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Profiling of Fatty Acid Compositional Alterations in Edible Oils Upon Heating Using Gas Chromatography

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Abstract: *Edible oils are recognised as essential nutrients in the human diet as they are one of the concentrated sources of energy that provide essential fatty acids, the building blocks for hormones needed to regulate body systems. The intense frying of oils causes thermal reactions, including hydrolysis, oxidation, polymerisation and isomerisation, resulting in thermal degradation of the oils. Heating stimulates the formation of peroxides, triacylglycerol and carbonyl compounds, which decrease the nutritional value of the oils. This leads to destructive diseases such as cardiovascular diseases, colon cancer, atherosclerosis and others. In the present study, palm and groundnut oils are subjected to four cycles of heating to frying temperature, and changes in their composition are studied using gas chromatography-mass spectrometry (GC-MS). The variation of the oil viscosity with heating time is also observed and is found to correlate with the composition of fatty acids in the oils.*

Keywords: Palm oil, groundnut oil, fatty acid, gas chromatography-mass spectrometry, oil viscosity

1. INTRODUCTION

Oils are used for the frying of food in many countries around the world. The primary constituents of the oils are fatty acids, which play a vital role in determining the quality of food, and thus the health of people.[1] Therefore, it is very important to analyse the chemical composition of the fatty acids in oils. The risk factors associated with coronary heart disease and cholesterol levels correlate to the amount of fatty acids such as linoleic acid and other saturated and polyunsaturated fatty acids.[2–5]

Gas chromatography-mass spectrometry (GC-MS) is the single most important tool and a highly compatible technique for the identification and quantification of volatile and semi-volatile organic compounds in complex mixtures. This technique is very useful for the determination of the molecular weights and elemental composition of such compounds.[6] Gas chromatography

and liquid chromatography instruments are widely preferred in the real-time analysis of fatty acids, especially in micro-scale research.[7]

The chromatograph is used in the separation of a variety of common fatty acids and their related compounds encountered in animal and plant tissues.[8] It is also used in the quantification of pollutants in drinking water, wastewater and the atmosphere, quantification of drugs and their metabolites in blood and urine for both pharmacological and forensic applications, identification of unknown organic compounds in hazardous waste sites, identification of reaction products by synthetic organic chemists and analysis of industrial products for quality control.[9] The creation of open-tube capillary columns provided a critical advancement in gas chromatographic isolation of acids.[10] However, this method has the disadvantage of lower accuracy, which can be overcome by using fused silica capillary columns and liquid phases.[11–14]

In GC-MS, the sample is in the vapour phase, and both techniques utilise approximately the same amount of sample (typically <1 ng). Quantitative accuracy is controlled by calibration of the overall analytical method. Using isotopic internal standards, the reproducibility of the measured data using gas chromatography must be <1% relative standard deviation.[15] In the case of edible oils, this can be achieved in practice.[16,17] The GC-MS system is composed of two major components: the gas chromatograph and the mass spectrometer. As the name implies, GC-MS is the combination of two techniques to form a single method of analysing mixtures of chemicals. Gas chromatography, specifically gas-liquid chromatography, involves a sample being vaporised and injected onto the head of the chromatography column. The sample is transported through the column by the flow of an inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. The retention time (RT) parameter is defined as the time it takes for a compound to travel from the injection port to the detector.[18]

The calculation of rheological parameters provides valuable and predictive information regarding product attribute and flexibility. In order to measure the consistency and quality of a food product, viscosity is considered to be one of the key parameters in food industry. In the creation of products by the food industry, edible oils can act as one of the elementary components.[1] Viscosity commonly refers to the resistance offered by one section of a fluid moving relative to another section. Hence, viscosity is closely associated with the structural parameters of fluid particles.[10] The viscosity of oils is directly associated with a few chemical features of the liquids, which include the degree of unsaturation and the chain length of fatty acids comprising triglycerides.[10]

In the present work, GC-MS is used to identify and quantify the composition of oils before and after heating. This paper reports the investigation

and analysis of the degradation of saturated, unsaturated and antioxidant compounds in oils by determining the compositional changes before and after four cycles of heating to frying temperature. Kinematic viscosity of oils is observed before and after heating to four cycles of frying temperature and is correlated with saturated and unsaturated fatty acid composition.

