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Comparative Study of the Oxidative Stabilities of Palm Oil and Olive Oil.

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Abstract

Palm oil and olive oil samples were subjected to methylene blue sensitized photoxidation in 10% methanol in dichloromethane in order to compare their oxidative stabilities using duration for oxidation to be observed and extent of product formation. Progress of the reaction was monitored by thin layer chromatography (TLC) which indicated that products were formed in the palm oil after 13 hours of irradiation and 10 hours for the olive oil. Chemical analysis of the reaction mixtures with triphenylphosphine (TPP) and potassium iodide/ starch indicator/thiosulphate solution indicate that peroxy compounds are formed during the sensitization. TLC results indicate that the TPP-reduction products showed lower R_f values than their precursors suggesting that alcohols are obtained from hydroperoxides. TLC also shows a dark polar spot indicating the formation of triphenylphosphine oxide. Column chromatographic separation of reaction mixtures on silica gel isolated reaction products for both oils. The proton NMR spectra of the unoxidized oils samples and their reaction products show that the major components of the oils are triacylglycerols. The spectra of the reaction products showed reductions in intensities in the peaks due to the protons of olefinic (5.2-5.4ppm), allylic (1.9-2.0 ppm) and bis-allylic (2.2-2.3 ppm) groups, indicating that oxidation preferentially affected these double bonds systems in the structures of the triacylglycerols. Additional peaks were observed at 5.7-5.8 ppm and 7.0-7.3 ppm in the spectra of the palm oil and at 5.6-5.7 ppm and 6.8-8.0 ppm in the spectra of olive oil. The reduction in peaks and heights of new peaks observed were more pronounced in the spectra of the olive oil indicating its higher abundance in unsaturated fatty acids and a corresponding lower oxidative stability.

Keywords: Palm oil, Olive oil, Methylene blue, Photoxidation, Dye-sensitizer, Hydroperoxide, Singlet Oxygen.

Introduction

Oils and fats are valuable natural products to man on account of their energy value, nutrient content in human nutrition and numerous industrial applications. Despite their usefulness, one major problem in the handling and utilization of these products is their ease of oxidation.

The oxidation of edible oils is influenced by energy input such as light or heat, composition of fatty acids in the constituent triacylglycerols, type of oxygen available and the presence of minor components in the oil such as metal ions, pigments and antioxidants (Dauqan et al 2011; Saber-Tehrani et al 2013). Generally, lipid oxidation can proceed by either autoxidation or photosensitized oxidation depending on the type of oxygen that reacts with the oil. In autoxidation, atmospheric oxygen is the oxidant while in sensitized oxidation singlet oxygen is the oxidizing species. Singlet oxygen which is more energetic than triplet oxygen is generated in the presence of light, triplet oxygen (^{3}O2) and a sensitizer (Lhotakova et al 2013, Kruft and Greer, 2011; Min and Boff, 2002).

In the utilization and storage of oils, oxidation reactions are important as it affects palatability, nutritional quality via the destruction of essential fatty acids and some fat-soluble vitamins and the formation of toxic compounds. The initial products obtained are unstable and decompose to give various low molar mass compounds that are responsible for off-flavours and undesirable odours encountered in oxidized oils. As a result, oxidation renders oils less acceptable to consumers or for industrial use especially as a food ingredient.

The stability of oils to oxidation is an important indicator in determining oil quality and shelf-life (Choe and Min, 2006). Resistance to oxidation can be expressed as the period of time necessary to achieve the critical point of oxidation such as sensory change or a sudden acceleration of the oxidative process. Several authors have observed that the oxidative stability of vegetable oils depend on the fatty acid composition as well as the TAG composition and structure. Neff et al (1992) examined the oxidative stability of soya bean oil in relation to TAG composition and structure. It was noted that the oxidative stability of the oil was dependent on the fatty acid composition of the constituent triacylglycerols (TAGs) as well as the triacylglycerol (TAG) structure, that is, the position occupied by the fatty acids in the glycerol moiety. Their studies show that oxidative stability of oils decreased with increase in the amount of easily oxidizable fatty acids such as linolenic and linoleic acids and increased with increase in the amount of oleic and saturated fatty acids. Some of the oxygenated decomposition products are implicated in degenerative diseases such as aging, membrane damage, heart disease and cancer; as a result the study of lipid oxidation has received great attention recently.

The aim of the present study is to access the oxidative stabilities of palm oil and olive oil as edible vegetable oils, based on their ease of oxidation and structural changes in the constituent triacylglycerols as monitored by changes in their proton NMR spectra after being subjected to an accelerated oxidation process.

