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Oxidation of Pear Seed Oil During Storage

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Abstract: The effects of storage on the oxidative deterioration of crude Pear Seeds Oil (PSO), obtained by soxhlet extraction, were studied. Oxidation was monitored after 6 months via several analytical techniques: peroxide value, iodine value, free fatty acids, Fourier-transform infrared spectroscopy (FTIR), and Gas chromatography–mass spectrometry (GC-MS). The slow detorioration of the oil was an indication that oxidation was strongly dependent on temperature, oxygen availability and the presence of Cu^{2+}. The mass spectra from the GC-MS results reveal the formation of secondary oxidation products, thereby denoting a transformation of primary hydroxyperoxides. This assertion is further strengthen based on the increasing value of the total Saturated Fatty Acids (SFA) from 26.09 to 29.44 % and a corresponding decrease for Unsaturated Fatty Acid (UFA) from 71.28 to 68.80 %. Other physicochemical analysis, shows that an average Iodine value recorded a reduction from 40.377 to 39.193 at a rate of 2.9 %, free fatty acid also recorded an increase at 15.18 %. Furthermore, the acid value also recorded a 2.60 % reduction. An increase in the peroxide value from 6.243 to 7.452 at a rate of 16.22 %, is a confirmation of possible formation of secondary oxidation products as revealed by the GC-MS mass spectra. Therefore the need to pay close attention during production, transportation as well as the presence of possible Cu^{2+} is key to addressing slow oxidation that is capable of impairing the quality of the oil.

Keywords: Oxidation, fatty acids, oil, physicochemical, peroxide value, storage

1. Introduction

The quest for an alternative source of industrial material from green sources for a sustainable environment has attracted several research findings related to natural waste sources. Continuous focus on the use of waste plants seeds oil as an alternative is based on their availability, renewability and environmentally friendly properties [1]. The choice of this oil is due to its high consumption within the Niger Delta region of Nigeria. Furthermore, PSO is currently considered as one of the main source of waste oil with diverse industrial applications [2]. However, its good lubricating property has prompted the need for extensive findings geared towards its industrial sustainability [3]. One of the disadvantages for a complete utilization of this oil, is the structural transformation via oxidation that has impacted on its level of stability [4]. The drawback of this oxidation process is evident in the reduce durability of the oil and also posed a threat to health through pollution in the environment via spills, leakages, evaporation and disposal routes. The oxidative stability of oils is the resistance to oxidation during processing and storage [5]. Oil oxidation is an undesirable series of chemical reactions involving oxygen that degrades oil quality resulting to rancidity characterized by off flavours and smells that is attributed to a changed in the essential fatty acids resulting to the production of other

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compounds and oxidized polymers. Also as nutritional guide, several oil oxidation products may be absorbed and metabolized in humans [6]. Among these secondary oxidation products is the highly reactive and cytotoxic 4-hydroxy-2-alkenals that has been reported to be toxic to the human cell [7]. In a related study as confirmed by Pillon el al. [8], who noted that such compounds were toxic to L6 muscle cells and also affecting its viability even at low concentrations.

Due to variation in temperatures from the point of extraction to storage and even during the process of transportation, resulting to possible autoxidation which is the major cause of quality losses in crude and refined oils [9]. The oxidative stability is accessed based on deterioration of the oil depending on initial composition, concentration of minor compounds with antioxidant characteristics, degree of processing, and storage conditions [10]. The fatty acid alkyl chain contained in most plant seed oil is susceptible to oxidation both at the double bonds and adjacent allylic carbons [11]. The presence of high polyunsaturated fatty acids from studies has been reported to have influence on the oxidative stability of PSO [12]. Findings have also reported that autoxidation and photosensitized oxidation, are responsible for the oxidation of oils during processing and storage depending upon the types of oxygen. These reactive oxygen are, atmospheric triplet oxygen, $^3O_2$, and the singlet oxygen, $^1O_2$ [7]. The formation of hydroperoxides is a key product of autoxidation of unsaturated oil through free-radical chain reaction mechanism in a catalytic process [13]. The oxygen availability and the temperature of the environment are important parameters that change the oxidative potentials of the oil [10]. The reactivity between the oil peroxy radical and hydroperoxide produces a nonradical species that stops the reaction [13]. The susceptibility of fatty acids to hydrogen abstraction, strongly depends on the degree of unsaturation and can be explained by the bond strength of the hydrogen of the α-methylene group in the fatty acid molecule [13]. The main pathway to the short-chain volatile compound has been reported to proceed via the hemolytic β-scission of a carbon-carbon bond to produce oxo-compounds as an alkyl or alkenyl radical [14]. Fig. 1 as described by Choe and Min [7], explained a possible route for the breakdown of hydroperoxide either spontaneously or in the presence of metal traces.

