

Effect of Oxidative Degradation on Vegetable Oils and Its Estimation Using Different Methods

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ABSTRACT: The use of fats and oils by man dates back to antiquity. Oil because of their chemistry is prone to oxidative damage. During the process of oxidative rancidity undesirable changes are seen in oil composition making it unsafe for consumption. One such change is production of free fatty acid. In order to quantify such changes in oil, a sensor can be design which can detect the same. For making such sensor we qualitatively and quantitatively analysed oil samples (crude and degraded) through titration studies for determining free fatty acid content in degraded and photolysed oil, TLC for comparing standards with free fatty acids obtained. UV spectroscopy and fluorescence spectroscopy are used for analysis of spectral properties of free fatty acids in crude and degraded samples. Thus, this project aims at detection of oxidative degradation of oils and a sensor which could sense such degradation according to the data and standards we obtained. We contribute our work to food based and petrochemical industries where oil is used for quality check, for home based use of oil, and places where oil is frequently used as a key ingredient.

KEYWORDS: Oxidative rancidity, TLC, UV spectroscopy, Fluorescence spectroscopy

I. INTRODUCTION

The term oil is used in generic sense to describe all substances that are greasy or oily fluid at room temperature [1]. Their chemical composition and specific properties have allowed them to find use as foods, fuels and lubricants. Their sources are numerous, encompassing vegetable, animal, and marine sources. As it is with all matter, their usefulness to man is determined by their chemical nature and all fats and oils have certain characteristics in common.

Fats and oils are naturally occurring substances which consist predominantly of mixtures of fatty acid esters of the trihydroxy alcohol or glycerol. Different fats and oils come about due to the fact that there are numerous fatty acids of various kinds and these can be combined in an infinite number of ways on the hydroxyl centres of glycerol. Moreover, the physical properties of fats and oils are dependent on the nature of fatty acids involved in the ester.

Vegetable oils are sensitive to oxidative conditions and produce many degradation products including aldehydes, ketones, epoxides, hydroxyl compounds, etc. which are now considered as toxic and potentially carcinogenic [2].

Oils because of their chemistry are susceptible to oxygen damage. Oxidation of unsaturated fatty acids is the main reaction responsible of the degradation of lipids [3]. During the process of oxidative rancidity, oxygen molecules interact with the structure of the oil and change its odour, its taste, and its safety for consumption, along with that bio fuels if degraded can affect the efficiency of engines. In this process the long-chain fatty acids are degraded and short-chain fatty acids compounds are formed.

Oxidation primarily occurs with unsaturated fats by a free radical-mediated process. These chemical processes can generate highly reactive molecules in rancid foods and oils. The purpose of this project is to observe the degradation of vegetable oil and to find technique for the detection of degradation. For correct estimation of degradation level, detection (TLC) of free fatty acid, quantification (Acid value) and spectral studies (UV Spectroscopy, and Fluorescent Spectrophotometry) were done.

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II. MATERIALS AND METHODS

1. Degradation

Initially four oil samples were taken namely, sunflower, sesame, mustard, groundnut each of 5ml were kept for the degradation using 0.5 ml of hypochlorite. In another method the Oil Samples were kept under sunlight for the required period.

2. Estimation of Free Fatty Acid Value

The free fatty acid in oil is estimated by titrating it against KOH in the presence of phenolphthalein indicator. The acid number is defined as the mg KOH required for neutralizing the free fatty acids present in 1g oil. Titration of the crude as well as degraded samples was done with 0.1N and 0.5N KOH and the acid value were estimated.

$$\text{Acid Value} = \frac{\text{Titration Value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of sample (g)}}$$

3. Thin layer chromatography

The purity of the separated lipid fractions was confirmed by TLC [4]. TLC was performed for the separation and detection of Fatty acids. Hexane: Ether: Acetic acid in the ratio of 60:40:1 was used as solvent. Firstly, Silica plate was activated by keeping it at 60°C for 1 hour. Then different oil samples dissolved in isopropanol were loaded on the plate with 1µl, 2µl, 3µl, 4µl, 5µl concentration and allowed to run. For the visualisation, iodine vapour was used.