Materials and Methods

Extra virgin olive oil, a product of Andalucia, Spain was purchased from a local supermarket in Port Harcourt, Rivers State, Nigeria and the palm oil was obtained from Swali market in Yenagoa, Bayelsa State, Nigeria. Hexane (60-80^oC), ethyl acetate, and methanol were purchased from BDH, dichloromethane was purchased from Sigma Aldrich. Silica gel, (Kieselgel 60 Merck, and silica gel 60 – 200 mesh, Burgoyne Bisrbridges, India; Methylene blue (M & B).The palm oil was purified before use. The oil samples were stored in a freezer prior to usage.

Irradiation/Analysis of Photoxidized Mixtures.

The oil samples were photoxidized in the presence of methylene blue and the resultant reaction mixtures analyzed for their peroxy content by reaction with TPP and potassium iodide/thiosulphate solution as in our previous work (Fekarurhobo et al 2013). Each reaction mixture was concentrated by evaporation under a fan to a small volume and chromatographed on silica gel column. The column was eluted with 3% ethyl acetate in hexane. The fractions collected were analyzed by thin layer chromatography for their product content.

Spectral Analysis

The proton Nuclear Magnetic resonance (NMR) spectra of the oil samples and their isolated products were run on a Varian Mercury 200 NMR spectrophotometer.

RESULTS AND DISCUSSION

Initial oxidation was observed for palm oil after 13 hours of irradiation, while for olive oil oxidation was observed after 10 hours. The duration for oxidation to be observed is an indication of their oxidative stabilities. This observation is in line with the fatty acid compositions of the oil samples under study; olive oil contains 90% oxidizable fatty acids and should oxidize faster than palm oil which contains 54% oxidizable fatty acids (Poiana and Mincone, 2004). Henna Lu and Tan (2009) made similar observations while studying the storage stability in virgin coconut oil (VCO) and extra virgin olive oil (EVOO). These authors observed that VCO with less oxidizable fatty acids was more resistant to oxidation than EVOO that contains the polyunsaturated fatty acid, linolenic acid. Smith et al (2007) while studying the oxidative stability of high oleic acid sunflower oil obtained from genetically modified sunflower demonstrated that the high oleic acid sunflower which contains 5.5% linoleic acid was more thermally stable compared to the regular sunflower oil which contains 71.6 % linoleic acid. The authors also observed that the high oleic acid oil showed better thermally stability than most conventional oils such as soya bean, corn and peanut oils.

Potassium Iodide/Starch Indicator Test:

The photoxidation reaction mixtures of both oils reacted with potassium iodide to give brown solutions which on addition of starch indicator turned blue-back. Further addition of thiosulphate solution turned the reaction mixture colourless. A variation of this reaction was used by Asakawa and Matsushita for the determination of peroxide values lipids. (Asakawa and Matsushita, 1980).

Triphenylphosphine Test:

The reaction mixture reacted rapidly with the triphenylphosphine giving products as indicated on the thin layer chromo-plates.

Chromatographic Separations

The chromatographic separations of the photoxidized and triphenylphosphine reduced reaction mixtures gave oxidized and reduced products. The isolated oxidation products also reacted with the test reagents, potassium iodide/Starch indicator/thiosulphate solution and triphenylphosphine indicating their peroxy nature.

Thin layer Chromatographic Analysis of the Reaction Mixtures

The plates show that the spots in the reaction mixtures are more than those in the corresponding controls and also have lower R_f values. This indicates that the reaction mixtures contain more substances, suggesting that reaction products are formed. The fact that the products were slower running on the TLC plates implies that they had incorporated oxygen into their molecular composition in the course of the reaction. Thin layer chromatographic analysis of the reduction reaction mixtures (Figure 1) showed that the oxidation products reacted with triphenylphosphine, showing that they were oxidation products.

Tlc analysis of dye-sensitized photoxidation of palm oil after 13 hours of irradiation
Key
A Unphotoxidized oil (control)
B Photoxidized oil

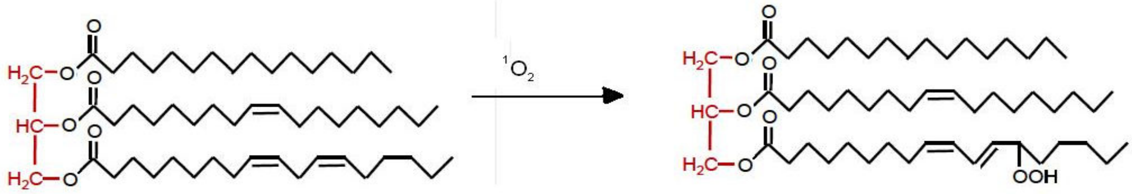
Tlc plate showing reacton of TPP with oxidation reaction mixture of palm oil.
Key
A Photoxidized oil +TPP
B TPP
C Photoxidized oil