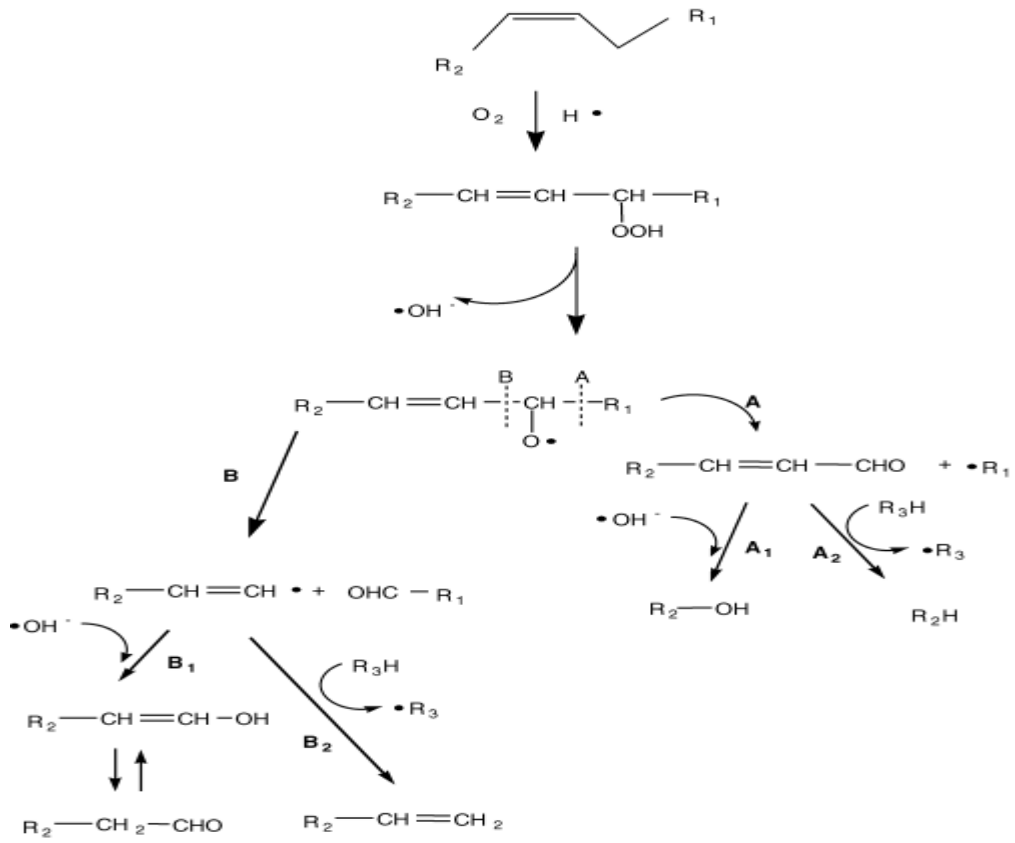

Fig. 1 - Mechanisms of hydroperoxide decomposition to form secondary oxidation products [7]

The inability to control the auto oxidation of oil resulting to possible rancidity and low quality. The need to study the oxidation pattern will inform production firms on the best practices to address this short falls. Several methods have been applied to evaluate the oxidation products of oil such as peroxide value (PV), weight gain, loss of unsaturated fatty acids, conjugated diene value, *p*-anisidine value (AV), thiobarbituric acid test, and chromatographic techniques [15]. Furthermore, the following are therefore fundamental to the oxidation of oil; an available energy in the form of

light or heat, composition of fatty acids, types of oxygen, and minor compounds such as metals, pigments, phospholipids, free fatty acids, mono- and diacylglycerols, thermally oxidized compounds, and antioxidants [7].

The primary oxidation products, lipid hydroperoxides, are relatively stable at room temperature and in the absence of metals. However, in the presence of metals or at high temperature they are readily decomposed to alkoxy radicals and then form aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons [7, 13]. The most likely pathway of hydroperoxide decomposition is a homolytic cleavage between oxygen and the oxygen bond, in which alkoxy and hydroxy radicals are produced [16]. The main objective of this work is to study the oxidative deterioration and possible secondary products formed during storage of PSO.