2. EXPERIMENTAL

2.1 Frying of Oil

Refined and branded palm oil and groundnut oil, which are most commonly used for cooking in southern India, were purchased from a local source. 50 mm of the sample oil is placed in a copper beaker, heated by an electric heater, and stirred manually with a glass rod. A microcontroller-based temperature sensor has been designed and used to monitor the temperature of the sample oil. To mimic the oil oxidation processes during frying, the sample is heated to 210°C for five cycles.

2.2 GC-MS Instrument Setting

An Agilent gas chromatograph from Hewlett-Packard (Palo Alto, California, U.S.A), equipped with an HP 5971 MS detector, is used for the determination of fatty acid composition. Separations are carried out on an Agilent (Hewlett-Packard) HP-5 fused silica capillary column (30 m × 0.25 mm I.D.; 0.25 μm film thickness) (Folsom, California, U.S.A). The GC-MS interface temperature is maintained at 210ºC. 1 μl of an unheated sample is injected manually in splitless mode with the injector port temperature at 200ºC. The helium carrier gas flow rate is 1 ml min^{-1}. The column temperature programme is as follows: 90ºC, held for 1 min; 12ºC min^{-1} to 150ºC, held for 1 min; 2ºC min^{-1} to 210ºC, held for 3 min; and 10ºC min^{-1} to 210ºC, held for 25 min. The selective ion mode is used in the analysis. The retention time and abundance of confirmation ions relative to that of quantification ions are used as criteria for identification. The start button and the injection of a sample are synchronised to have consistent RT values. The mass-to-charge range is 50–500 amu. An oven temperature programme is used to maintain the temperature at 50°C–210°C. The spectra are recorded on an interfaced computer. The same procedure is repeated for each heated sample.

3. RESULTS AND DISCUSSION

3.1 Palm Oil

Table 1 shows the composition and retention time of unheated and heated palm oil. Figures 1–6 show the mass spectra of the peaks in the chromatogram before and after heating palm oil to its smoke point. The unsaturated alkenes decadiene (20.55%), palmitic methyl ester (15.67%) and palmitic ethyl ester (13.91%) take part in thermal chemical reactions to increase the percentage of the saturated fatty acid palmitic acid to 77.28%.

Table 1: Fatty acid composition of palm oil before and after heating.

S. no.	Peak name	Unheated oil		Heated oil	
		RT	% peak area	RT	% peak area
1	2,8-Decadiene – $C_{10}H_{18}$ MW: 138	2.18	20.55	_	_
2	Palmitic acid, methyl ester – $C_{17}H_{34}O_2$ MW: 270	2.45	15.67	_	_
3	Palmitic acid, ethyl ester – $C_{18}H_{36}O_2$ MW: 284	5.18	13.91	_	_
4	Palmitic acid – $C_{16}H_{32}O_2$ MW: 256	6.83	15.38	5.87	77.28
5	á-Sitosterol – $C_{29}H_{50}O$ MW: 414	7.60	18.34	_	_
6	Linoleic acid – $C_{18}H_{32}O_2$ MW: 280	11.98	10.65	11.02	5.73
7	β Carotene – $C_{40}H_{56}$ MW: 536	14.37	16.16	14.37	9.07