Tlc analysis of dye-sensitized Photoxidation of Olive oil after 10 hours of irradiation
Key
A Unphotoxidized oil(Control)
B Photoxidized oil

Tlc analysis of dye-sensitized Photoxidation of Olive oil after 10 hours of irradiation
Key
A photoxidized oil
B TPP
C Photoxidized oil + TPP

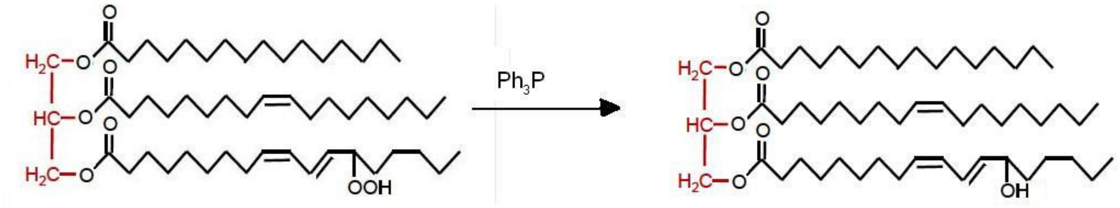
Figure 1: TLC Plates of the Unoxidized Oils and Reaction Products.

The oxidation products are likely to be hydroperoxides derived from the reaction of unsaturated triacylglycerols of the oil with $^1O_2$ in 'ene' reactions such as the one in scheme 1 as isolated alkenyl bonds are known to do.



Scheme 1: Reaction of Singlet Oxygen with Unsaturated Triglyceride

Marmesat et al 2009, Maduelosi et al 2012,Steenhort-Slikkerveer et al 2000 and Vasilecu et al 2013, had similarly explained the formation of hydroperoxides in the oxidation reaction of vegetable oils. The reducibility of the oxidation products by reaction with triphenylphosphine further alludes to their peroxy nature. The observation that the reduction products had lower R_f values (on TLC) than their precursors suggests that alcohols had been formed from hydroperoxides in the reactions. The reduction of hydroperoxides of triacylglycerols to their corresponding alcohols had been observed by Nakamura and Maeda (1991) and the reaction can be illustrated as in Scheme 2.



Scheme 2: Reduction of Triglyceride Hydroperoxide with Triphenylphosphine

Spectral Analysis

The proton NMR spectrum of the palm oil (unoxidized) , Figure 4 show the following signals: - a multiplet between 5.26 -5.40, a group of two multiplets between 4.10-4.30, a multiplet between 2.70-2.80, a doublet centred at 2.10, a triplet at 2.3, a singlet between 1.5-1.7, a singlet at 1.2 and a triplet centred at 0.85 (0.895, 0.885 and 0.883) ppm.

Similarly the proton NMR spectrum of the unoxidized olive oil, Figure 2 show chemical shifts in the region 1.0 -5.5 ppm. The major peaks are similar to those observed in the spectrum of palm oil: a multiplet between 5.2-5.4, multiplets between 4.2-4.3, 2.7-2.8, 2.0-2.1 and 1.9-2.0, a singlet at 1.7, 1.3 and a triplet at 0.80 ppm. Both spectra are typical of those of triacylglycerols, the major constituents of oils and fats.

The observed peaks in the spectra were assigned to specific groups in the structure of the triacylglycerol in comparison to existing literature (Carneiro et al 2005; Guillen and Ruiz, 2005; Guillen et al 2003; Sacchi et al, 1997; Vlahov, 1999). 5.2-5.4 (olefinic), 1.9-2.0 (allylic group of unsaturated acyl groups), 2.7-2.8 (bis-allylic group), 4.2-4.3 (1,3 of glyceryl group), 2.0-2.1 (methylene group alpha to carbonyl), 1.7 (methylene group beta to carbonyl), 1.3 (methylene envelope of all acyl groups) and 0.85 ppm terminal methyl group.

The spectra of the oxidized and reduced products are similar to those of the unoxidized oils but with reductions in intensities in the peaks due to the protons of easily oxidizable groups (olefinic, allylic and bis-allylic groups): the triplet at 2.2-2.3 ppm, the multiplet between 5.26-5.40 ppm and the multiplet between 2.7-2.8 ppm. Also additional peaks are observed between 5.7-5.8 ppm and between 7.0-7.3 ppm in the spectrum of the palm oil and between 5.6-5.7 ppm and between 6.8-8.0 ppm in the spectrum of the olive oil. The new peaks are more pronounced in the spectra of the triphenylphosphine-reduced products. The peak at 5.6-5.7 ppm is assignable to olefinic protons of hydroperoxydienes (Sacchi et al 1997), while those at 6.8-8.0 ppm which were not baseline resolved may be due to a combination of a hydroperoxydienyl and hydroperoxyl protons. Similar results were obtained by Wasasundara and Shahidi (1993) while monitoring the oxidative stability of canola and soya bean oils by proton NMR. Guillen and Ruiz (2005) also observed a similar reduction in peak intensities while monitoring the oxidation of vegetable oils by proton NMR. These authors noted that the intensities of peaks due to bis-allylic protons diminish faster than those due to olefinic protons. Also that as the intensities of these peaks gradually diminish, peaks due to hydroperoxides and conjugated dienes were observed. Schnitzler et al 2013, while monitoring the thermal oxidation of sesame oil samples noted a similar reduction in the peak intensities due to olefinic, allylic and bis-allylic protons. The authors noted that the reductions affected the bis-allylic protons more than the allylic and olefinic protons.

