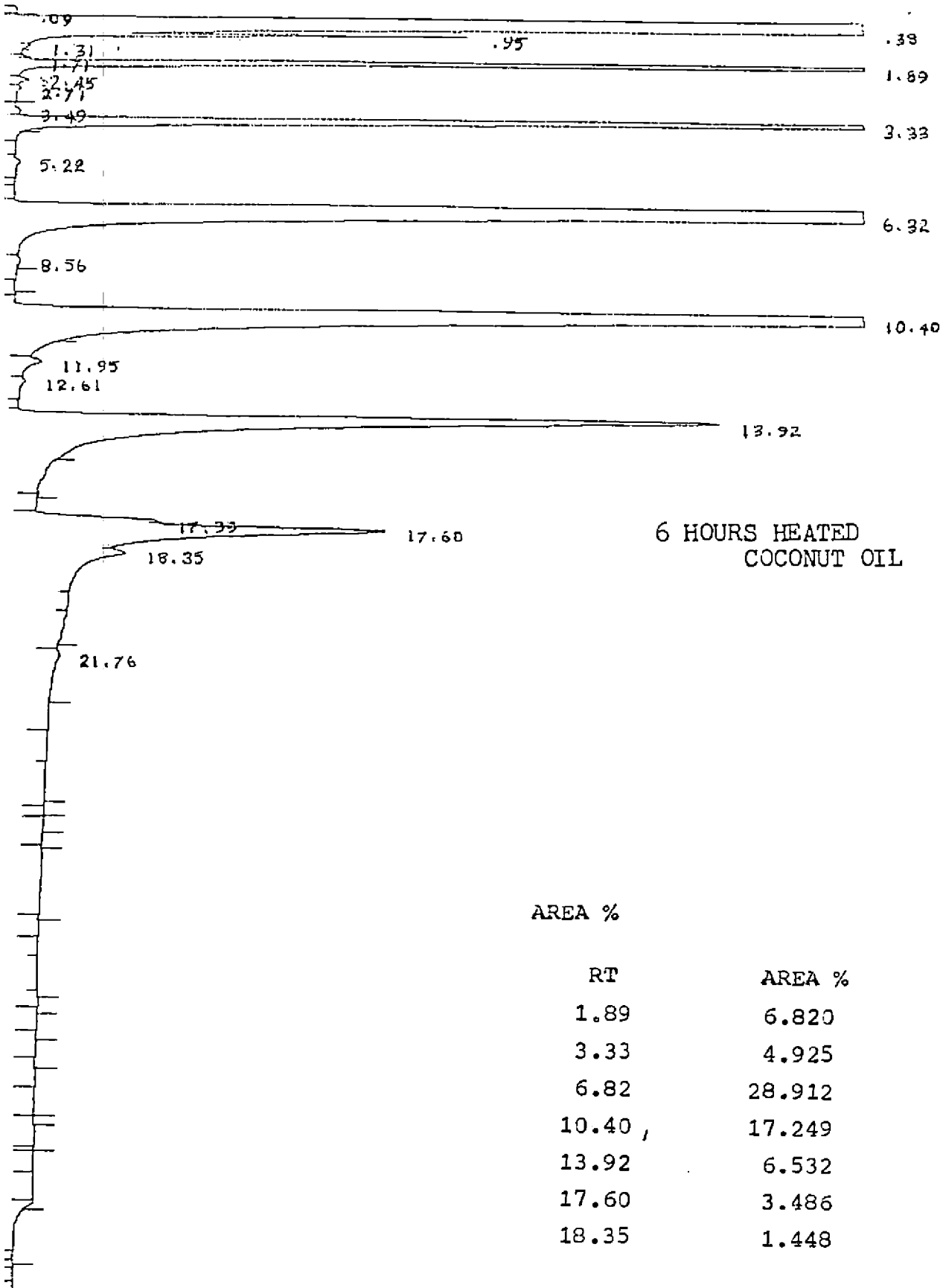
Fig. 2. Fatty acid Profile of Coconut Oil heated for 2 hours



AREA %

RT	AREA %
1.87	6.079
3.81	4.394
6.79	22.602
10.34	16.917
18.87	8.200
17.26	4.922
18.30	2.746

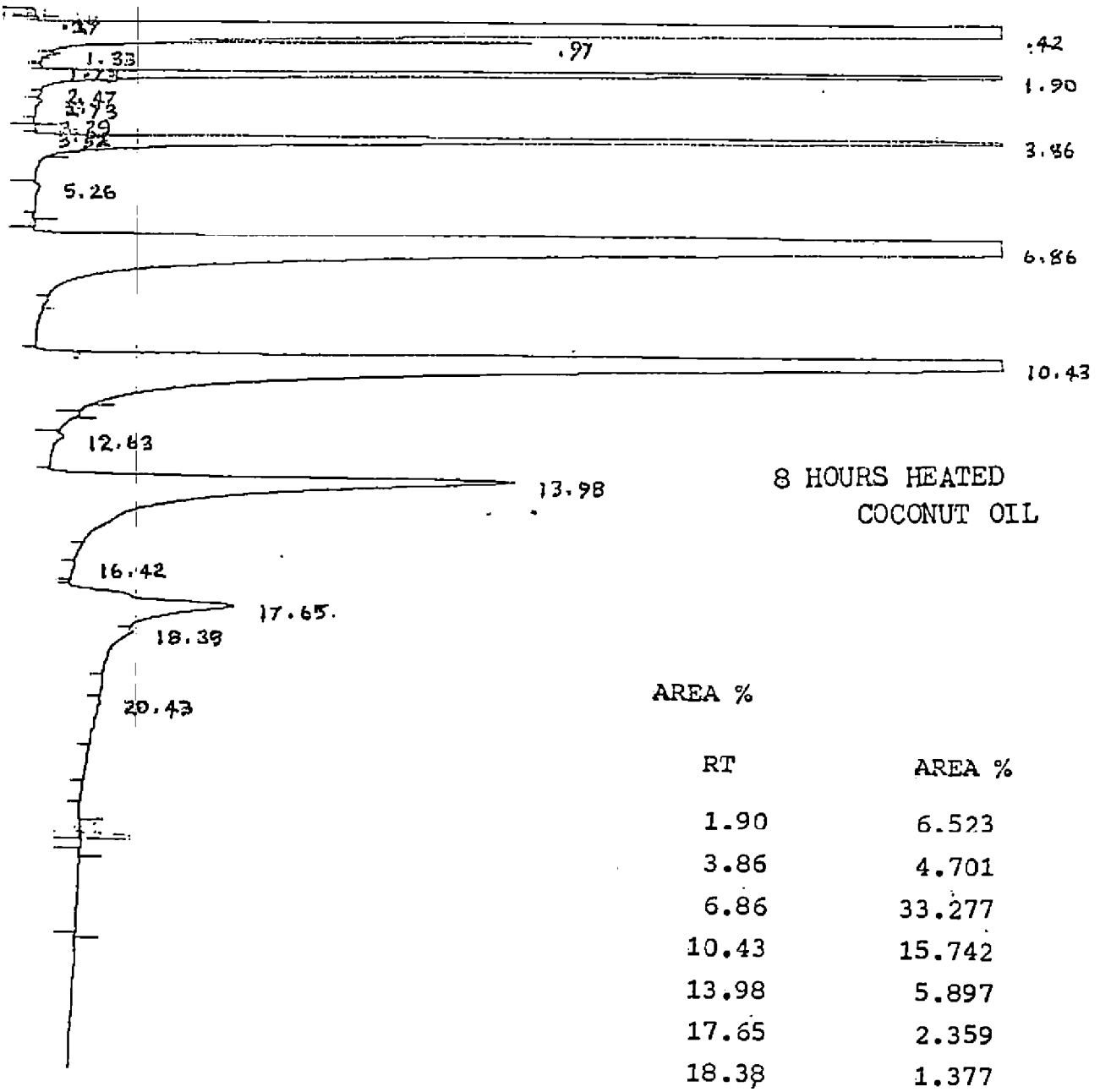
Fig. 3. Fatty acid Profile of Coconut oil heated for 4 hours



AREA %

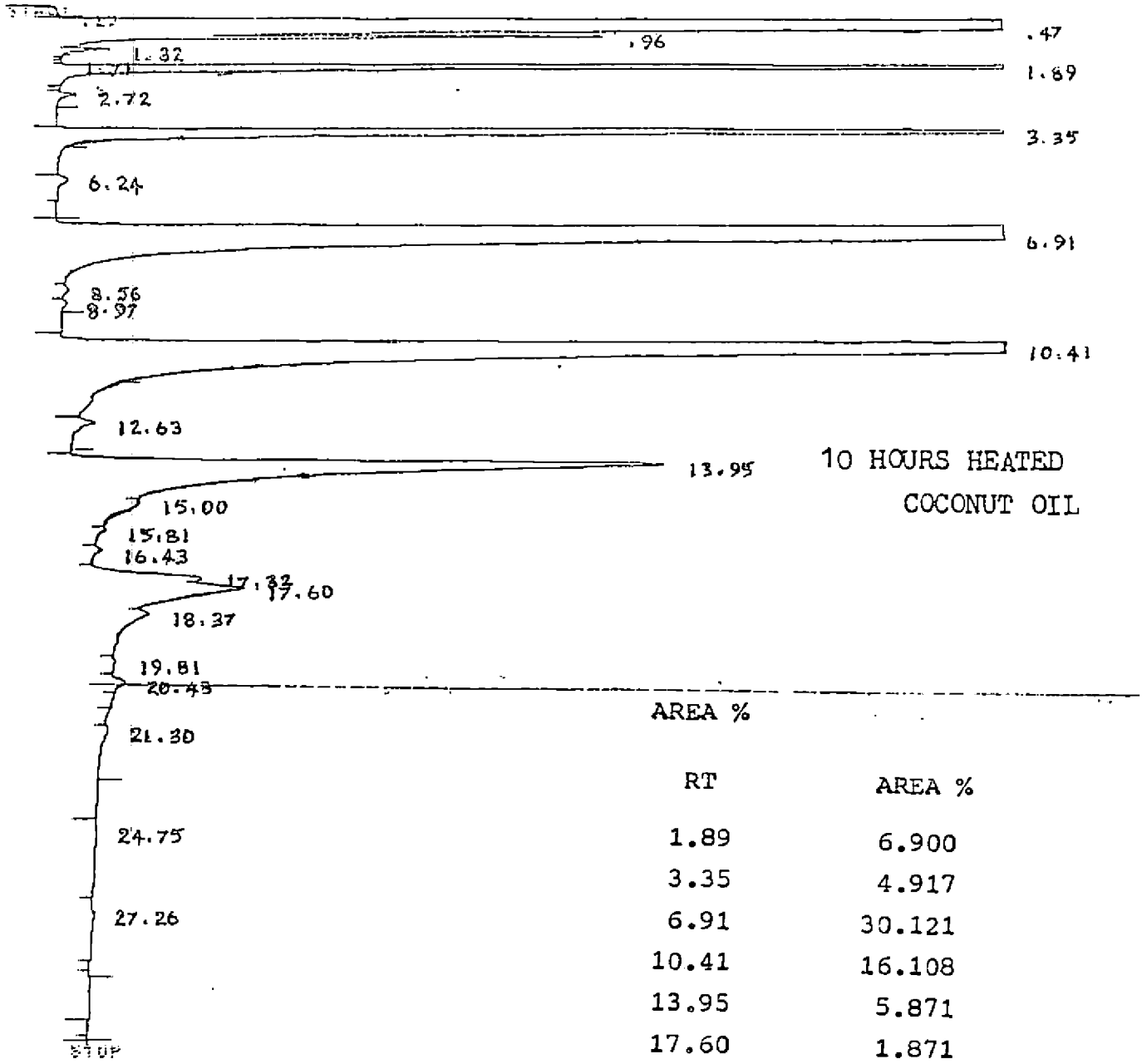
RT	AREA %
1.89	6.820
3.33	4.925
6.82	28.912
10.40	17.249
13.92	6.532
17.60	3.486
18.35	1.448