4. UV Spectral Studies

Spectroscopic analysis is based on measuring the amount of radiation produced or absorbed by molecular or atomic species to identified [5].

A dual beam UV-spectrophotometer was used to study the transmission characteristic of oil. As a preliminary step all sample of oil were dissolved in isopropanol (10µl of oil in 3ml of isopropanol) then taken in a UV transparent quartz cell. The transmission characteristics of oils were taken using a dual beam UV spectrophotometer. The measurements were taken one by one after cleaning the quartz cell before taking each sample between 200-800 nm wavelength.

5. Fluorescence Studies

Fluorescence (photoluminescence) is the emission of light by a substance that has absorbed electromagnetic radiation. Fluorescence usually appears as visible light and occurs when the exciting radiation is in the ultraviolet or blue light region of the spectrum. Wolfbeis and Leiner (1984) [6], firstly applies this technique to edible oil. Fluorescence technique has been abundantly used for exploring oil fluorescence. The phenomenon occurs when an orbital electron of a molecule relaxes from its first excited state (S1) to a ground state (S0) by emitting a photon of light after being excited to any higher quantum state.

III. RESULTS AND DISCUSSION

1. Free Fatty Acid Value

The effect of Hypochloride and Photolysis treatment were shown in Table No. 1 and 2. According to the results obtained, we can say that considerable amount of degradation occurred after 2 days of hypochlorite treatment. There was no significant difference in day 1 of Hypochloride treatment. Along with that, degradation through photolysis take long time, it occurred after 23 days, samples were kept under light continuously. Oxidation of unsaturated fatty acids via Thermal energy is accompanied by considerable isomerization of double bonds leading to products containing *trans* double bonds and conjugated double bond systems [7]. Amount of fatty acids produced differs from sample to sample because of their net composition and presence of saturation. As per the results, with the degradation free fatty acids increased.

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Table No. 1 Free Fatty Acid Produced by Hypochlorite Treatment

Samples (Hypochlorite)	Amount of Fatty Acids (mg/gm)			
	Sunflower Oil	Sesame Oil	Groundnut Oil	Mustard Oil
Crude Sample	2.24	9.53	1.12	1.6
Day 0	2.24	10.64	2.8	2.8
Day 1	2.8	12.88	5.6	3.36
Day 2	5.6	12.88	7.84	3.36

Table No. 2 Free Fatty Acid Produced by Photolysis Treatment

Samples	Amount of Fatty Acids (mg/gm)			
	Sunflower Oil	Sesame Oil	Groundnut Oil	Mustard Oil
Day 6	5.6	2.8	8.4	5.6
Day 23	8.4	8.4	11.2	5.6

2. Separation of Free Fatty Acids by Thin Layer Chromatography

2.1. TLC of crude Groundnut oil

According to the TLC data shown in Figure No. 1, we observed four distinct band and a spot, the lower bands are composed of Monoacylglycerols, Diacylglycerols and above we have Free Fatty Acids. The TLC results give the conformation of composition of the groundnut oil upon which the further results were depends.

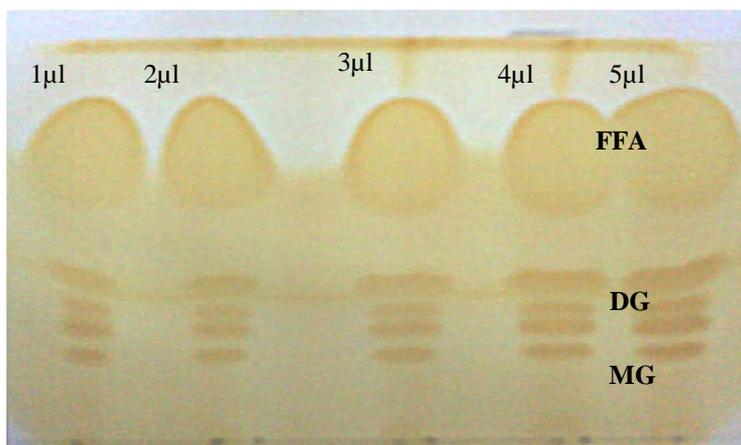


Fig.1 Thin Layer Chromatography of Crude Groundnut oil with Different Concentration

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2.2. TLC of degraded groundnut oil

TLC performs with hypochloride degraded ground samples reveals that, the intensity of spot on TLC plate were increased with the increment in free fatty acids concentration. The iodine vapour stains the free fatty acids and the colour got intensified. While comparing the Fig. 1 and Fig. 2 it was clear that the intensity of colour were increased in Fig.2 at 3 μ l samples concentration, however at the same concentration the colour was not that much intense in Fig.1.