2.0 Materials and Methods

2.1 Materials and Apparatus

African Pear Seeds (*Dacryodes Edulis*), Wij's solution, n-hexane and other chemicals were products of Sigma-Aldrich, USA. The methanol used (99% pure) is of analytical grade with boiling point of 78°C; while the NaOH, Potassium dichromate and Potassium iodide used were of analytical grade and purchased from Aldrich Chemical Co. Ltd. Sodium sulfate, Hydrochloric acid, starch, sodium thiosulphate, Phenolphthalein used were also an analytical grade product of Merck Co Ltd. Laboratory oven (DHG 9030) magnetic stirrer with hotplate (UNICON), three necks round bottom flask, measuring cylinder, beaker, separating funnel, burette, density bottle, funnels, pet-bottle thermometers and measuring flask were used.

2.2 Sample Collection and Treatment

Samples used in this process were purchased from Otovwodo market in Ughelli North Local Government Area of Delta State, Nigeria. The seeds were dehulled with a sharp stainless knife to remove the seed from the pulp. The prepared seeds samples was then dried at a temperature of 70 ^{0}C in a Gallenkamp hot air oven for 48 hrs. The dried samples were ground into uniform powder.

2.3 Soxhlet Extraction of Seeds Oil

One hundred grams (100 g) of the powdered sample were wrapped with whatmann filter paper and transferred into a thimble of Soxhlet extractor. The thimble was carefully fixed on a 1-litre capacity round-bottomed flask. 700 ml of n-hexane (B.p. 40-60 ^{o}C) was poured to about two-third of the volume of the flask and heated at 60 ^{o}C on a thermostatically controlled heating mantle and allowed to reflux continuously for 6 hrs. Percentage oil yield was determined as expressed and replicate extraction process was performed [17]. This process of extraction is captured in Fig. 2.

$$\text{Seed oil content (\%)} = \frac{Wo}{Ws} \times 100$$

Where Wo = weight of the oil extracted
Ws = weight of the sample

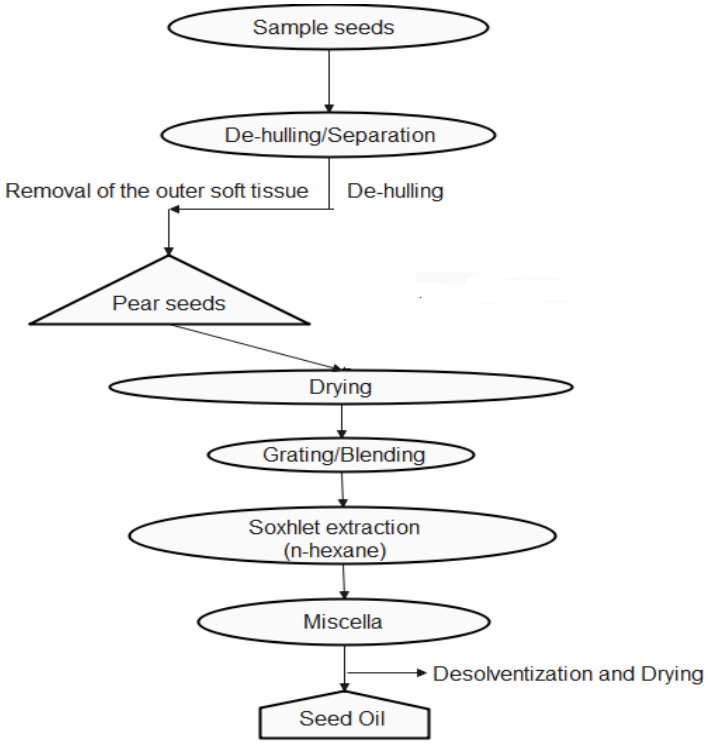

Fig. 2 - Flow chart for extraction of seed oil [17]

2.4 Iodine Value

The iodine value is a measure of the degree of unsaturation of oils and determines the stability to oxidation. Standard AOAC [18] official methods of analysis by Enferadi et al., [19] and Wij's iodine method was used for this analysis: 0.52 g of oil sample was dissolved in 10 ml of cyclohexane. 20 ml of Wij's solution (Iodine monochloride) was added, the stopper flask was allowed to stand for 30 min in the dark at room temperature, and 20 ml of 10% potassium iodide solution was added. The resulting mixture was then titrated with 0.1 M $Na_2S_2O_3$ using starch as indicator. Iodine value was calculated using equation [18].

$$IodineValue = \frac{[\rho^o - \rho] \text{ x } M \text{ x } 12.69}{W}$$

Where M = concentration of sodium thiosulphate used;
ρ^o = volume of sodium thiosulphate used as blank;
ρ = volume of sodium thiosulphate used for determination.
W = Weight in g of the material taken for the test.