Fig. 4. Fatty acid Profile of Coconut oil heated for 6 hours



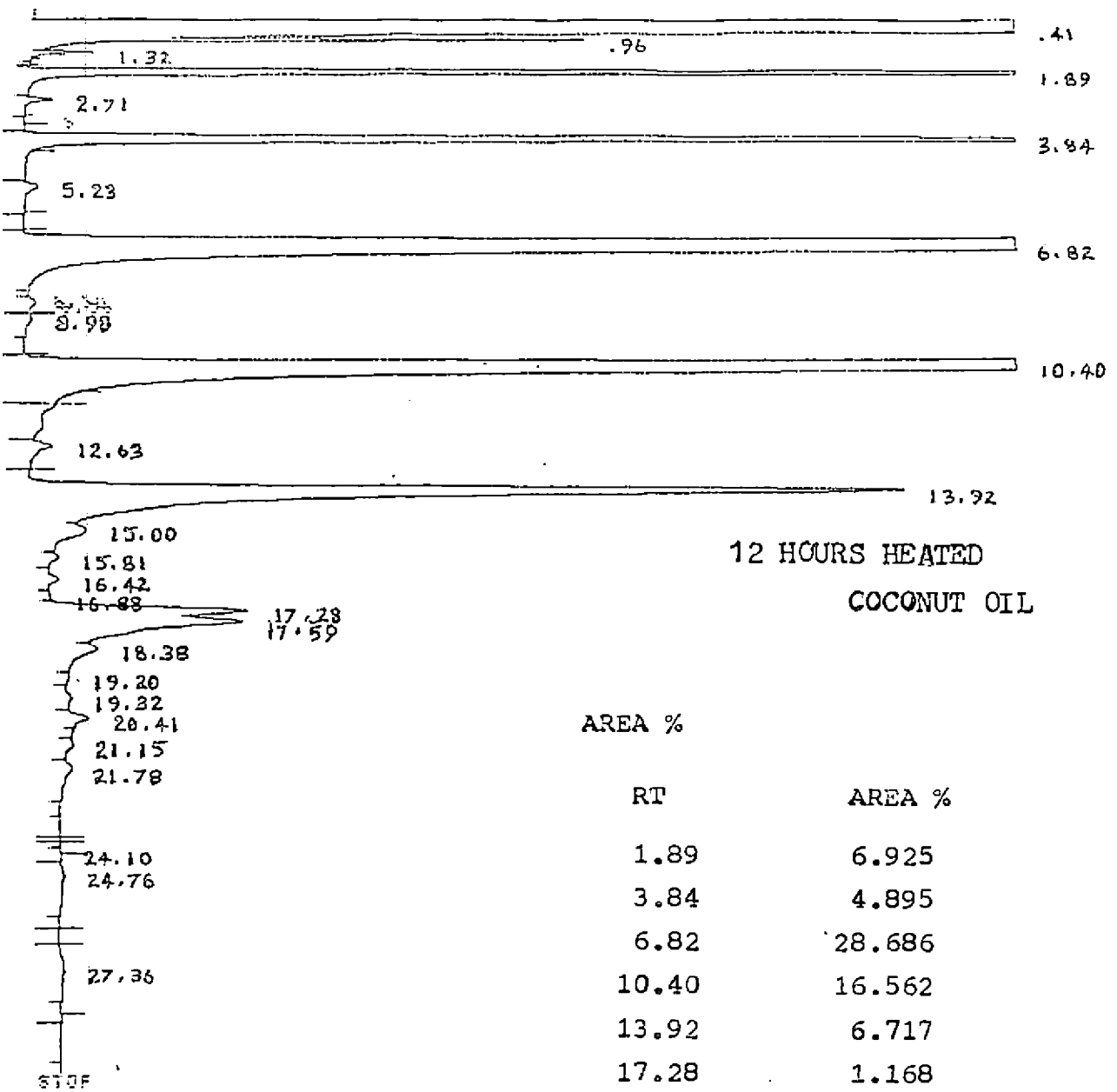
RT	AREA %
1.90	6.523
3.86	4.701
6.86	33.277
10.43	15.742
13.98	5.897
17.65	2.359
18.38	1.377

Fig. 5. Fatty acid Profile of Coconut oil heated for 8 hours.



RT	AREA %
1.89	6.900
3.35	4.917
6.91	30.121
10.41	16.108
13.95	5.871
17.60	1.871
18.37	1.143

Fig. 6. Fatty acid Profile of Coconut oil heated for 10 hours



AREA %

RT	AREA %
1.89	6.925
3.84	4.895
6.82	28.686
10.40	16.562
13.92	6.717
17.28	1.168
17.59	1.864
18.38	0.635

Fig. 7. Fatty acid Profile of Coconut oil heated for 12 hours

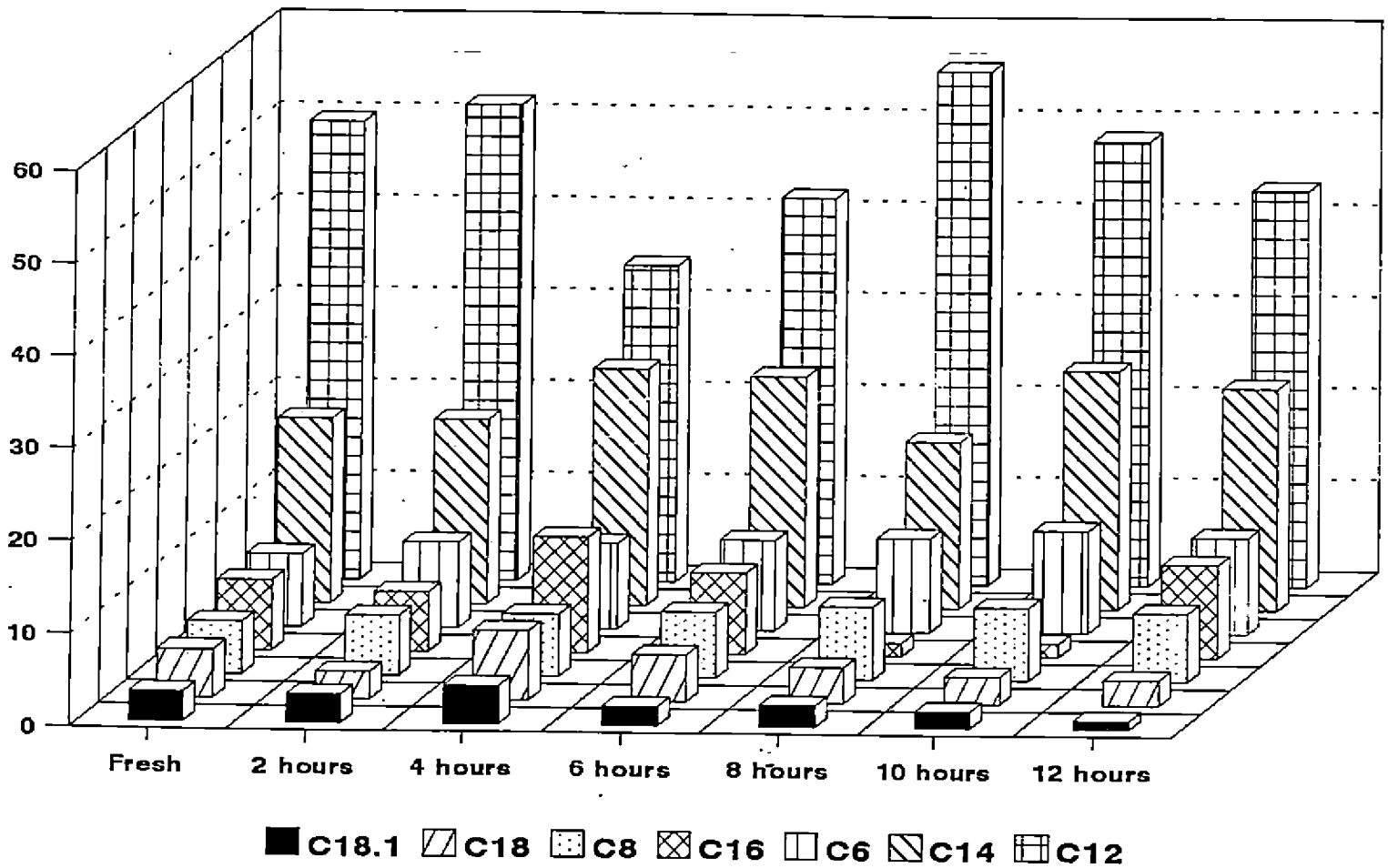


Fig. 15. Fractionation of fatty acids in 12 hours reheated coconut oil with 2 hours intervel

and reheated samples. Lauric acid (C_{12}) which has registered an initial value of 49.65 per cent in the fresh sample had given 55.9 per cent after 8 hrs of reheating. However the values reported after 12 hrs of cumulative heating was only 43.03 per cent. Myristic acid (C_{14}) content was 20.05 in the fresh sample and consequent to reheating it has increased to 24.09. In the case of C_{16} and C_{18} though these was a drastic increase in their absolute contents after 4 hrs of reheating, the values decreased subsequently towards the last sample. (See Fig. 15). However palmitic acid content had registered an increase towards last observation when compared to the fresh sample. Stearic acid content in the fresh sample had decreased from 5.24 to 2.79 after 12 hrs of heating.