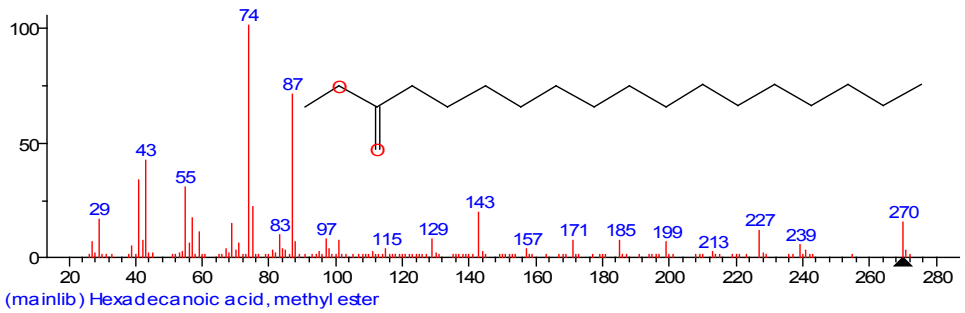

Figure 1: Mass spectrum of methyl ester.

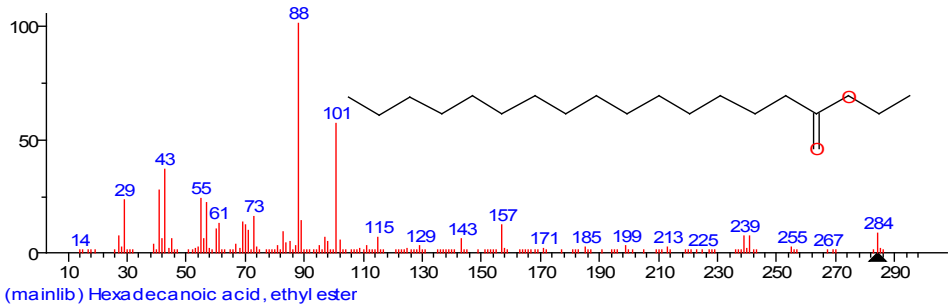

Figure 2: Mass spectrum of ethyl ester.

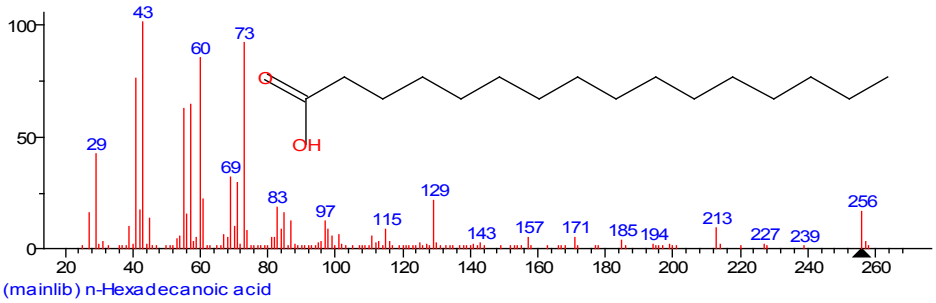

Figure 3: Mass spectrum of palmitic acid.

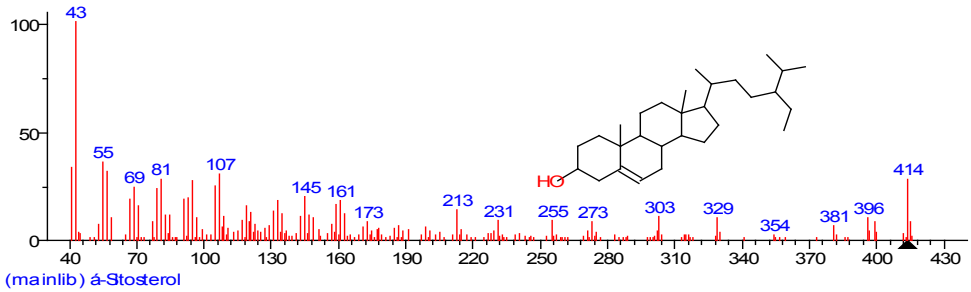

Figure 4: Mass spectrum of α sitosterol.