The spectra of the oxidation products in the present work indicate a similar reduction in intensities in the peaks due to the protons of olefinic, allylic and bis-allylic groups. Figure 5, the expanded spectra of the oxidized product of palm oil confirms that oxidation affects the bis-allylic protons more than other oxidizable groups. The peak due to the bis-allylic protons is almost unobservable. The reductions were more prominent for the olive oil (more unsaturated) than for the palm oil. This can be attributed to the fact that palm oil is more saturated than olive oil and also contains more antioxidants in the form of carotenoids and the E vitamins (tocopherols and tocotrienols) than olive oil. These antioxidants exhibit singlet oxygen quenching activities thereby rendering it more resistant to singlet oxygen oxidation. Correspondingly, the new peaks observed were more prominent in the spectra of the olive oil compared to those of palm oil. A similar observation was made by Guillen and Ruiz (2005) that intensities of signals due to hydroperoxides and conjugated diene protons are higher in the spectra of polyunsaturated oils than in oils rich in oleic acyl groups. Palm oil contains 43% of MUFA and 11% of PUFA while olive oil contains 78% of MUFA and 12% PUFA. Therefore olive oil oxidizes faster than palm oil under similar conditions.

Park et al (2010) similarly observed that the polyunsaturated fatty acids are affected more than monounsaturated fatty acids in both thermal and sensitized oxidations. These authors subjected soya bean oil to both thermal and methylene blue sensitized photoxidation. The results showed that the relative percentage of linoleic and linolenic acids decreased by 0.87 and 0.65% respectively after 60 minutes of thermal oxidation. On the contrary the relative percentage of oleic acid increased by 1.3%.

Neff et al (1997) while studying the resistance to oxidation of canola oil used normal and modified varieties of the oil observed that oxidative stability decreases with the presence of readily oxidizable fatty acids such as linoleic and linolenic acids. Similarly, Lee and Choe (2013) while investigating the effect of fatty acid composition of oils in chlorophyll-sensitized oxidation of oil/water emulsions demonstrated that oxidation rate was higher for sunflower oil or soya bean oil whose polyunsaturated fatty acid content was higher compared to canola oil or olive oil.

Conclusion

Methylene blue is a well-known Type II sensitizer and can generate singlet oxygen upon visible light irradiation. Due to its electrophilic nature, singlet oxygen attacks TAGs containing olefinic systems and the higher the electron density in the acyl chains the faster will be the attack. This is observed in the present work as the duration for oxidation is shorter for olive oil (contains more unsaturated fatty acids) than for palm oil. Also the reduction of peaks due to the oxidizable groups in the proton NMR spectra and the new peaks are more prominent for olive oil than palm oil, confirming that palm oil is more stable to oxidative degradation than olive oil.