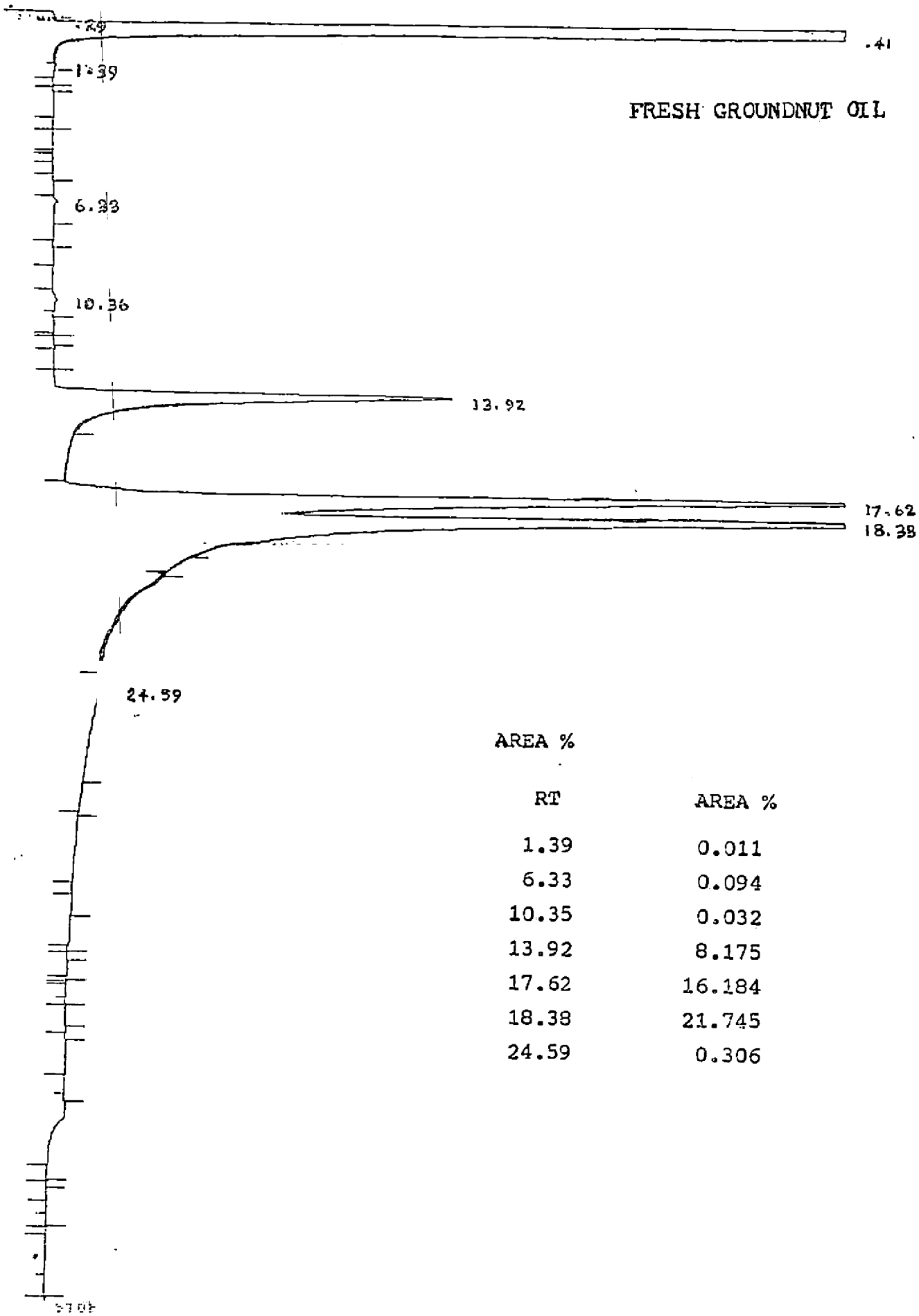
Table 8 and figures 8-14 represent data on changes in fatty acid composition of groundnut oil consequent to reheating. Nearly 95 per cent of the fatty acids present in groundnut oil was contributed by palmitooleic ($C_{16.1}$), stearic (C_{18}) and oleic acids ($C_{18.1}$). Individual contributions were 46.5 per cent, 34.6 per cent and 17.5 per cent for oleic, stearic and palmitooleic acids respectively. (See fig. 16). Short and medium chain fatty acids of chain length C_6, C_{12}, C_{14} and poly unsaturated fatty acids like

hrs, 4 hrs, 6hrs, 8hrs and 10hrs had given saturated fatty acid content of 96.87 per cent, 95.93 per cent, 97.6 per cent, 97.14 per cent and 97.7 per cent respectively. Saturated fatty acids mostly comprised short and medium chain acids like caproic (C_6), Caprylic (C_8), Capric (C_{10}), Lauric (C_{12}) and myristic acids (C_{14}). The content of long chain fatty acids represented by C_{16} and C_{18} (Palmitic and Stearic) was only a very small fraction of the total saturated fatty acid content. The unsaturated fatty acid present was mostly contributed by oleic acid, linoleic acid and linolenic acids which were present only in traces.

A critical analysis of the data reveals an increase in the content of short and medium chain fatty acids and a decrease in the content of unsaturated fatty acids with increase in heating time. According to Iwaoka and Perkins (1978) higher levels of cyclic fatty acids or cyclic monomers of aromatic compound causing toxicity in biological systems had been isolated from repeatedly reheated edible oils. However values for long chain C_{16} and C_{18} fatty acids have shown erratic values without any uniform trend. In the case of caproic acid (C_6) the increase was 7.872 to 10.4 while for caprylic acid (C_8) it ranged from 5.73 to 7.34 between fresh

Table 8. Groundnut oil - Percentage of each fatty acid in oil samples (GLC method)

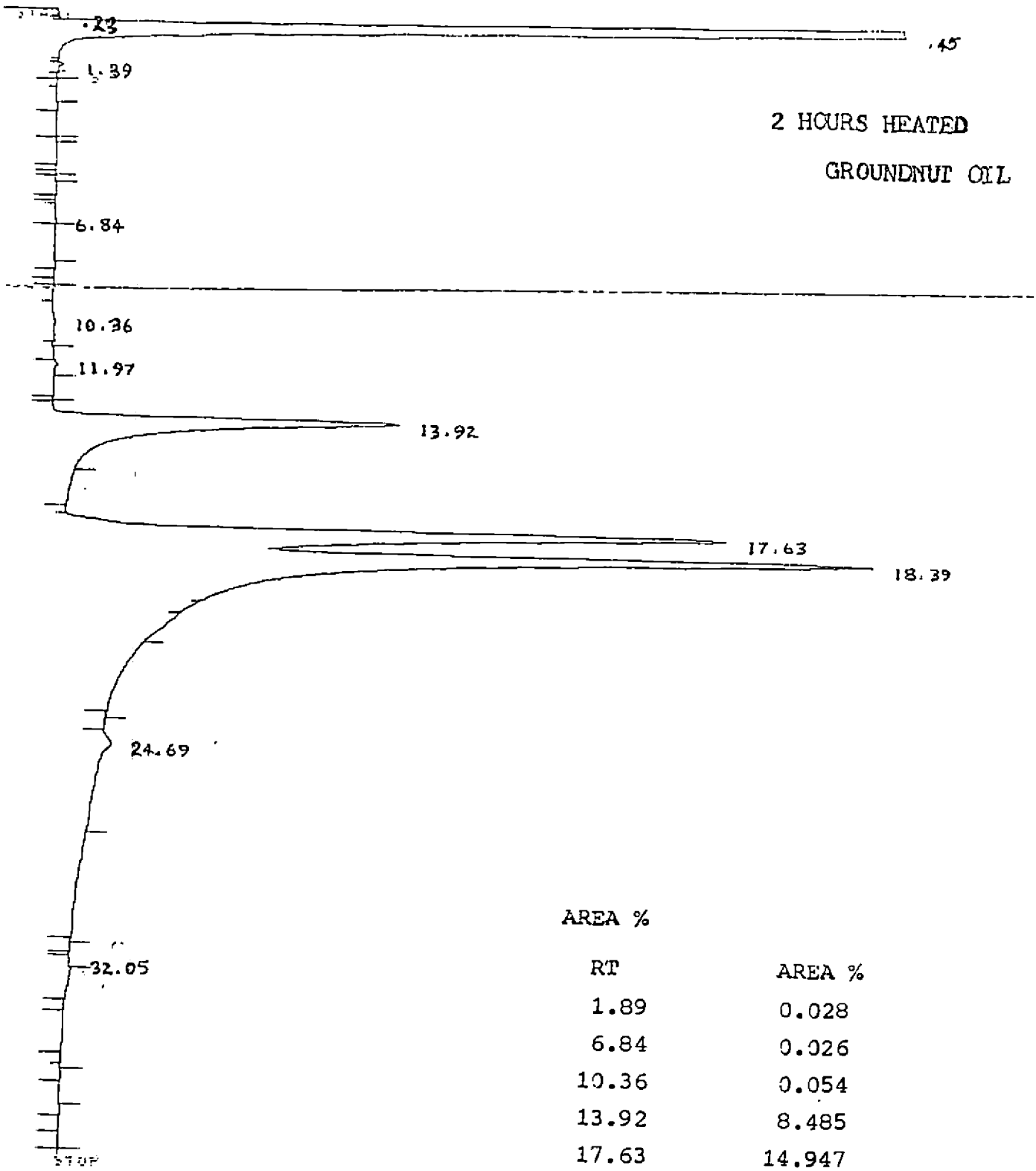
Sample No.	C_6	C_{12}	C_{14}	C_{16}	$C_{16.1}$	C_{18}	$C_{18.1}$	$C_{18.2}$	$C_{18.3}$	Saturated Fatty acid	USFA
FG	0.023	0.20	0.19	—	17.50	34.6	46.5	--	0.65	35.013	64.65
2G	0.05	0.05	0.16	--	16.10	28.4	56.6	—	0.53	28.66	73.23
4G	0.02	0.041	0.21	--	16.98	27.8	56.8	--	0.36	28.07	74.14
6G	0.02	0.44	0.28	0.41	20.20	37.3	40.3	—	0.02	38.45	60.52
8G	0.22	0.25	0.24	0.30	19.34	38.6	40.7	--	0.02	39.61	60.06
10G	0.58	0.33	0.30	0.24	20.10	34.8	42.6	—	0.24	36.25	62.94
12G	0.87	0.30	0.16	0.61	23.30	29.7	44.7	--	0.30	31.64	68.30



FRESH GROUNDNUT OIL

AREA %

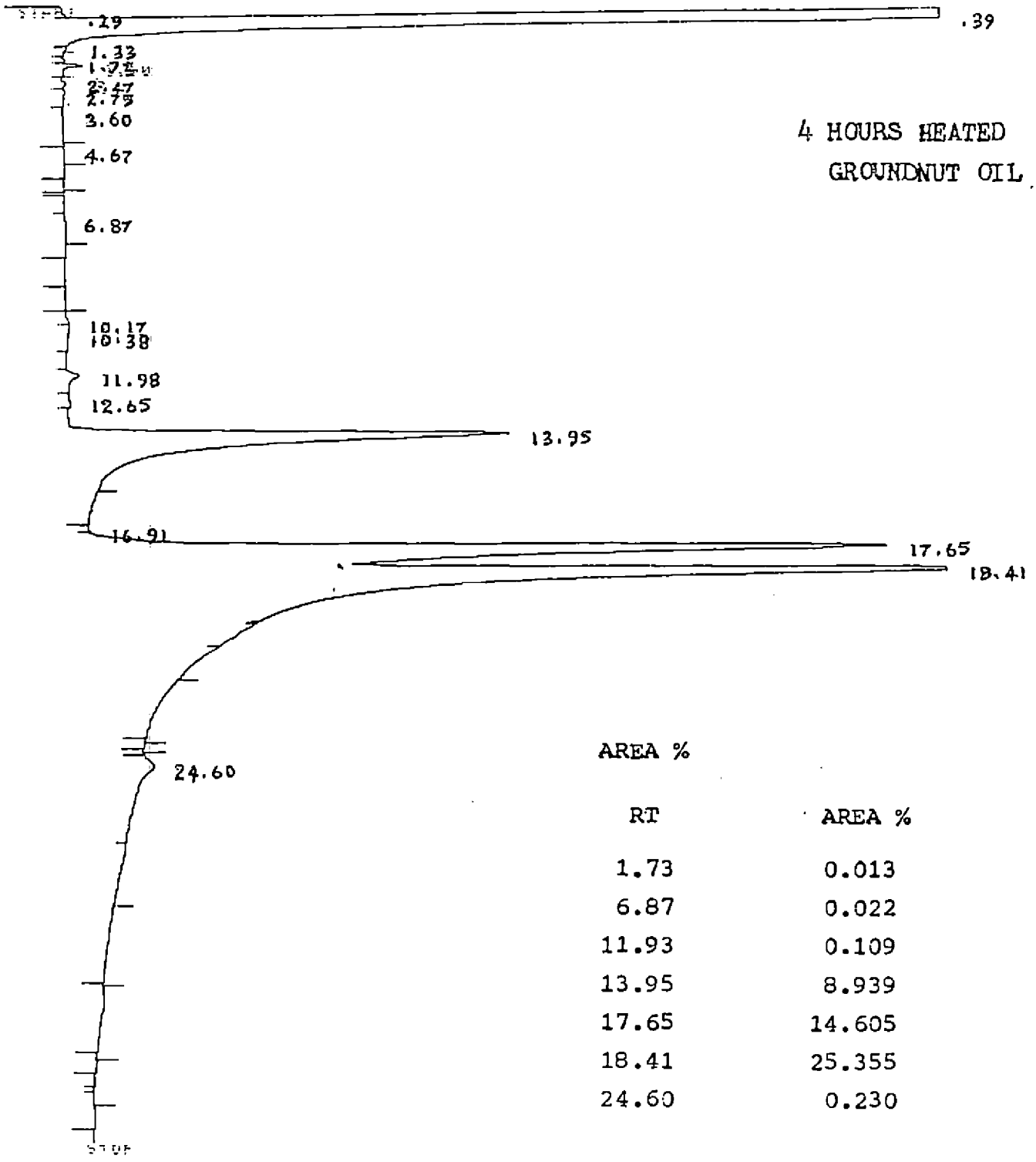
RT	AREA %
1.39	0.011
6.33	0.094
10.35	0.032
13.92	8.175
17.62	16.184
18.38	21.745
24.59	0.306