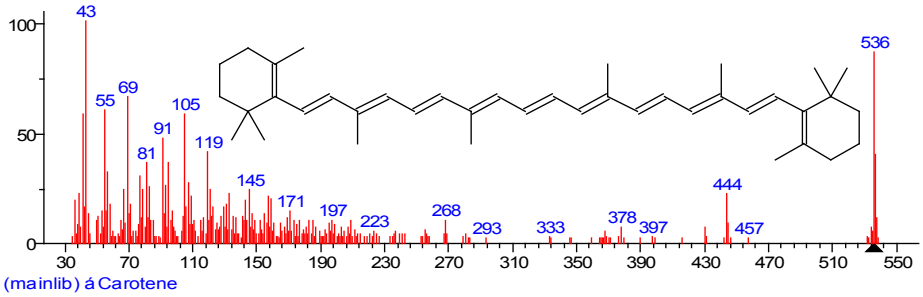

Figure 5: Mass spectrum of carotene.

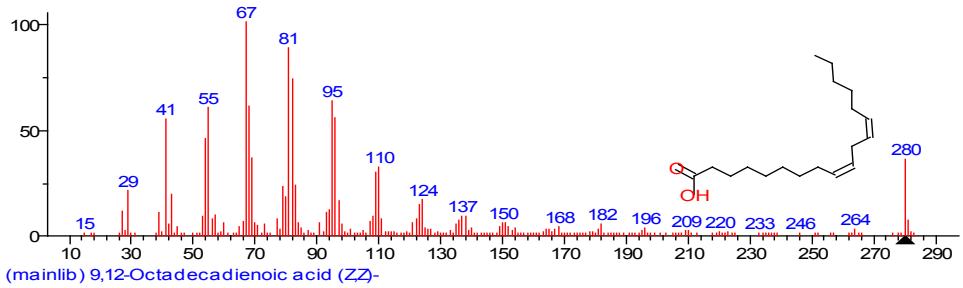

Figure 6: Mass spectrum of linoleic acid.

The antioxidant sitosterol (20.16%) is completely evaporated upon heating. Palm oil is naturally reddish in colour due to the presence of a large amount of β-carotene.[19] Palm oil is considered to be the richest natural source of carotenoids (approximately 15 times greater than that in carrots). The human body uses carotenoids as a source of vitamin A, which enhances eye health. Carotenoids also play a potential important role by acting as biological antioxidants, thereby protecting cells and tissues from the damaging effect of free radicals.[20] The antioxidant β-carotene is evaporated to some percentage due to heating. Frying conditions, however, cause the saturation of unsaturated fatty acids, and the ratio of saturated to unsaturated fatty acids will also change due to the degradation and polymerisation of the unsaturated fatty acids. For a diet containing heated *trans* fatty acids, the health effects would be unfavourable due to an increase in low-density lipoprotein (LDL) cholesterol and decrease in high-density lipoprotein (HDL) cholesterol. The increase in the percentage of palmitic acid upon heating increases the adverse effects on human health.[21–23]

3.2 Groundnut Oil

Groundnut oil is edible oil derived from peanuts that contains a large quantity of protein and antioxidants and is noted to have a slight aroma and the taste of its parent legume. It is often used in Chinese, south Asian and southeast Asian cuisine as much as olive oil is used in the Mediterranean. Peanut oil is appreciated for its high smoke point relative to many other cooking oils.[24]

Table 2 illustrates the composition and retention time of the components in unheated and heated groundnut oil. Caproic acid, caprylic acid, nonanoic acid are saturated fatty acids whose quantities have not changed upon heating, illustrating that they do not take part in any chemical reactions. The unsaturated fatty acids oleic acid and linoleic acid, as well as palmitic methyl ester, react strongly upon heating to increase the percentage of the saturated fatty acids stearic acid, arachidic acid and behenic acid. The antioxidant vitamin E is completely evaporated at a high temperature. It is observed that the quantity of

the antioxidant vitamin B is reduced upon heating to one fifth of its unheated value. The highly potent, antioxidant polyphenolic acids are found to be stable upon heating and have not undergone many changes.

Table 2: Fatty acid composition of groundnut oil before and after heating.