2.5 Determination of % Free Fatty Acid (FFA)

The % FFA of the hydrolyzed seed oil was determined according to Mahesar et al., [20]. Approximately 50 mL of isopropanol was placed into the flask, and about 0.5 mL phenolphthalein added and then neutralized by addition of sodium hydroxide (NaOH, 0.02N) until a permanent pink colour is obtained. The neutralized isopropanol was added to the 5 g of FFA, which will be placed into an Erlenmeyer flask, and about 0.5 mL of phenolphthalein was added. After shaking the mixture gently, the mixture is neutralized by the addition of NaOH, 0.02N until the first permanent pink colour is obtained. The FFA% was calculated by using the equation.

$$\%\text{FFA as oleic} = \frac{28.2 \times N \times V}{W}$$

Where; V = Volume in ml of 0.5N NaOH required for titration in ml.
W = Weight in g of sample taken.
N = Normality of Sodium hydroxide solution

2.6 Acid Value

Accurately 10 ml of the cooled oil sample was weighed into a 250 ml conical flask and 50 ml of the freshly neutralized hot ethyl alcohol and about 1 ml of phenolphthalein indicator solution was added to the content in the flask. Boil the mixture for about five minutes and titrate while hot against standard alkali solution shaking vigorously during the titration. The weight of the oil/fat taken for the estimation and the strength of the alkali used for titration is such that the volume of alkali required for the titration does not exceed 10 ml [18].

Acid Value = Percent fatty acid (as oleic) x 1.99

2.7 FTIR Spectroscopic Analysis

FT-IR spectra of samples as described by Liang et al., [21] was adopted for this study, using SHIMADZU FTIR-8400S equipped with deuterated triglycine sulphate (DTGS) as detector, potassium bromide (KBr) as beam splitter. The measurements were carried out on a HATR surface at room temperature in the IR region of 4000–450 cm^{-1}, by accumulating 40 scans with a resolution of 4 cm^{-1}.

2.8 GC-MS Analysis

Gas chromatography-mass spectrometry analysis was performed on a GCMS-3800 system (Shimadzu, Tokyo, Japan). This technique was adopted from Adams [22]. Twenty microliters of sample (extract or essential oil) was diluted to 1 mL with hexane (≥99%, Sigma–Aldrich, Germany). The column used was a 30 m × 0.25 mm i.d. × 0.25 µL film thickness RTX-5MS column. Flow rate of helium (99.999%, AGA Lithuania) carrier gas was set at 1.23 mL/min. The oven temperature was maintained at 40 °C for 2 min after injection and then programmed at 3 °C/min to 210 °C, at which the column was maintained for 10 min. The split ratio was 1:10. The mass detector electron ionization was 70 eV. Identification of volatile compounds was carried out using mass spectra library search (NIST 14).

3.0 Results and Discussion

3.1 Physicochemical properties

Table 1 - Fatty acid composition of PSO

S/N	Fatty acids	Systematic names	Composition (%) Fresh	Storage after 6 months
1	Capric acid; C10:0	Decanioc acid ($C_{11}H_{12}O_2$)	0.07	0.11
2	Lauric acid; C12:0	Dodecanoic ($C_{12}H_{24}O_2$)	0.23	1.61
3	Myristic acid; C14:0	Tetradecanoic $C_{14}H_{28}O_2$	1.77	2.36
4	Palmitic acid; C16:0	Hexadecanoic ($C_{16}H_{32}O_2$)	9.23	9.38
5	Palmitoleic acid; C16:1	cis-9-hexadecenoic $C_{16}H_{30}O_2$	4.77	4.83
6	Stearic acid; C18:0	Octadecanoic ($C_{18}H_{36}O_2$)	11.56	12.09
7	Oleic acid; C18:1	cis-9-octadecenoic ($C_{18}H_{34}O_2$)	51.43	48.12
8	Linoleic acid; C18:2	cis-9-cis-12-octadecedianoic ($C_{18}H_{30}O_2$)	10.06	10.64
9	Linolenic acid; C18:3	cis,cis,cis-9,12,15-octadactrienoic $C_{18}H_{30}O_2$	5.02	5.21
10	Arachidic acid; C20:0	Eicosanoic $C_{20}H_{40}O_2$	3.23	3.89
	SAFA		26.09	29.44
	UFA		71.28	68.80
	others		2.63	1.76

SFA = Saturated Fatty Acid, UFA = Unsaturated Fatty Acid

The impact of sunlight and oxygen availability on the composition of fatty acids, as captured in Table 1, reveals that the changes for oil stored after 6 months shows a slow form of deterioration. This is described by the reduction in oleic acid (C18:1) from 51.43 % to 48.12 % but recorded an increase for stearic acid; C18:0 from 11.56 % to 12.09 %.