AREA %

RT	AREA %
1.89	0.028
6.84	0.026
10.36	0.054
13.92	8.485
17.63	14.947
18.39	25.796
24.69	0.275

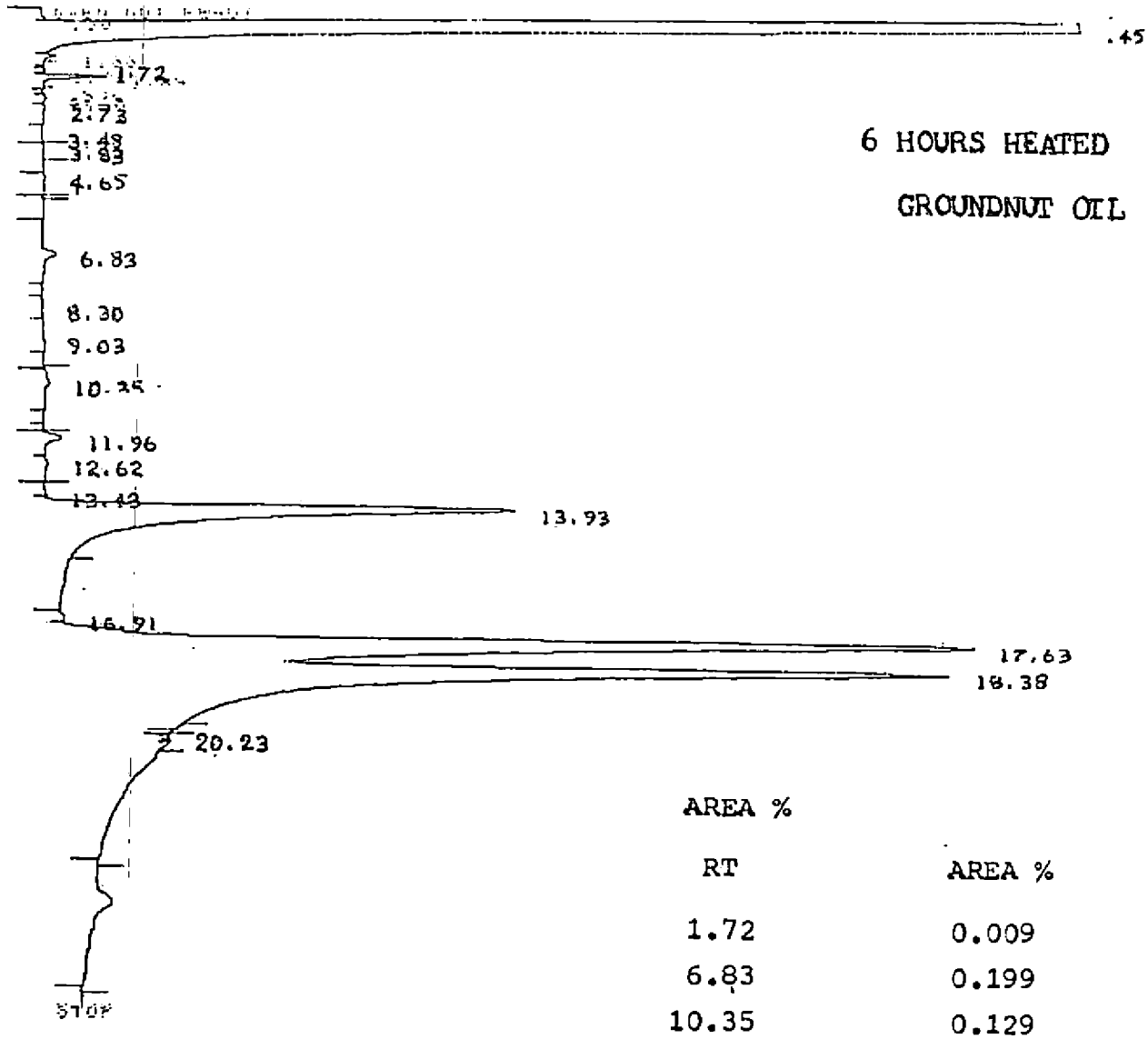
Fig. 9. Fatty acid Profile of groundnut oil heated for 2 hours



AREA %

RT	AREA %
1.73	0.013
6.87	0.022
11.93	0.109
13.95	8.939
17.65	14.605
18.41	25.355
24.60	0.230

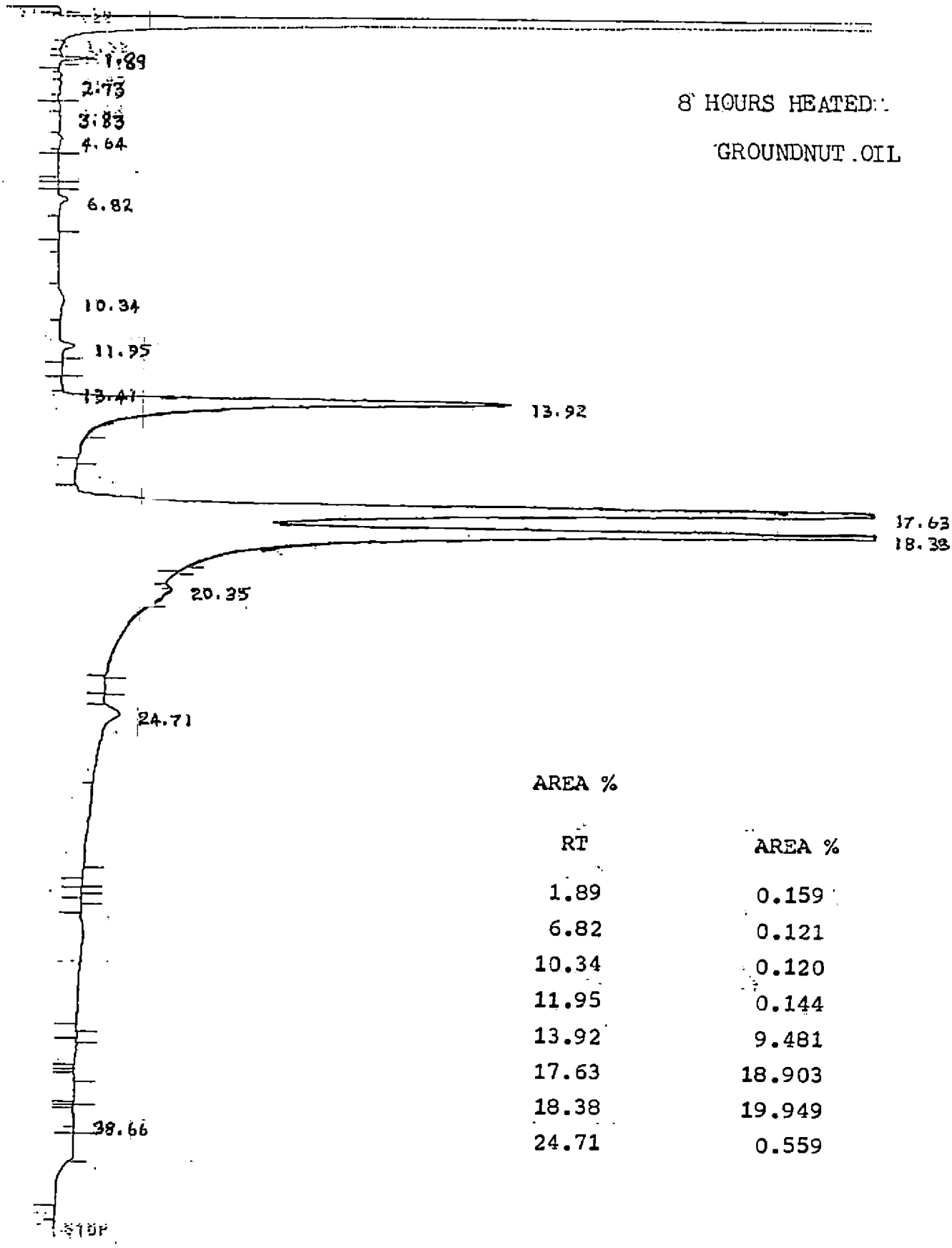
Fig. 10. Fatty acid Profile of groundnut oil heated for 4 hours



AREA %

RT	AREA %
1.72	0.009
6.83	0.199
10.35	0.129
11.96	0.186
13.93	9.131
17.63	16.537
18.38	18.310
20.23	0.009

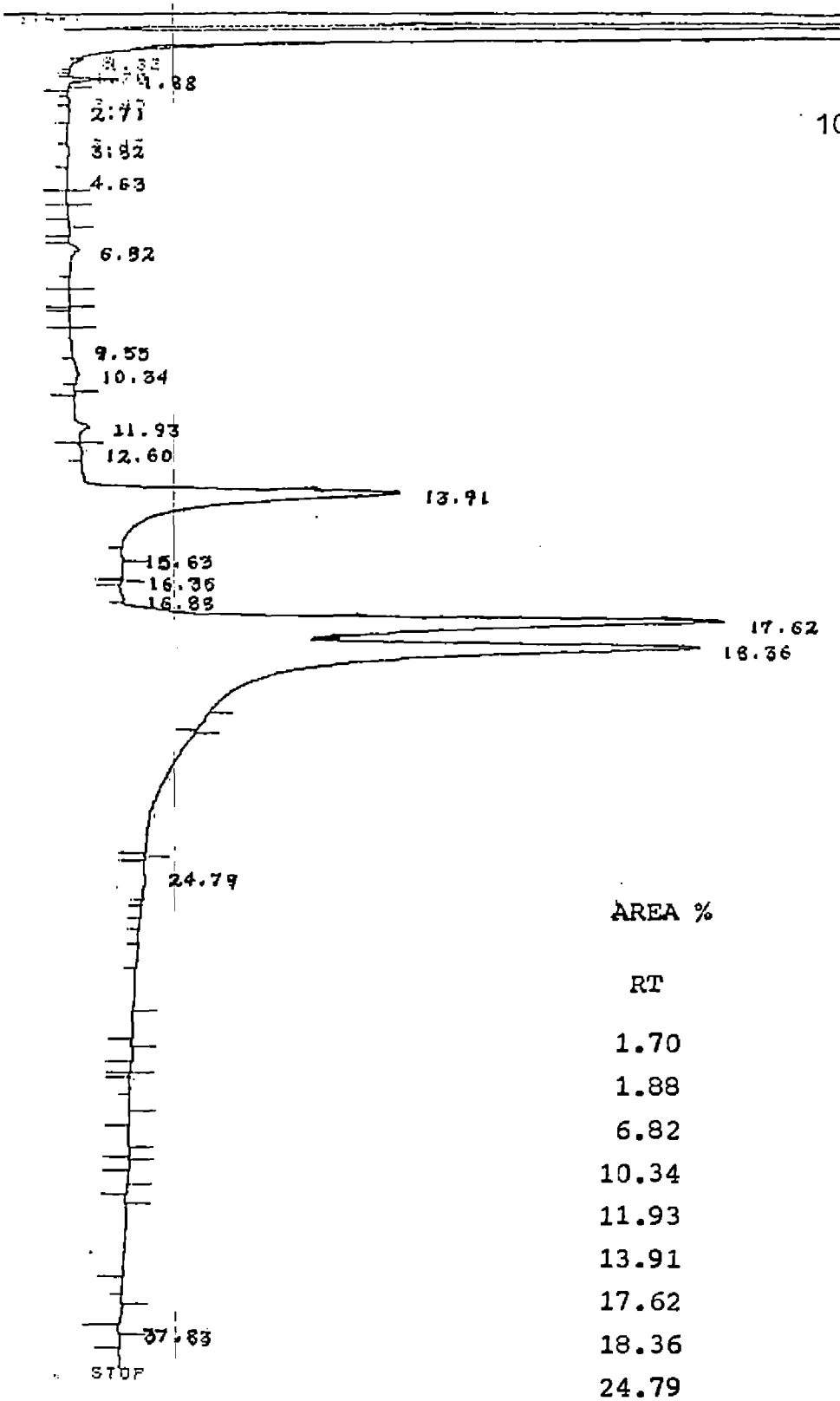
Fig. 11. Fatty acid Profile of groundnut oil heated for 6 hours