S. no.	Peak name	Unheated oil		Heated oil	
		RT	%P.A	RT	%P.A
1	Caproic acid – C6H12O2 MW: 100	2.35	1.47	2.35	1.46
2	Caprylic acid – C8H12O2MW: 144	6.62	0.38	7.06	0.37
3	Nonanoic – C9H18O MW: 142	7.06	0.16	7.08	0.19
4	Palmitic methyl ester – C17H34O2 MW: 270	–	–	7.25	5.47
5	Linoleic acid – C18H32O2 MW:280	7.37	24.88	7.37	12.03
6	Vitamin E – C29H50O2 MW: 430	8.22	4.06	–	–
7	Oleic Acid – C18H34O2 MW: 282	9.13	56.06	9.13	30.13
8	Vitamin B – C9H17NO5 MW: 153	10.58	5.64	10.58	1.34
9	Polyphenolic – C15H10O2 MW: 252	17.04	2.67	16.98	2.42
10	Stearic acid – C18H36O2 MW: 284	–	–	13.28	39.48
11	Arachidic acid – C20H40O2 MW: 313	22.78	2.48	22.76	4.36
12	Behenic acid – C22H44O2 MW: 341	22.95	1.41	22.95	3.10

Figures 7–15 show the mass spectra of the peaks in the corresponding chromatograms. Frying fat for a long time is discouraged for it causes excessive foaming of the hot oil, the fat tends to smoke excessively, or an undesirable flavour or dark colour develops.[25–27] Any or all of these qualities associated with heating fat can decrease the quality of the fried food. Groundnut oil is widely used in southern India for cooking. Groundnut oil contains 56% and 25% monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively. An increase in the level of polar compounds (mono- and diglycerides, free fatty acids, and other polar transformation products) formed during frying/heating of foodstuffs in the oil is observed. The evaporation of antioxidants and the formation of saturated fatty acids illustrates that this oil is not suitable for multiple cycles of heating to frying temperature.[25]

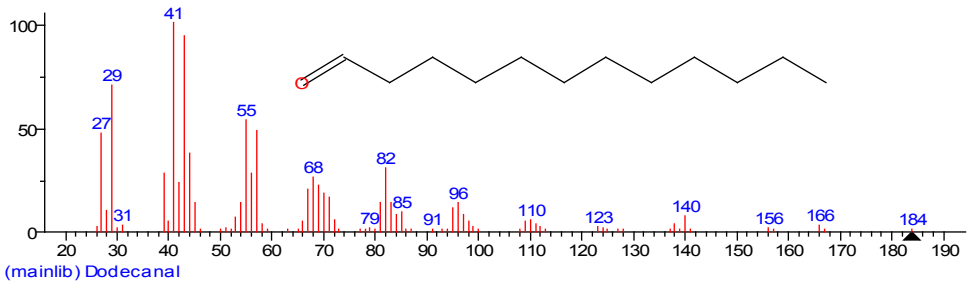

Figure 7: Mass spectrum of caprylic acid.

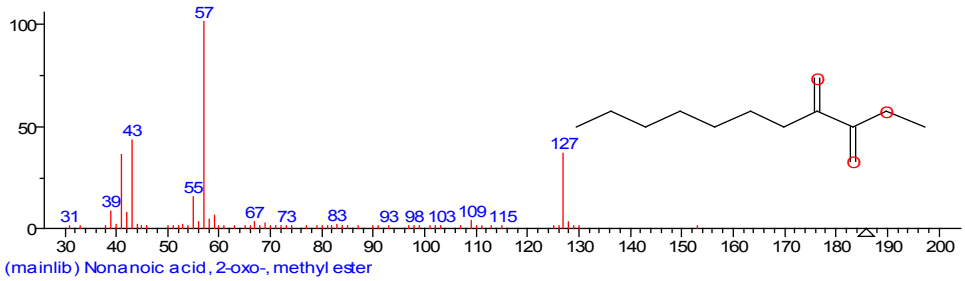

Figure 8: Mass spectrum of nonanoic acid.