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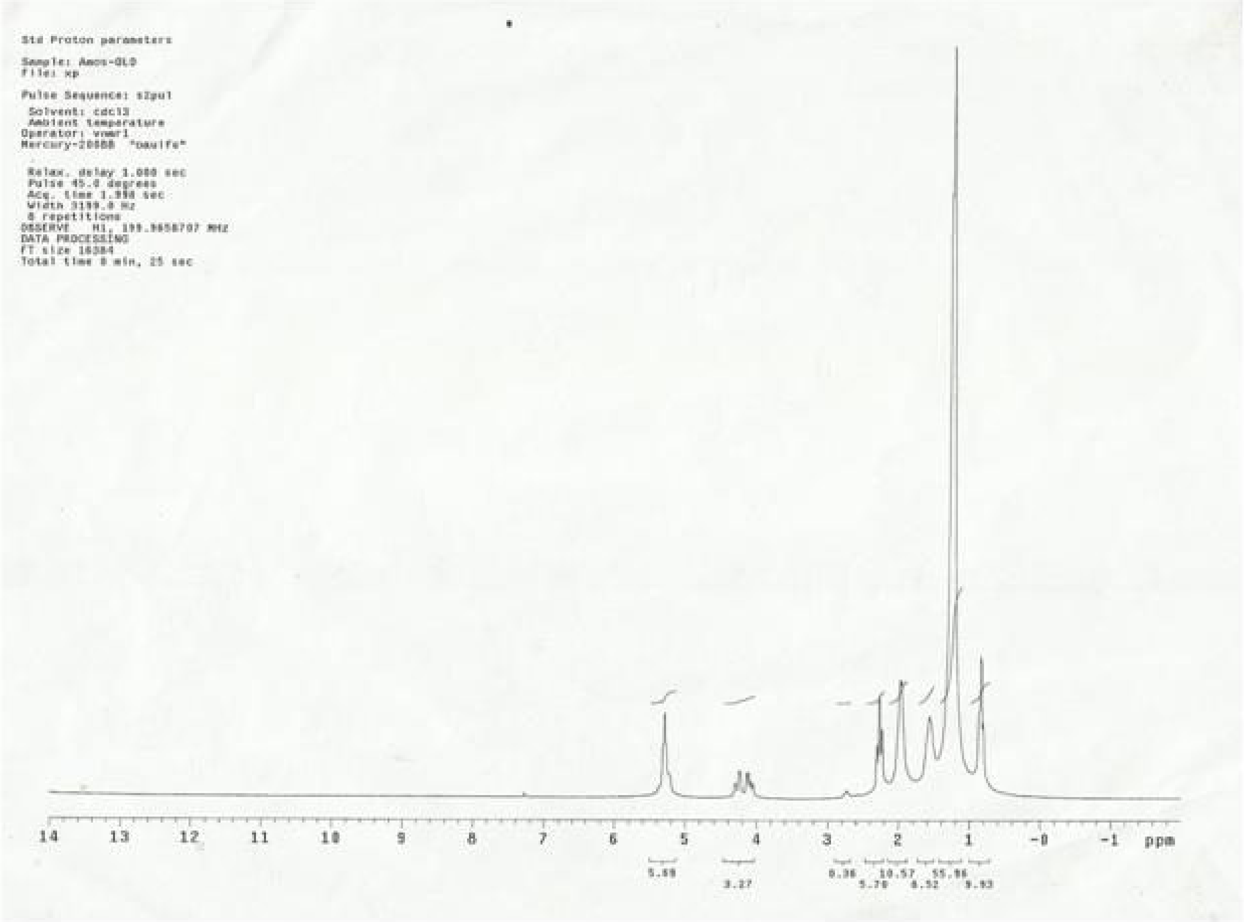
APPENDIX:



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Std Proton parameters

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File: xp

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 Ambient temperature
Operator: vnmr1
Mercury-200BB "oauife"

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Figure 2 : Proton NMR Spectrum of Unoxidized Olive Oil.