AREA %

RT	AREA %
1.89	0.159
6.82	0.121
10.34	0.120
11.95	0.144
13.92	9.481
17.63	18.903
18.38	19.949
24.71	0.559

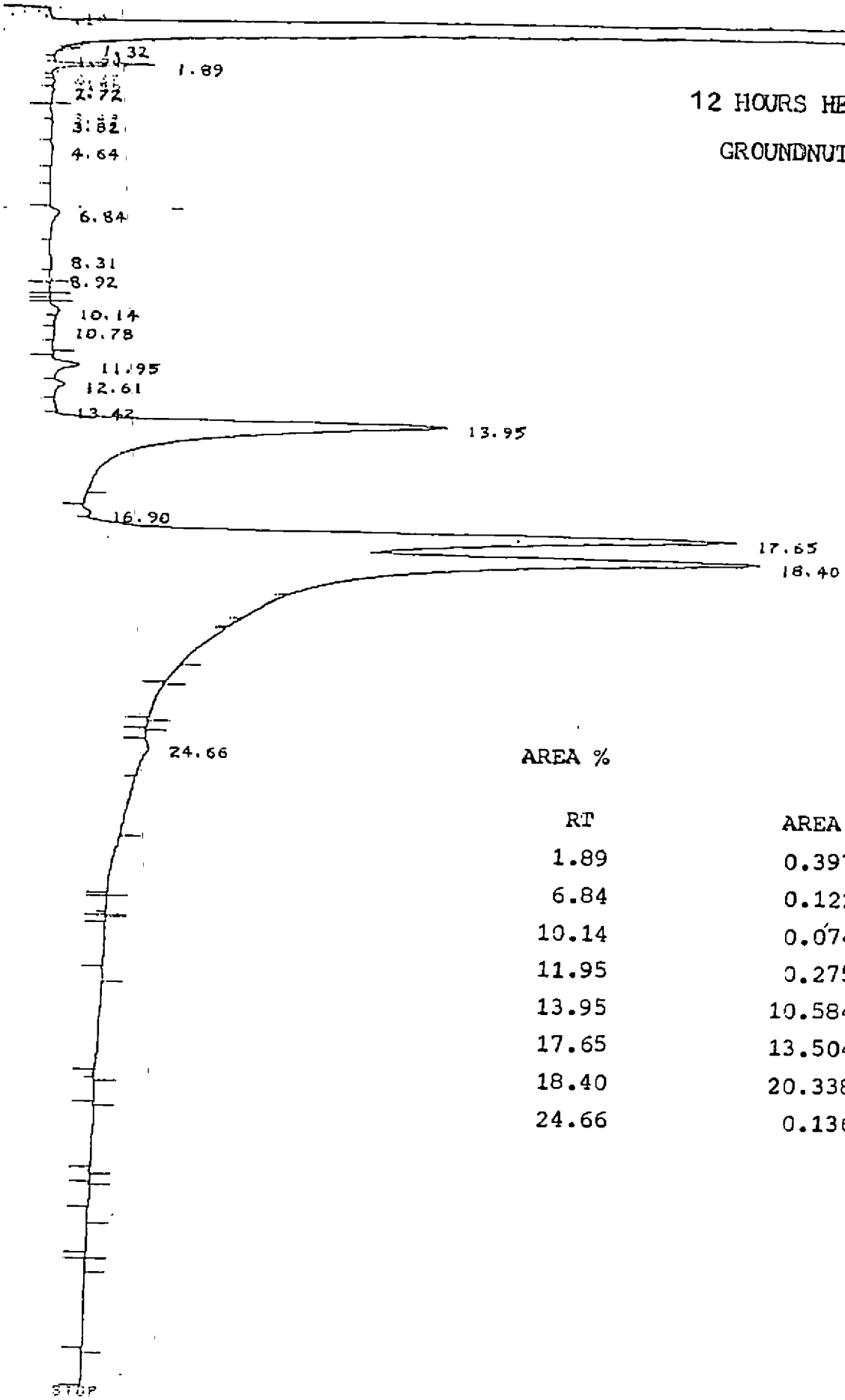
ig. 12. Fatty acid Profile of groundnut oil heated for 8 hours



AREA %

RT	AREA %
1.70	0.005
1.88	0.176
6.82	0.101
10.34	0.086
11.93	0.074
13.91	6.098
17.62	10.546
18.36	12.930
24.79	0.074

Fig. 13 Fatty acid Profile of groundnut oil heated for 10 hours



AREA %

RT	AREA %
1.89	0.397
6.84	0.122
10.14	0.074
11.95	0.275
13.95	10.584
17.65	13.504
18.40	20.338
24.66	0.136

Fig. 14. Fatty acid Profile of groundnut oil heated for 12 hours

$C_{18.2}$ and $C_{18.3}$ totalled less than 5 per cent of the fatty acid composition. Appreciable change in percentage composition could not be observed in the case of C_6, C_{12}, C_{14}, C_{16} and C18.3 fatty acids due to reheating with respect to groundnut oil. Percentage of C_{18} and $C_{18.1}$ (stearic acid and oleic acid) registered a decrease due to reheating. The initial value of 46.5 had decreased to 44.7 after 12 hours of heating in the case of oleic acid. Stearic acid content showed a decrease from 34.62 to 29.7 between fresh and the last sample. However palmito oleic acid content registered an increase from 17.5 to 23.3 between the fresh and 12 hrs reheated sample. The observed increase in the content of oleic acid after 2 hours of heating (56.6 per cent) and 4 hours of heating (56.8 per cent) might be due to the relative increase in their contents owing to the escape of short and medium chain volatile fatty acids (see Fig. 16). The decrease observed after 4 hours of reheating is attributed to the decomposition and peroxidation of this unsaturated fatty acid. It is finally stabilised to a value of 44.7 per cent. In the case of stearic acid the above said changes have occured only after 6 and 8 hours of heating. The final value obtained was 29.7 less than the original values of 34.6 per cent. Palmitic acid have shown a marginal increase to 23.3 per cent from an initial value of 17.5 per cent.

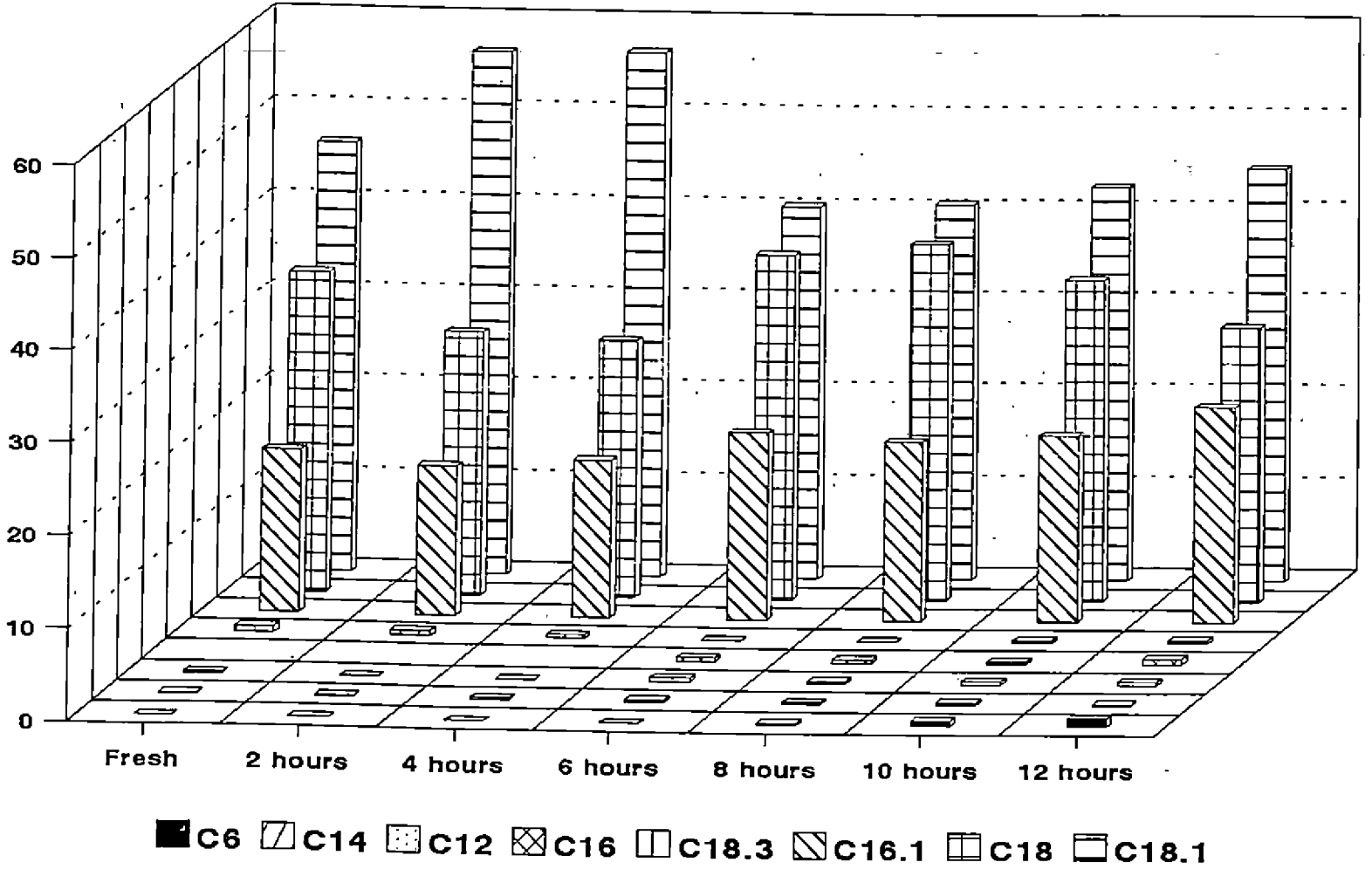


Fig. 16. Fractionation of fatty acids in 12 hours reheated groundnut oil with 2 hours intervel

A critical study of the data obtained from the present investigation reveals a decrease in the content of unsaturated fatty acid with a slight increase in the amounts of saturated fatty acids consequent to reheating in the case of coconut oil. This change is attributed to decomposition and associated chemical transformations, the unsaturated fatty acids undergo during the heating process. Similar changes in fatty acid composition of oils due to deep frying has also been reported by Sukhdev Singh (1986). According to his report the levels of essential fatty acid linoleic and linolenic acids were decreased with a corresponding increase in levels of short chain fatty acids which have no nutritional significance. Variations observed with respect to individual fatty acids can not be generalised with the data generated through this study as it requires a detailed investigation with more number of samples of the oil fractionated for all the observations. Thus further experimentation is required for such a detailed investigation. Further the time lag between sampling and fractionation of oil was not uniform throughout the study and hence any effects accrued on this account was not taken into consideration.