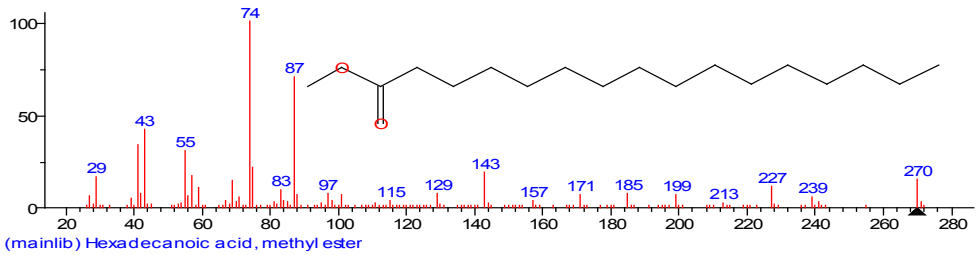

Figure 9: Mass spectrum of palmitic methyl ester.

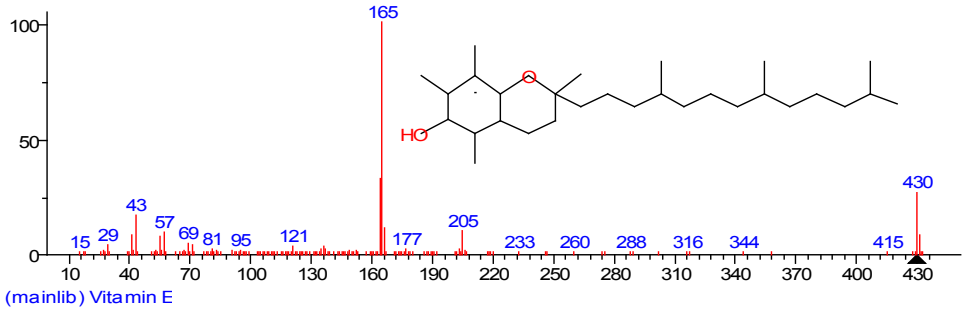

Figure 10: Mass spectrum of vitamin E.

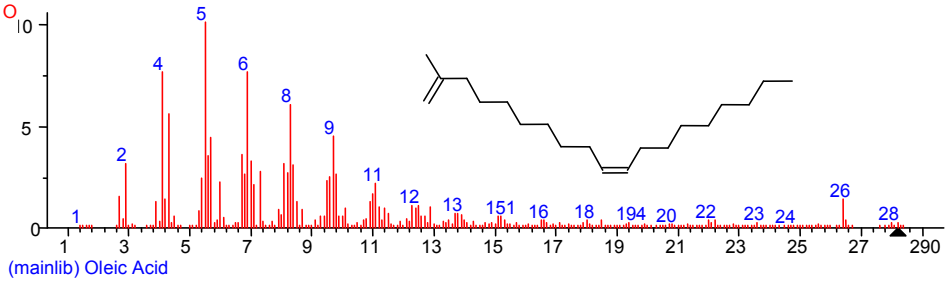

Figure 11: Mass spectrum of oleic acid.

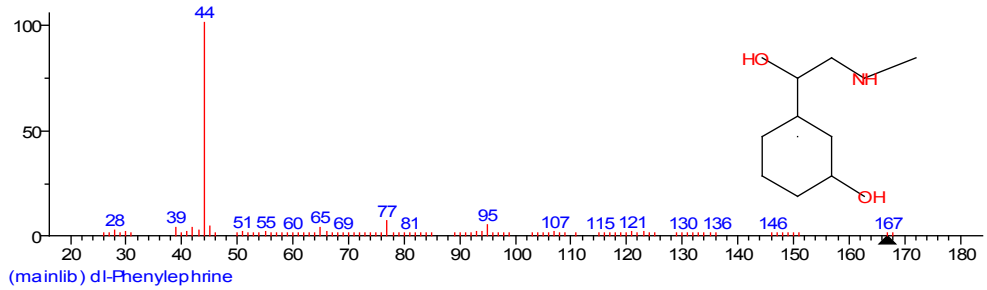

Figure 12: Mass spectrum of vitamin B.