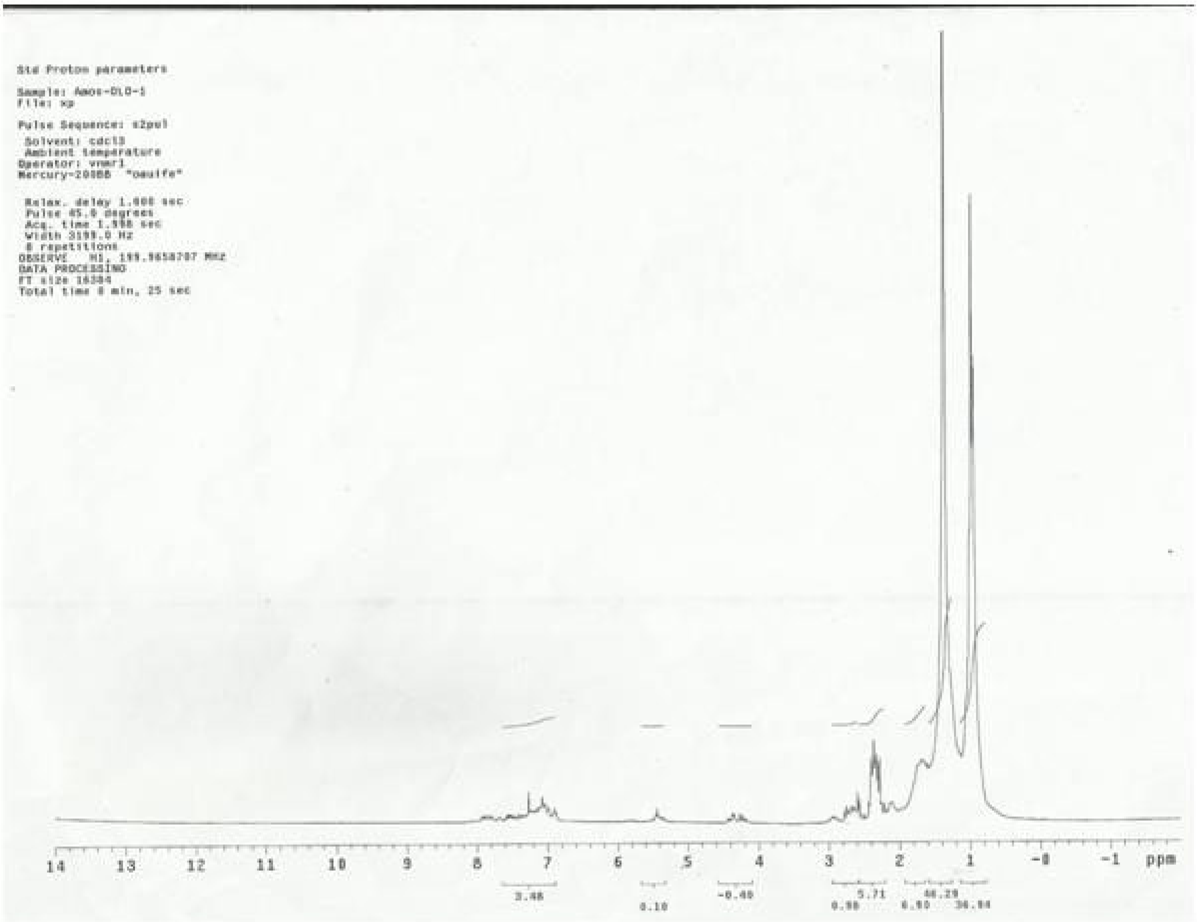


Figure 3 : Proton NMR Spectrum of Isolated Oxidized Product of Olive Oil.