With respect to groundnut oil the data generated on the fractionation does not give a conclusive picture to arrive at a generalisation. Values obtained for total unsaturated fatty acid content increased for the second and third observations and decreased drastically to lower values by the sixth observation. This may be attributed to the escape of volatile saturated fractions and its decomposition products during the first few observations and thus resulting in a relative increase in contents of unsaturated fatty acids. However towards the last observation the content of unsaturated fatty acid increased to 68.6 per cent as compared to 64.65 for the first sample, though this effect has not been reflected in Iodine value. In the case of saturated fatty acids also the cumulative total decreased for the second and third observations and decreased subsequently for the next three observations. The final value of 31.64 per cent was lesser than the result reported for the first sample (35.01). More or less the same trend has been observed with respect to individual fatty acids for both groundnut oil and coconut oil. Perkins (1979) conducted animal studies with saturated fractions of heated fats or used frying oils oxidized under controlled conditions resulted in nutritional toxicity.

Though there was considerable decrease in the volume of oil used consequent to reheating unusual decrease and increase in the content of certain fatty acids could be explained with the present data. Seperate investigations are to be carried out to expedite this behaviour by taking more number of samples at frequent intervals for fractionation.

From nutritional point of view also the results obtained by fatty acid fraction revealed an increase in saturated fatty acids with decrease in essential fatty acids to a minimum. According to Nawar (1978) destruction of unsaturated lipids such as carotene, vitamin A and tocopherols will directly affect nutritional level resulting in reduced efficiency and nutritional value.

4.3. Assessing the toxicological evaluation of fresh and reheated oil through animal feeding experiments.

Effect of fresh and reheated oils at 9 per cent level in normal diets were assessed through animal experiments of six months duration. The results of the feeding experiment are discussed under the following lines.

4.3.1 Changes in body weight

Changes in Mean body weights of experimental animals in this experiment are presented in Table 9.

As revealed in Table 9, animals fed in group I (control I-9 per cent fresh groundnut oil) attains maximum weight gain followed by group II (control II - 9 per cent fresh coconut oil). In experimental groups, group III and group IV, the gain in weight was less compared to control groups with values of 178 g. and 184.5 g respectively. Studies by Alfin slater (1959) showed that highly saturated fats heated 10 to 40 hours at 225 - 250^{o}C and fed at a 20 per cent level cause weight loss and high mortality rates in rats. But in this study mortality rate was very low which may be due to low percentage level of feeding abused oils. The weight gained by the animals in the control group was highest. The difference in weight gain could be due to the difference in rate of feed consumption. Iwaoka and Perkins (1978) reported that incorporation of cyclic monomers ie, oxidation products of saturated fats into the diet of rats caused low weight gains and low feed consumption in animals.

Table 9. Effect of fresh and reheated oils upon mean body weights of rats fed with fresh and reheated oil in normal diets

Particulars	Groups I	II	III	IV
Mean initial body weight	67.5	70.00	70.50	70.00
After				
1st month	138.75 (+71.25)	123.75 (+54)	122.50 (+52)	105.00 (+35)
2nd month	165.00 (+26)	142.50 (+19)	140.00 (+18)	136.25 (+31)
3rd month	177.00 (+11)	168.75 (+26)	143.75 (+4)	146.25 (+10)
4th month	209.25 (+32)	204.25 (+36)	150.00 (+7)	165.00 (+20)
5th month	238.75 (+30)	233.50 (+29)	171.25 (+21)	178.75 (+31)
6th month	251.25 (+13)	243.00 (+9.5)	178.00 (+6)	184.50 (+7)
Mean gain in weight after 6 months (gms)	183.75	173.00	107.50	117.00

Figures in parenthesis gives increase in weight

Group I - Control I - 9 per cent fresh groundnut oil

Group II - Control II - 9 per cent fresh coconut oil

Group III - Experimental I - 9 per cent 12 hrs reheated coconut oil

Group IV - Experimental II - 9 per cent 12 hrs reheated coconut oil.

The diet followed for controls and experimental groups were the same with exception in type of oils used like fresh groundnut oil, fresh coconut oil and 12 hours reheated coconut oil respectively. Even the fraction or percentage of oil fed in the diet were the same. All other nutrients supplied in the diet were also is the same percentage for all the groups (NIN, 1984).

Statistical analysis of mean body weight of rats are presented in Table 10.

From the table mean body weight of 4 groups were 178.9 g, 169.39 g, 139.43 g and 140.82 g. Mean body weight of group I and group II were on par. ie the gain in body weight of rats fed with fresh ground nut oil and those fed with fresh coconut oil were on par. This indicates that there was no significant effect on feeding fresh coconut oil and fresh ground nut oil. Mean body weight of group III and group IV were also on par. Statistical analysis of mean body weight (gain in body weight) showed that mean body weight of the control groups (GI and GII) was significantly different from experimental groups (GIII and GIV).

Table 10. Mean body weight of groups in rrelaton to months

Groups (4)	Months (B) B_1	B_2	B_3	B_4	B_5	B_6	B_7	Mean A
Group I	67.50	138.75	165.00	177.50	209.25	238.75	251.25	178.29
Group II	70.00	123.75	142.50	168.75	204.25	233.50	243.00	169.39
Group III	70.50	122.50	140.00	143.75	150.00	171.25	178.00	139.43
Group IV	70.00	105.00	136.25	146.25	165.00	178.75	184.50	140.82
Mean B	69.50	122.50	145.94	159.06	182.13	205.56	214.18	--
SE	A = 4.43	B = 3.37	AB = 6.74					
CD	A = 13.66	B = 9.53	AB 19.06					

Plate 6. Comparison of animals between control and experimental group in appearance and size.

From the above table it is indicated that the feeding period had significant effect on gain in body weight.

Statistical analysis of gain in body weight against period of feeding showed no significant change in the initial body weight but after one month it showed that Group II. GroupIII and Group IV were on par. During second month also Groups II, III and IV were on par. From third month onwards control groups had significant gain in weight against experimental groups. Table 6 shows that gain in body weight were significantly lower in rats fed with fresh oils. According to fred (1979) rats fed with used frying fat had an unkept appearance and they did not gain as much weights as rats fed with fresh unheated fat. In this study also the reheated oils had adverse effect on gain in body weight. This is visible in their external appearance also. (See Plate 6).

4.3.2 Changes in histopathological observations

Effects of fresh and reheated oils in causing toxicological changes were examined by feeding it at 9 per cent level in normal diets through animal experiments of six months duration. Histopathological changes were observed in animal tissues at the end of third month and also at the end of study ie, after six months.

Histopathological changes observed after 3 months feeding are presented in Table 11. Histopathological changes in kidney, liver & intestine observed after 6 months feeding are presented in Table 12, Table 13 and Table 14 respectively.

Liver, kidney and Intestines from controls and experimental animals were examined at the end of 3 months to see the changes. All the organs were appeared normal (both controls and experimentals) with some exceptions which may be attributed to various other causes. Control I fed with 9 per cent fresh ground nut oil in normal diet showed normal liver, kidney and Intestine with some parasitic Ova in the intestine. This may be due to some infections or due to worm infestation which might have occured through contamination. Control 2 fed with 9 per cent fresh coconut oil in normal diet showed normal liver, kidney and Intestine. In Experimental, group III fed with 9 per cent 12 hours reheated oil liver appeared more or less normal with slight inflammatory cell infiltration. This change may be due to various other factors along with effect of reheated oils. In case of kidney, tubules appeared normal but glomeruli showed increase in cellularity (Table 11). In regard to intestine

Table 11. Histopathological changes observed after 3 months feeding

Groups	Liver	Kidney	Intestine
G_I	N	N	N with parasitic ova
G_{II}	N	N	N
G_{III}	N with slight inflammatory cell infilteration	N-tubules Glomeruli increase in cellularity	N
G_{IV}	N with slight inflammatory cell infilteration	N-tubules Glomeruli increase in cellularity	N

G_I - Control 1 fed with 9 per cent fresh groundnut oil

G_{II} - Control 2 fed with 9 per cent fresh coconut oil

G_{III} - Experimental 1 fed with 9 per cent 12 hours reheated oil

G_{IV} - Experimental 2 fed with 9 per cent 12 hours reheated oil

N - Normal condition of the organs.

section of stomach with gastrooesophageal junction appeared normal without any change. In the case of experimental, group IV fed with 9 per cent 12 hours reheated coconut oil, liver appeared normal with slight increase in cellularity as in case of experimental group III. Kidney also appeared same as experimental group III with normal tubules and increased cellularity in glomeruli. Section of stomach and gastro oesophageal junction appeared normal (Table 11).

From these results we can assume that oils reheated upto 12 hours could no longer bring any change in organs observed under histopathological observation when animals were fed only in 9 per cent level these oils in normal diet for such a short durations. According to Andrew et al. (1960) in general the feeding of highly oxidized fats (at levels of 10-20 per cent in the diet) results in loss of appetite, growth retardation, enlargement of liver, kidney and accumulation of peroxides in adipose tissue. But in this study the weight difference between the groups showed their loss of appetite and enlargement of organs were not considered so that it is sure that these change is not so evident. Artman (1969) in hi[illegible]dy observed a negative effect upon feeding used oils [illegible]gical system, when oils

heated upto 180°C for repeated frying incorporated at a 10 per cent level in diet of rats. Conclusions could be taken only after further analysis ie., after 6 months which also appears very short duration to produce evident changes in organs causing toxicity in animals.

Changes observed after 6 months duration of the feeding experiment

Liver, kidney and intenstines from control and experimental animals were examined. All the organs showed congestion (both control and experimental) and that could be explained by the hypoxia produced as a result of the mode of killng of animals adopted.