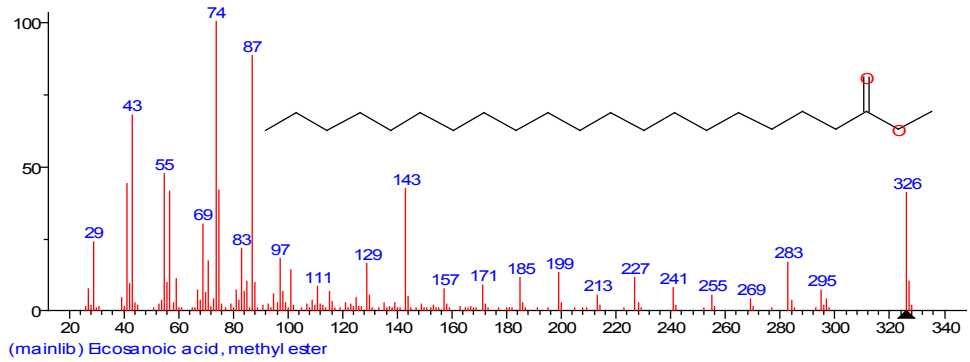

Figure 13: Mass spectrum of arachidic acid.

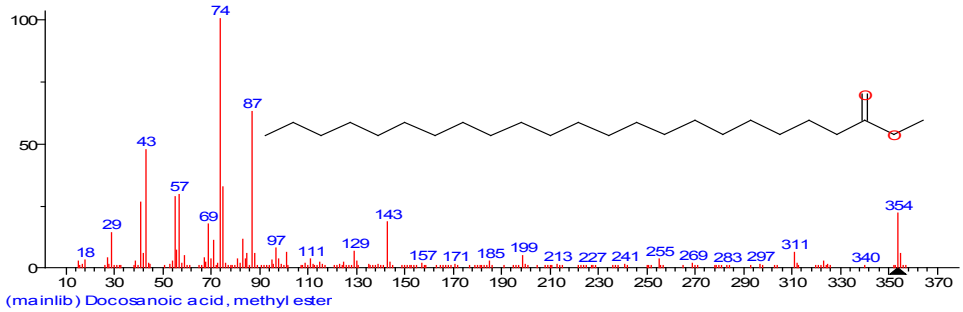

Figure 14: Mass spectrum of behenic acid.

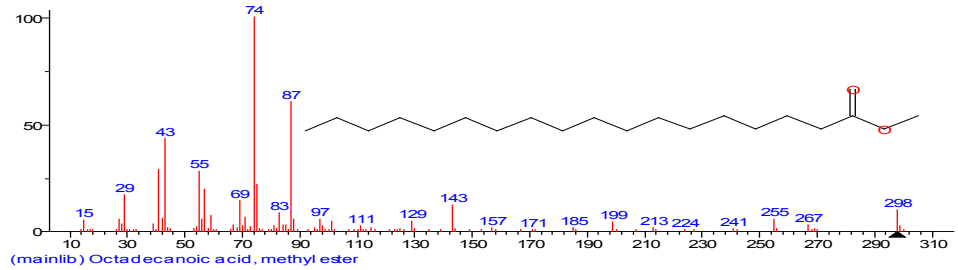

Figure 15: Mass spectrum of stearic acid.