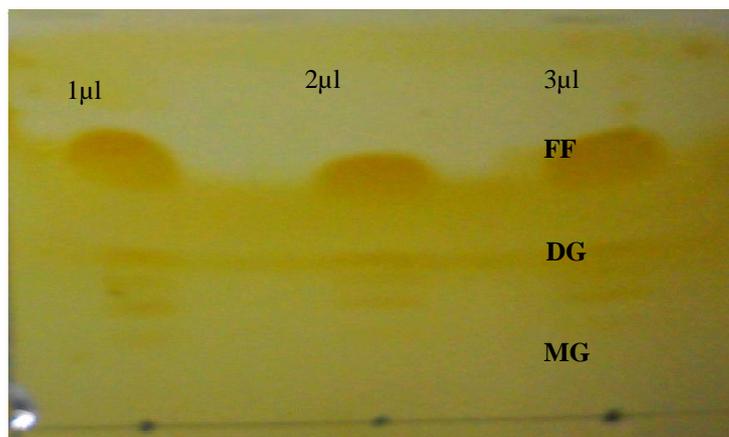


Fig.2. Thin Layer Chromatography of degraded Groundnut oil with different Concentration

2.3. TLC of crude sesame oil, mustard oil, and sunflower oil

The result of present study reveals that, the TLC of Sesame, Mustard and Sunflower oil loaded on plate shows different bands patterns of free fatty acid, and glycerol's content, and it was vary from oil to oil. The composition of Glycerol's and FFA changes according to oil samples, and also the intensity of spot. There was one spot observe in sunflower oil that is triglycerides, but there is no significant difference in free fatty acids content in oil.

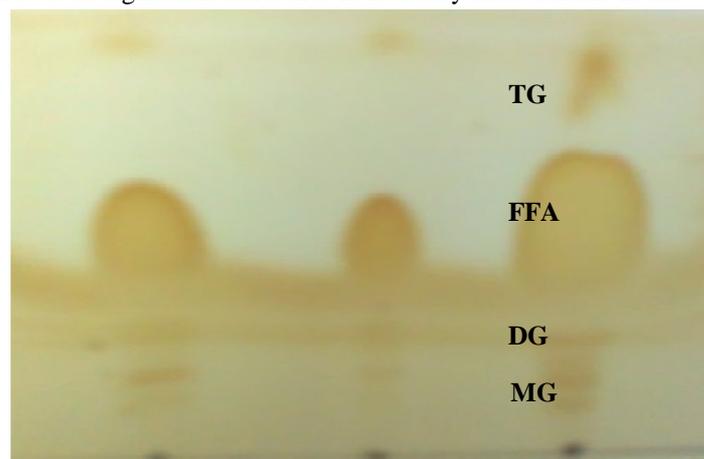


Fig.3. Thin Layer Chromatography of degraded Sesame, Mustard and Sunflower oil.

2.4. TLC of standard free fatty acid and fatty acid in oil sample

The findings of experiment are shown in Fig. 4. The comparative results of Standard FFA and FFA contained in oil samples were spotted at same position shown in figure. By comparing bands we can say that bands obtained in previous results were of FFA and not on others. It also signifies the Presence of oleic acid in sunflower oil.