Liver was the organ which showed some changes other than congestion. Histo pathological changes observed in liver are presented in Table 12. As shown in the Table 12 the hepatocytes showed microvesicular fatty change of varying degrees in the different cases (plate 7). According to Raju *et al*. (1965) coconut oil heated to 270°F (132°C) for 8 hours in an open pan fed to albino rats at 15 per cent level resulted in depressed growth of rats, reduced liver weights

Plate 7. Liver showing microvesicular fatty changes and appearance of sinusoids represented by thick red patch and dark blue parts respectively. (60 x 2.5)- high power

Plate 8. Normal appearance of liver showing normal liver cells without any fatty changes. (4 x 2.5)- low power

Table 12. Histopathological changes observed in liver after 6 months feeding

Group No.		Central Vein	Sinusoids	Hepatocytes cytoplasmic	Nucleus	Kupffer cells	Portal area
G_I	1.	Congestion	N	Mitochondrial microvesicular	N	N	Scattered area (+)
	2.	Congestion	Congestion	Fatty change	N	N	Scattered area (+)
	3.	Congestion	Congestion	Fatty change	N	N	Scattered area (+)
G_{II}	1.	Congestion	Congestion	Fatty change	N	Prominent	++
	2.	++	++	Fatty change	N	N	+
	3.	++	++	N	N	N	N
G_{III}	1.	+	+	N	N	N	++
	2.	+	Mild congestion	++	N	N	++
	3.	+	Mild congestion	Mild	N	N	Occasional
	4.	+	Mild congestion	Mild	N	N	N
G_{IV}	1.	+	Mild congestion	Mild congestion	N	N	Occasional
	2.	+	+	N	N	N	+
	3.	+	++	Fatty change (++)	N	Prominent	++
	4.	+	Mild congestion	N	N	N	+

Mild - Represents very feeble or mild

N - Normal

\+ - Increase in number of each condition

++ - Increase in number of each condition

and showed fatty liver. This change also occurs as a result of hypoxic damage of liver cells. The fact that this change also was found in both controls and experimental animals makes it clear that the changes are due to hypoxia (Change produced as a result of mode of killing) The central veins and the sinusoids were congested in majority of cases (plate 7 & 9). The Kupffer cells didnot show much alteration. According to details of Table 12 the portal region showed lymphocytic infiltration. Shalini Rao (1985) conducted animal studies with thermally oxidised oils showed enlargment and leisions of liver and increased susceptability to certain diseases. But in this study leisions were not evident. Only lymphocytic infiltration was seen in liver (plate 9 & 10). This finding also was not specific to any particular group. Normal appearance of liver were presented in plate 8.

Histopathological changes observed in kidneys are presented in Table 13.

According to Table 13 kidneys showed congestion of the interstitial blood vessels (plate 11 & 12). This was also found in both controls and experimental animals. Increase in cellularity in glomeruli were observed in earlier

Table 13. Histopathological changes observed in kidney after 6 months feeding

Groups	No.	Tubules	Glomeruli	Interstitum	Vessels
G_1	1	N	N	N	Congested
	2	N	N	N	Congested
	3	Occassional casts	N	N	Congested
G_2	1	N	N	N	Congested
	2	Occassional casts (+)	N	N	Congested
	3	N	N	N	Congested
G_3	1	N	N	N	Congested
	2	N	N	N	Congested
	3	N	N	N	Congested
	4	N	N	N	Congested
G_4	1	N	N	N	Congested
	2	N	N	N	Congested
	3	N	N	N	Congested
	4	N	N	N	Congested

Plate 9. Liver showing congested blood vessels and lymphocytic infilteration denoted by large congested veins and red thick patched area. (4 x 2.5) - low power

Plate 10. Liver showing lymphocytic infiltration where there is scattered appearance of lymphocytes as red dotted area. (20 x 2.5)

tests after 3 months feeding was not much prominent in this case. So that this change which observed earlier cannot be considered as change due to consumption of reheated oils. In some of the cases few tubules showed hyaline casts in the lumen (Plate 13). This finding also was not confined to any particular group. Also hyaline casts can form as a result of minimal injury to the kidney. Because of these reasons this finding also cannot be considered significant in this particular experimental work. The glomeruli and interstitium was normal in all cases.

Histopathological changes observed in intestine are presented in Table 14. According to Table 14 intestine was the organ which showed minimum change. The mucosa and the rest of the wall of the intestine were normal in all cases (Plate 14). Congestion was noted insome cases. But a study by Hageman (1991) showed that frying oils like satuarated fatty acid rich coconut oil and PUFA rich ($\geq$ 60 per cent PUFA) vegetable frying oil which had been used repeatedly were fed to groups of rats at 10 per cent level with control group reported that short term consumption of heated deep frying oils which contain oxidation products of PUFA can cause increased cell proliferation in the gastro intestinal

Plate 11. Kidney showing congested vessels denoted by blue and red patched area. (4 x 2.5)- low power

Plate 12. Kidney showing congested vessels denoted by blue and red crowded area. (20 x 2.5)- high power

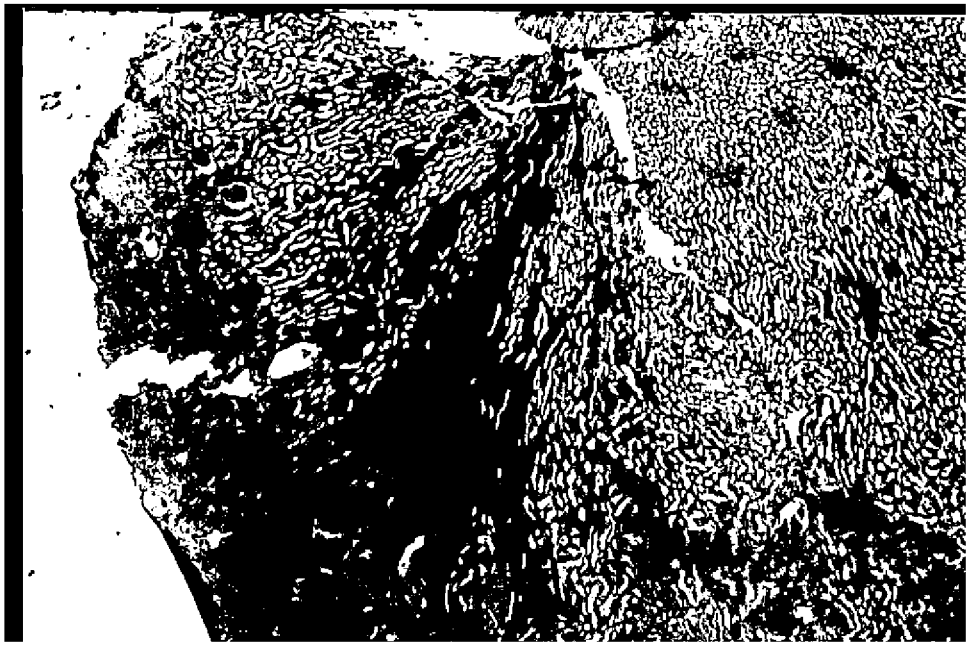


Plate 13. Kidney showing occasional hyaline casts in lumen denoted by empty vesicles within space. (40 x 2.5) - high power

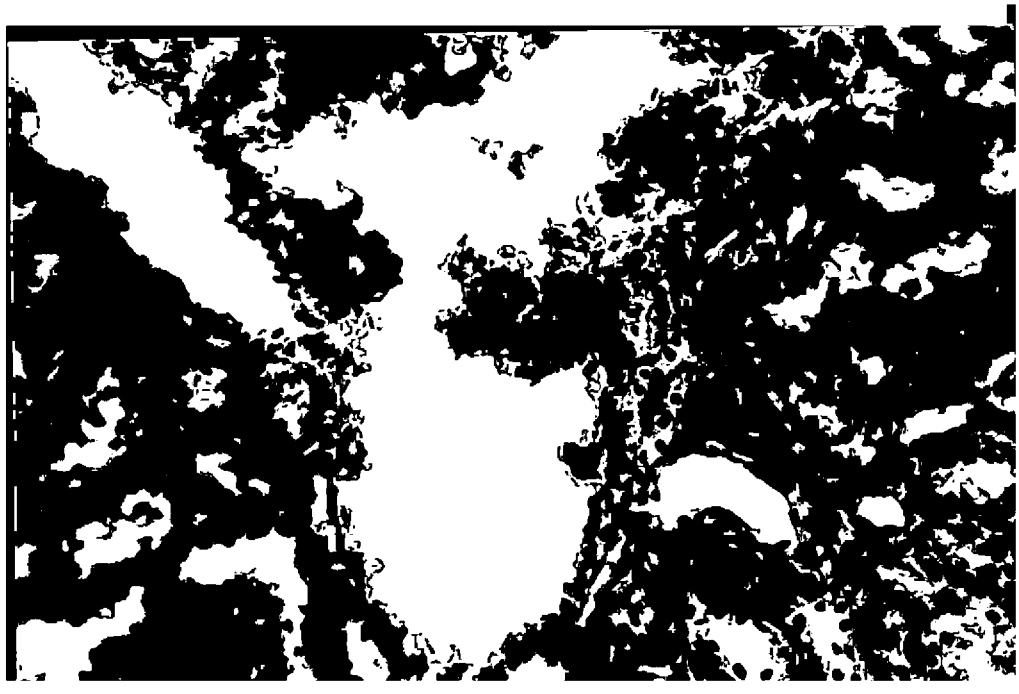


Plate 14. Normal appearance of intestine with villi which are in perfect condition

Table 14. Histopathological changes observed in intestine after 6 months feeding

Groups	No.	Mucosa	Muscular wall	Vessels
G_1	1	N	N	N
	2	N	N	N
	3	N	N	N
G_2	1	N	N	N
	2	N	N	N
	3	Congested	Congested	Congested
G_3	1	N	N	N
	2	N	N	N
	3	N	N	N
	4	N	N	N
G_4	1	N	N	N
	2	Congested	Congested	Congested
	3	N	N	N
	4	Congestion and odema of stomach	Congested	Congested

tract and induced oxidative stress. Here in this study the intestines appeared more or less normal even without congestion.

In summary the pathological changes found in the different organ in this study can be explained by the hypoxia produced during the killing of animals. Behniwal (1991) reported the feeding of peroxidized oil at 10 per cent level supress the growth, increased osmotic fraction of erythrocytes leading to reduced haemoglobin (Hb). Some results by Huang et al. showed a high mortality rate in rats when they were fed a low protein diet containing 15 per cent deteriorated used frying fat. In this study the excess oil given in the diet does not seem to produce any significant change in the inner morphology of the various organs. With further studies using a higher percentage of oil in the diet and a longer time of study might help us to identify significant changes in the organs due to a high fat intake.

4.3.3. Assessing the toxicological evaluation in reheated coconut oil (oil heated upto 12 hours) by screening for mutagenicity in bacterial system

Effect of reheated oils in causing toxicological changes were examined by mutgenicity assay (Ames et al.,

1975). For assessing these changes the organism selected was salmonella typhimurium TA 1535 strains. Test was conducted without activation by S9 oxygenase enzyme. The results obtained are presented in the Table 15.

As revealed in the table the experiment with salmonella typhimurium TA 1535 was conducted following mutagencity assay by Ames et al. (1975) without activation. From the table it was concluded that the oils reheated upto 12 hours were not significant in causing any mutagenicity or carcinogenic effect. The positive control with known carcinogen NPDA elicited in developing 610 colonies which were much higher than the experimental carcinogen ie. reheated oils (Table 15). In experimental cases the colonies developed were very few ranging between 16-29 when compared with positive control with 610 colonies so that the chance of being a possible carcinoge can be eliminated (Table 15). This may be due to lack of activation to an extend which may otherwise induce carcinogenic effect in bacterial system as bacteria lacks the enzyme S9 oxygenase which is present in all mammals including man. Due to the task of extraction of S9 enzyme from rat liver being very tedius that process is avoided. Besides the lack of this enzymes the chemical

Table 15. Toxicological evaluation of reheated oils (heated upto 12 hours) using mutagenecity assay

(without activation by S9 oxygenase enzyme) because activation is a laborious and tedious task.