3.3 Variation of Viscosity with Time

A microcontroller-based redwood viscometer is used to measure viscosity at different times of heating.[1] A study of the variation in viscosity with time of heating is carried out by heating the oil to the frying temperature (up to 210°C) for 0.5 h, 1 h, 1.5 h or 2 h. After heating for the desired time, viscosity is measured at 30°C. Figure 16 illustrates the variation of viscosity with time. The diagrams depict the abrupt increase in the variation of viscosity with frying time for palm oil compared to groundnut oil, which is due to oxidation, isomerisation and polymerisation reactions.[10] Oxidation leads to the formation of hydrogen bonds that increase intermolecular forces, which causes the flux among molecules that increases viscosity. The increase in the viscosity of palm oil with frying time shows that, on successive heating, the polyunsaturated fatty acids have undergone molecular reactions to form saturated compounds such as triacylglyceride or triglycerides (addition of saturated fatty acids in glycerol whose molecular weight remains high and hard to be broken) as the antioxidants are evaporated. It is observed that a trivial change in viscosity for groundnut oil is due to a higher concentration of stable antioxidants. A correlation graph is drawn between viscosity and fatty acid composition of palm oil and groundnut oil is shown in Figure 17. It is observed that viscosity and fatty acid composition are

highly correlated (R^2 = 0.948) and that viscosity decreases with an increase in unsaturated fatty acids.

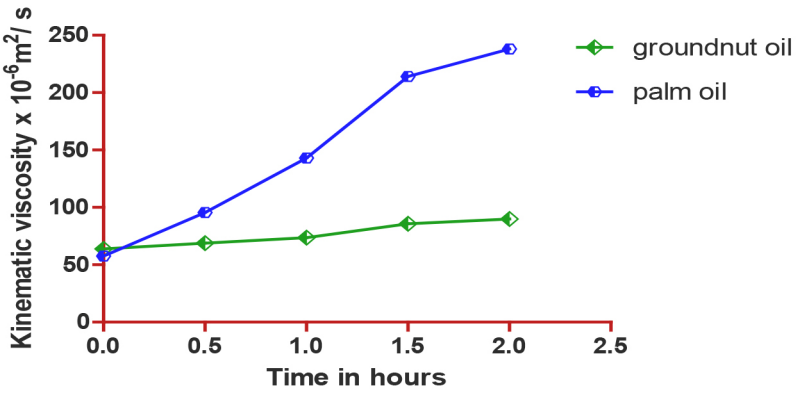

Figure 16: Variation of kinematic viscosity with time of heating.

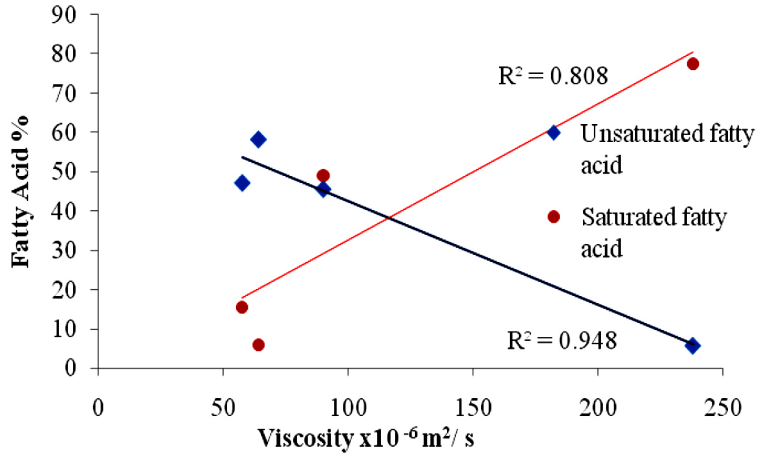

Figure 17: Correlation between viscosity and fatty acid.

4. CONCLUSION

The GC-MS analysis of palm oil shows the formation of linoleic and palmitic acid. The disappearance of sitosterol and the decrease in the percentage of β-carotene shows poor antioxidant stability in the oil. The formation of free fatty acids in the heated oil demonstrates thermal degradation of the components. The observation of a decrease in the concentration of unsaturated fatty acids in groundnut oil due to the evaporation of tocopherol (vitamin E) and the drastic

reduction in vitamin B levels reveals the instability of these antioxidants, and therefore this oil is not suitable for multiple heating cycles. The high correlation between viscosity and fatty acid composition shows that viscosity depends on the fatty acid composition of the oil. The viscosity increases with an increase in saturated fatty acids and decreases with an increase in unsaturated fatty acids.

5. ACKNOWLEDGEMENT

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