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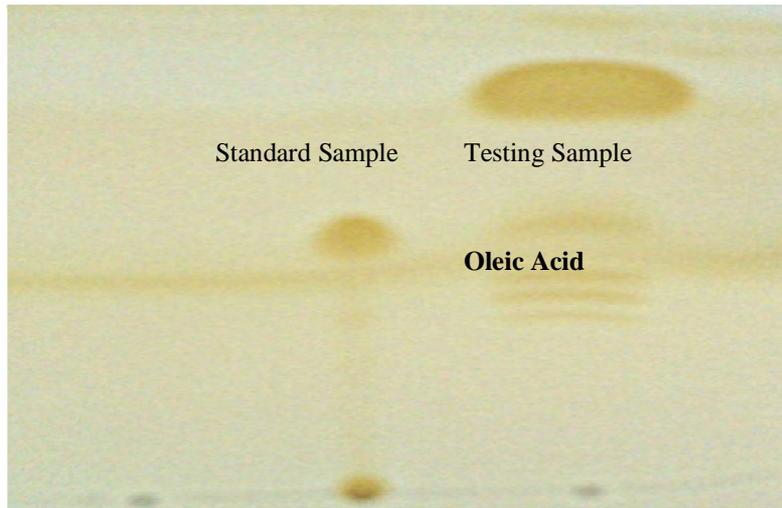


Fig.4. Thin Layer Chromatography of Standard FFA and FFA in oil

3. UV Spectra Analysis

The Ultra violet spectral analysis of Crude and Degraded samples of groundnut, sesame, mustard and sunflower oil were analysed at 240 nm wavelength and the results obtained are shown below in figures. The spectrum obtained at 240 nm for all the samples of crude and degraded oils are did not mach. The degraded sample of all oil shows more picks in graphs, i.e. it contains more FFA than crude oil samples shown in fig. 4. The samples were also compared with the standard like Stearic acid, Palmitic acid, which shows the same pattern.

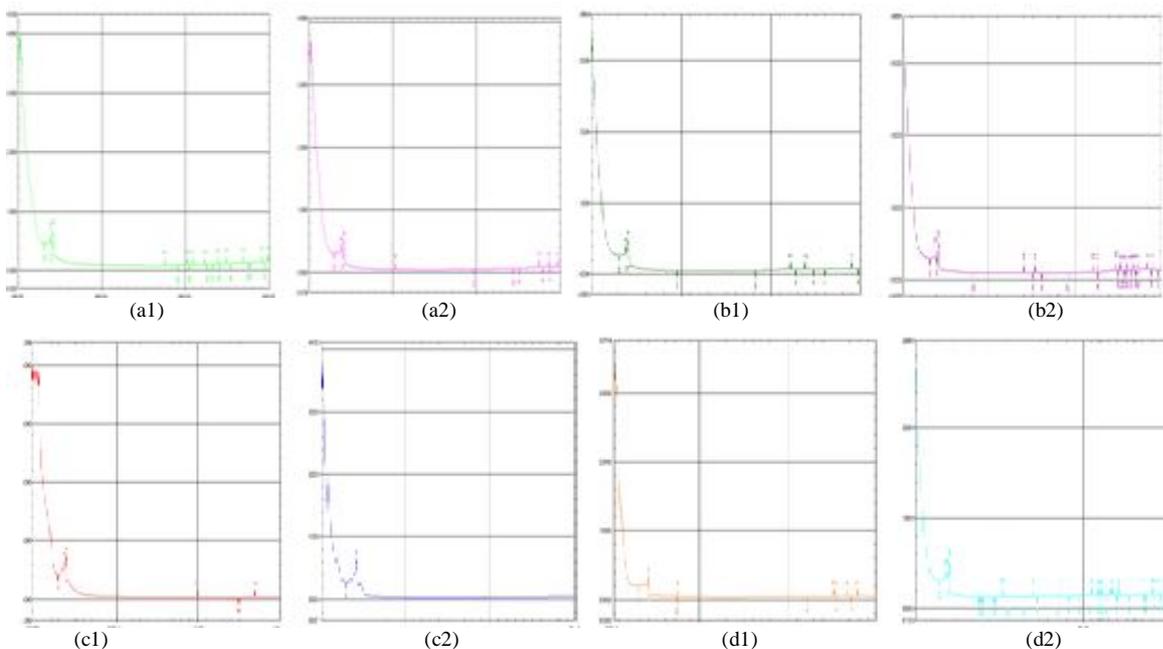


Fig.4. Thin Layer Chromatography of Standard FFA and FFA in oil: a1-Groundnut crude, a2- Groundnut degraded, b1-Mustard crude, b2-Mustard degraded, c1-Sesame crude, c2-Sesame degraded, d1-Sunflower crude, d2-Sunflower degraded

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4. Fluorescence Spectroscopy

The fluorescence Spectroscopy graph obtained from Oil samples were shown in Fig 5. It is seen from the graph that fluorescence emission peak is obtained at or near 420-430nm. The number of peak increased with the number FFA present in oil.