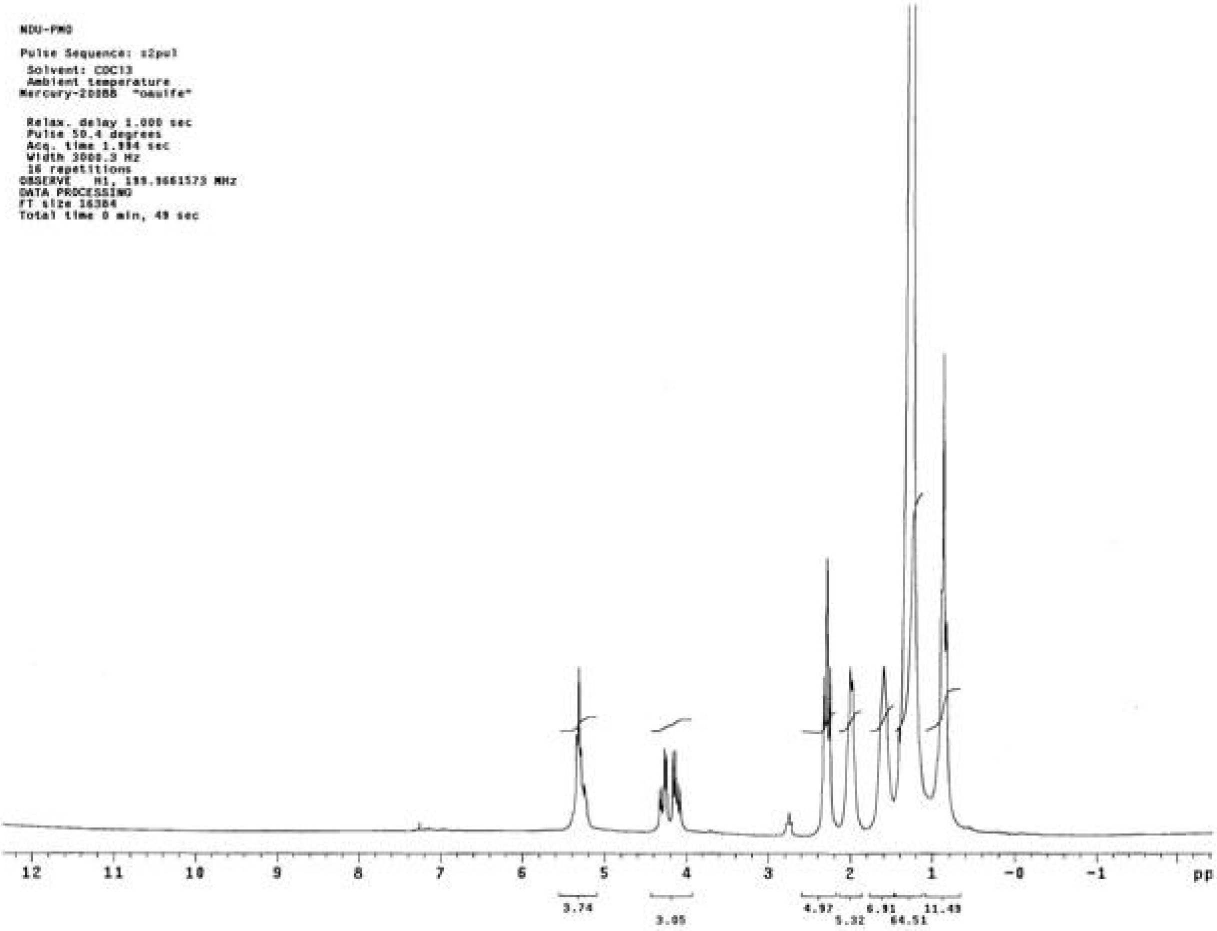
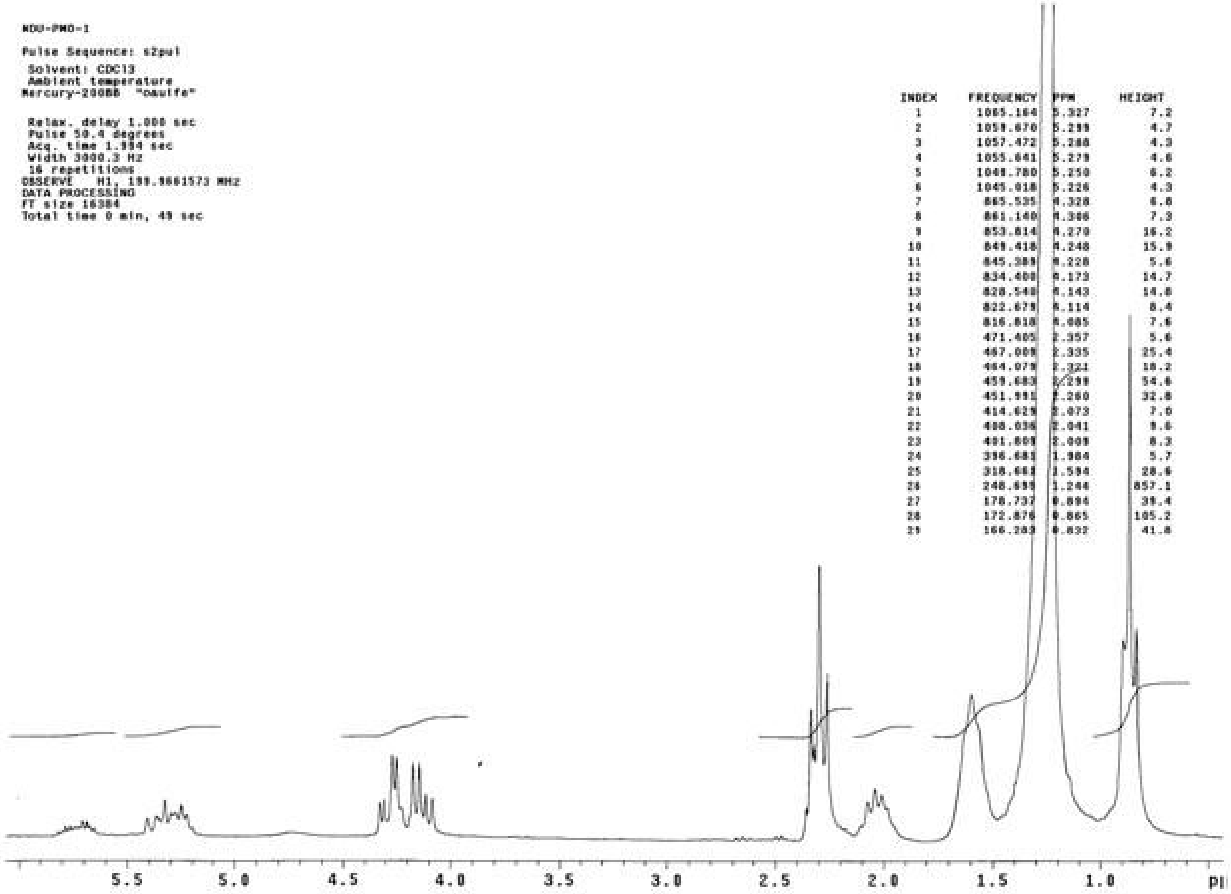


Figure 4 : Proton NMR Spectrum of Unoxidized Palm Oil.