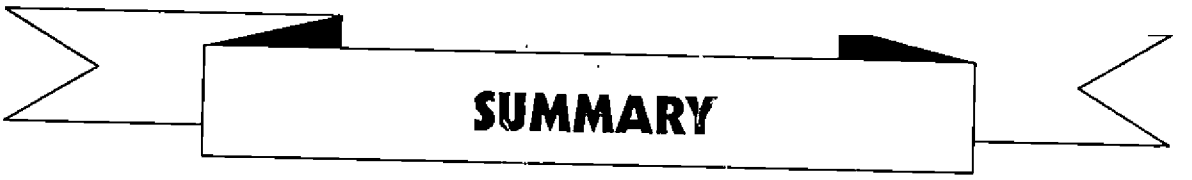
Sample	Amount of mutagen injected	No. of colonies developed
Negative control	No mutagen (spontaneous revertant)	16 colonies
Positive control	NPDA - 10 ug (carcinogen)	610 colonies
Oil I 1)	50 ul - Reheated oil	29 colonies
2)	75 ul - Reheated oil	17 colonies
Oil II 1)	50 ul - Reheated oil	16 colonies
2)	75 ul - Reheated oil	29 colonies

Oil I and Oil II are reheated coconut oil (upto 12 hours)

compounds formed may not be that much effective in causing a carcinogenic effect. A study by Scheutwinkel-Rich (1980) for testing mutagenicity of used frying oil negative result were obtained when a polar fraction of fatty acids were subjected to salmonella / microsome mutagenicity test. A contradictory study by Saleh et al., (1986) for mutagenic activity using salmonella typhimurium with and without activation 59.6 per cent of heated samples showed significant mutagenic activity. In that case peroxide, hydroxyl acid and conjugated diene values were much higher for mutagenic samples. In this study these dimensions are not measured.

A sample is considered to be mutagenic if it brings about a two fold or greater increase in the mutation rate. To assure the working ability of the system especially with regard to S9 activity positive mutagenic substance were run simultaneously. Test range limited by preparation of test substance and dose of toxic substance. Toxic effects can be seen when number of spontaneous revertants diminishes followed by disappearance of background lawn of nonrevertant bacteria. Toxic effects are well demonstrated with hydroperoxides of linolenic acid. But in contrast to standard mutagens tested substance do not increase mutation rates significantly.

From all the above experiments and datas observed during the study might help us to conclude that reheated oils to some extend are not much harmful to health though they may produce some internal changes due to changes or conversion of fatty acids to harmful ones. These oils also bring about some changes in our internal tissues of certain organs especially in liver. So it is frustrating to draw a decisive conclusion from this result to prove that highly thermally abused oils are detrimental to health in regard to causing liver and intestinal tumours or cancers.


SUMMARY

SUMMARY

A study of on "Physico Chemical, nutritional and toxicological evaluation of thermally oxidized edible oils" was conducted to assess the effect of reheating of edible oils in their physico chemical characterestics, fatty acid composition to see the nutritional changes and also to assess toxicological changes produced by reheating. The influence of long time, high temperature, deep frying foods are taken into consideration. Fresh oils (both coconut oil and ground nut oil) are taken as controls and compared with reheated samples to assess all these changes. Toxicological evaluation of fresh and reheated oils were conducted through animal experiments using only coconut oil since it is the most widely used frying oil in Kerala.

The Physico chemical characteristics of fresh as well as reheated coconut oils and ground nut oils were ascertained by estimating smoke point, Boiling point, acid value, iodine value and saponification value of oil samples drawn and kept after every hour of heating. The boiling point of both the oils were found to be decreased consequent

to reheating. The lowest boiling point was recorded in case of samples subjected to 12 hours of heating (cumulative). But in case of smoke point a reverse trend was observed. Fresh oil had given the minimum value with respect to smoking temperature. A high variation of 26^{o} C and 9^{o} C were observed in boiling point between the fresh and 12 hours reheated oil in coconut oil and ground nut oil respectively. But the variation of smoking temperature between fresh and 12 hours reheated sample was 11^{o} C for both the oils.

Acid value of coconut oil were found to be decreasing consequent to reheating. But the reverse trend was observed in ground nut oil. It was found to be increasing with reheating with least variation in their values. Iodine values of both coconut oil and ground nut oil showed similar pattern of change consequent to reheating. Iodine value were observed to be decreasing from the fresh sample to 12 hours reheated sample. In case of saponification number contradictory results were observed between the two oils. In coconut oil the saponification values were found increased with a small range difference, where as in ground nut oil it was found to be decreasing with a small range difference. The change was marginal for acid value where as it is substantial for iodine number.

Nutritional changes were studied by fractionation of fatty acids from fresh as well as reheated oil samples at two hours interval in both coconut oil and ground nut oil. Distribution pattern of individual fatty acids from different treatment in coconut oil indicated a total saturated fatty acid percentage of 96.17 in the fresh sample and 99.47 in reheated samples with cumulative heating time of 12 hours. The data observed were found to reveal an increase in the content of short and medium chain fatty acids and a decrease in the content of unsaturated fatty acids with increase in heating time. The fatty acid composition of ground nut oil consequent to reheating was contributed by oleic, stearic and palmitooleic with a percentage of 46.5, 34.6 and 17.5 respectively totalled to 95 percentage of total fatty acids. Short and medium chain as well as poly unsaturated fatty acids were contributed only to 5 percentage of total fatty acids. In this case values for total unsaturated fatty acid content increased and then decreased drastically due to escape of volatile saturated fractions. There was unusual increase and decrease in the content of certain fatty acids. From nutritional point of view the results obtained by fatty acid fractionation revealed an increase in saturated fatty acid with decrease in essential fatty acids to a minimum.

Toxicological evaluation of thermally oxidized edible oils was further assessed by conducting suitable animal experiments. A preliminary feeding of six months duration was conducted with two control groups fed with fresh coconut and ground nut oil at 9 per cent level and experimental group with reheated coconut oil at 9 per cent level. Monthly weight gain was recorded and the experimental conditions were maintained the same throughout the experiment. The results of the study revealed that the gain in body weight were significantly lower in rats fed with reheated oil compared to rats fed with fresh oils. But there was no significant effect observed on feeding fresh coconut oil and fresh ground nut oil.

Histopathological investigations were carried out by taking major organs liver, kidney and intestine at two periods during the course of experiment. In case of observation after three months kidney and intestine appeared normal. Liver showed slight inflammatory cell infiltration. After six months feeding all the organs showed congestion which might be due to hypoxia. Liver was the organ which observed more changes than others. The hepatocytes showed micro vesicular fatty change in varying degrees. The central

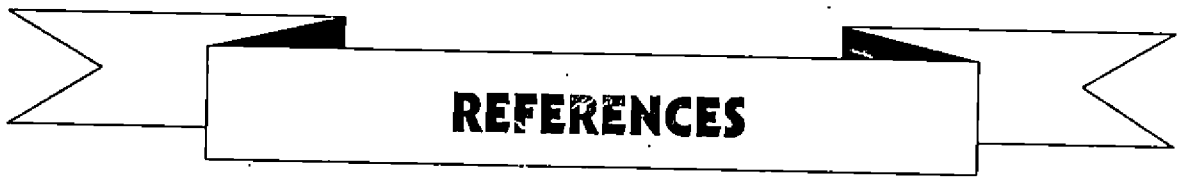
veins and sinusoids were congested in both controls and experimentals. The portal region also showed lymphocytic infilteration in few cases.

Kidneys showed congestion of intestial blood vessels irrespective of the group. In few cases both controls and experimental groups showed hyaline casts in the lumen. Intestines showed only minimal changes due to consumption of reheated oils. The mucosa and rest of the wall of intestine were normal in both the cases.

Toxicological evaluation of reheated coconut oil (heated upto 12 hours) were conducted by screening for mutagenicity in bacterial system. For assessing these changes the organism selected was salmonella typhimurium TA 1535 strains without activation by S_9 oxygenase enzyme. The positive control with known carcinogen NPDA elicited in developing 6IO colonies which were much higher than the experimental carcinogen ie reheated oils when injected in 50 ul or 75 ul the colonies developed in experimental cases were only 29 and 17 colonies respectively. This is very few when compared to positive control so that chance of being a possible carcinogen can be eliminated. Lack of activation by

the enzyme could be one reason for such a response. From these experiment we can conclude that reheated oils upto 12 hours were not that much effective in causing carcinogencity or mutagenicity though lack of enzyme activation may be a reason for this behaviour.

Based on all the above explanations and results it is sure that reheating of oils upto 12 hours have not much to do in causing mutagenicity or carcinogencity. Short term duration of the feeding experiment was also reporting that such a short duration of consumption of heated oils could not produce any tumour in the organism. Similarly in the case of humans also. Reheated oil consumption could not produce any ill effect outwardly but of course it will produce some changes interiorly organs. So it is always better to practice the correct and proper use of all foods especially regarding to cooking oils.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PHYSICO CHEMICAL, NUTRITIONAL AND TOXICOLOGICAL EVALUATION OF THERMALLY OXIDISED EDIBLE OILS

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Abstract of the Thesis
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ABSTRACT

"Physico chemical, Nutritional and toxicological evaluation of thermally oxidized edible oils" were determined by assessing their changes in physical and chemical properties, Nutritional changes ie changes in fatty acid composition and toxic as well as murtagenic effect in relation to reheated oils taking fresh oils as check.

Among the five factors considered for physico chemical analysis in both the coconut and groundnut oil the boiling point were found to be decreasing consequent to reheating. In case of smoke point a reverse trend was observed. Acidvalue of coconut oil were found to be decreasing consequent to reheating but it was vice versa in case of groundnut oil. Iodine values of both the oils were observed to be decreasing from the fresh to 12 hours reheated sample. Coconut oil showed a regular increase in saponification value where as in groundnut oil it is found to be decreasing.

Nutritional changes were observed by observing changes in fatty acid composition of fresh as well as reheated oils with 2 hours interval. The data observed ended in conclusion that as the time of heating increases there will be increase in content of short and medium chain fatty acid and a decrease in content of unsaturated fatty acids. Short, medium & Poly unsaturated fatty acids contribute only 5 percent of total fatty acid. From the nutritional point of view essential fatty acids were decreased to a minimum in case of groundnut oil with increase in saturated fatty acid.

A preliminary feeding of 6 months duration was conducted with two control groups fed with fresh coconut oil and groundnut oil at 9 per cent level and experimental group with reheated coconut oil in the same level for toxicological evaluation. The results of study revealed that gain in body weight were significantly higher for controls when compared with experimental groups.

The animal feeding experiment was undertaken also to estimate the effect of reheated edible oils with regard to histopathological changes in animals. Histopathological investigations was carried out by taking major organs

viz., liver, kidney and intestine at two periods during the course of experiment. Liver was the organ that showed changes other than congestion. Hepato cytes showed microvesicular fatty changes of varying degrees in both control and experimental groups. The central veins and sinusoids showed only congestion where as kupffer cells did not show much alteration in all cases. Kiney appeared normal in all cases with exception of few hyaline casts in the lumen. Intestine appeared normal in all cases.

Toxicological evaluation of fresh and 12 hours reheated coconut oil also was evaluated by mutagenicity screening in bacterial system salmonella typhimurium TA 1535 strains without activation. The study resulted in conclusion that the colonies developed as a result of induction of experimental carcinogen ie reheated oil were very few when compared with positive control. So that the chance of being possible carcinogen can be eliminated.

Thus in conclusion reheated oils upto 12 hrs are not much harmful to health, though they may produce changes in the physico chemical properties as well as nutritional aspects. Short term consumption of these oils also bring

about slight alteration in the internal tissues. Mutagenic and toxicological studies are to be conducted for longer periods with thermally oxidised edible oils to get final results. So it seems difficult to draw decisive conclusion from this study to prove that highly thermally abused oils are detrimental to health.