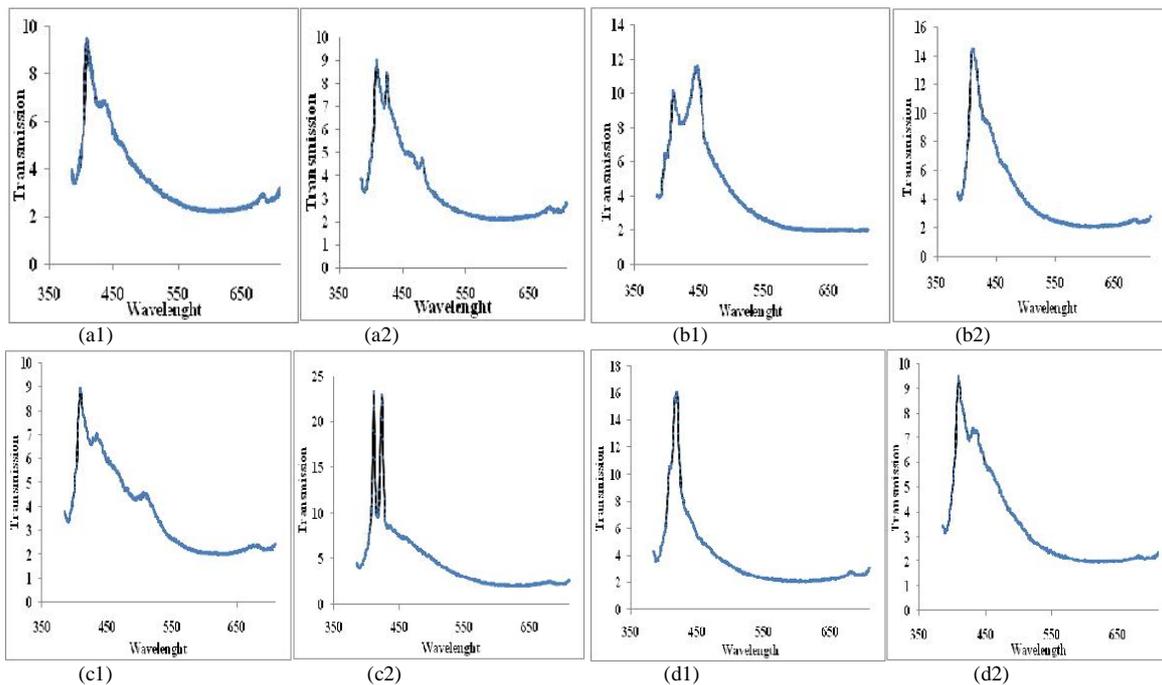


Fig.5. Fluorescence Spectroscopy of Standard FFA and FFA in oil: a1-Sunflower crude, a2-Sunflower degraded, b1-Sesame crude, b2-Sesame degraded, c1-Mustard crude, c2-Mustard degraded, d1-Groundnut crude, d2-Groundnut degraded

IV. CONCLUSION

Edible oil is oxidized during processing and storage via autooxidation and photosensitized oxidation. In this reactive oxygen species react with oil. Autooxidation forms acyl glycerols, dienes, and hydroperoxides are decomposed to produce off flavour compounds and the oil quality degraded [8]. As per our analysis the free fatty acid content increased when degradation was done chemically and by photolysis.

The formation of aldehydes was detected, saturated and unsaturated aldehydes could be identified such chemical changes are seen during lipid oxidation [3]. High oleic, linoleic acid and other fatty acid moieties functions as the active sites for various oxidative reactions [9].

The results suggest that increase in free fatty acid is due to oxidation. Thus, free fatty acid content indicates extend of oxidative damage in oil. The quantification and qualification of the free fatty acids is therefore important so as to determine how much free radical activity is, and where the most probable sites for damage are located.

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