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 Ambient temperature
Mercury-200BB  "oaulfe"

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 Width 3000.3 Hz
 16 repetitions
OBSERVE   H1, 199.9661573 MHz
DATA PROCESSING
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Total time 0 min, 49 sec
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3	1057.472	5.288	4.3
4	1055.641	5.279	4.6
5	1049.780	5.250	6.2
6	1045.018	5.226	4.3
7	865.535	4.328	6.8
8	861.140	4.306	7.3
9	853.814	4.270	16.2
10	849.418	4.248	15.9
11	845.389	4.228	5.6
12	834.400	4.173	14.7
13	828.540	4.143	14.8
14	822.679	4.114	8.4
15	816.818	4.085	7.6
16	471.405	2.357	5.6
17	467.009	2.335	25.4
18	464.079	2.321	18.2
19	459.683	2.299	54.6
20	451.991	2.260	32.8
21	414.629	2.073	7.0
22	408.036	2.041	9.6
23	401.809	2.009	8.3
24	396.681	1.984	5.7
25	318.682	1.594	28.6
26	248.699	1.244	857.1
27	178.737	0.894	39.4
28	172.876	0.865	105.2
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Figure 5: Proton NMR Spectrum of Isolated Oxidized Palm Oil (Expanded).

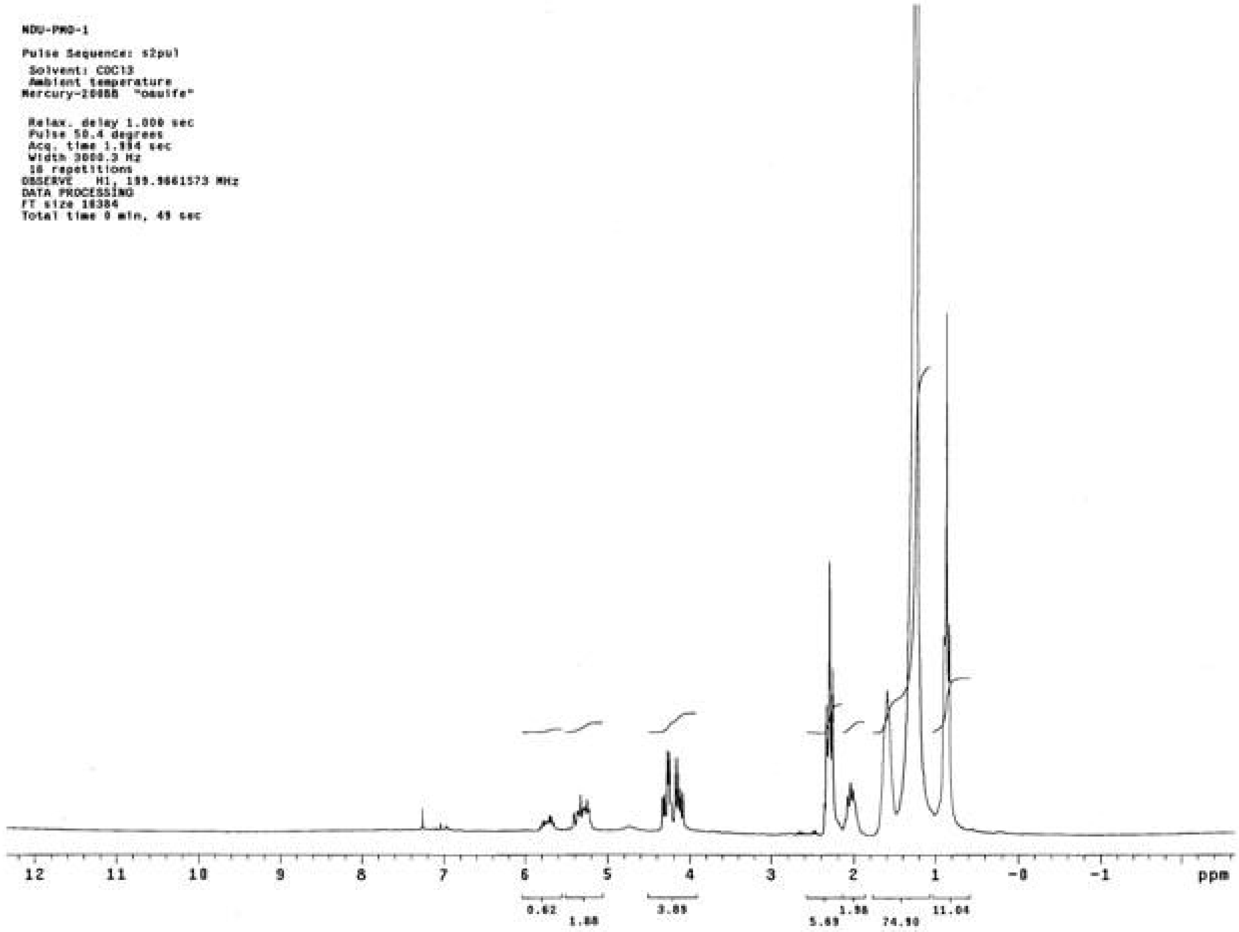

Figure 6 : Proton NMR Spectrum of Isolated Oxidized Product of Palm Oil.

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