Table 2 - Physicochemical properties of the extracted seed oil

Parameters	Units	Pear seed oil	Stored oil after 6 months
State at room temperature	-	Liquid	Liquid
Iodine value	g/100 g	40.377±.889	39.193±1.352
Free fatty acid	mgKOH/g	4.130±0.243	4.869±1.021
Peroxide value	meq O_2/Kg	6.243±0.290	7.452±0.290
Acid value	mgKOH/g	8.011±0.151	7.803±0.212

The average Iodine value as captured in Table 2 recorded a reduction from 40.377 to 39.193 at a reduction rate of 2.9 %, the free fatty acid also recorded an increase at 15.18 %. Furthermore, the acid value also shows a 2.60 % reduction. An increase in the peroxide value from 6.243 to 7.452 at a rate of 16.22 %, is a confirmation of possible formation of oxidation products. The result shows that the highly unsaturated PSO is more prone to oxidation resulting in poor oxidation stability.

3.2 FTIR

More emphasis on the FTIR results is focused on the degree of oxidation by a general response in the carbonyl (C=O) region of between 1,800 to 1,670 cm^{-1} as reflected in Fig. 3a & 3b. In this region, IR energy is absorbed due to the carbon oxygen bonds in the oxidized oil.

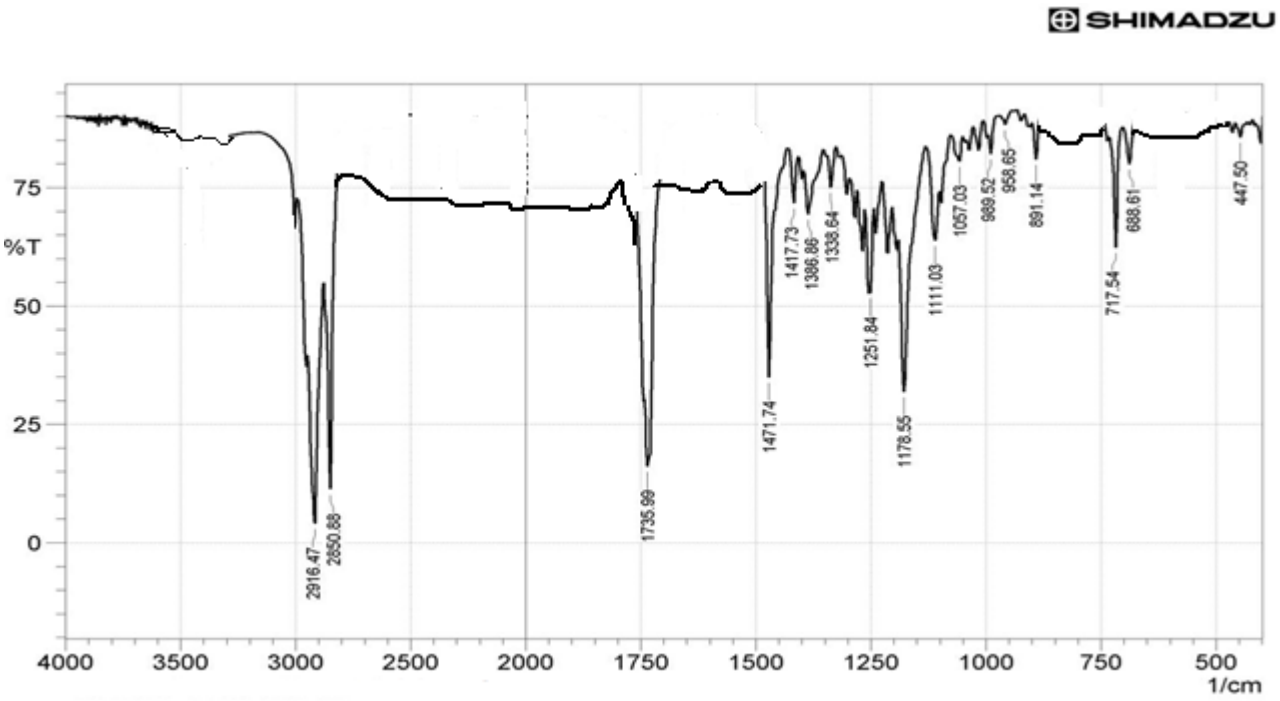

Fig. 3a - FTIR for fresh oil sample

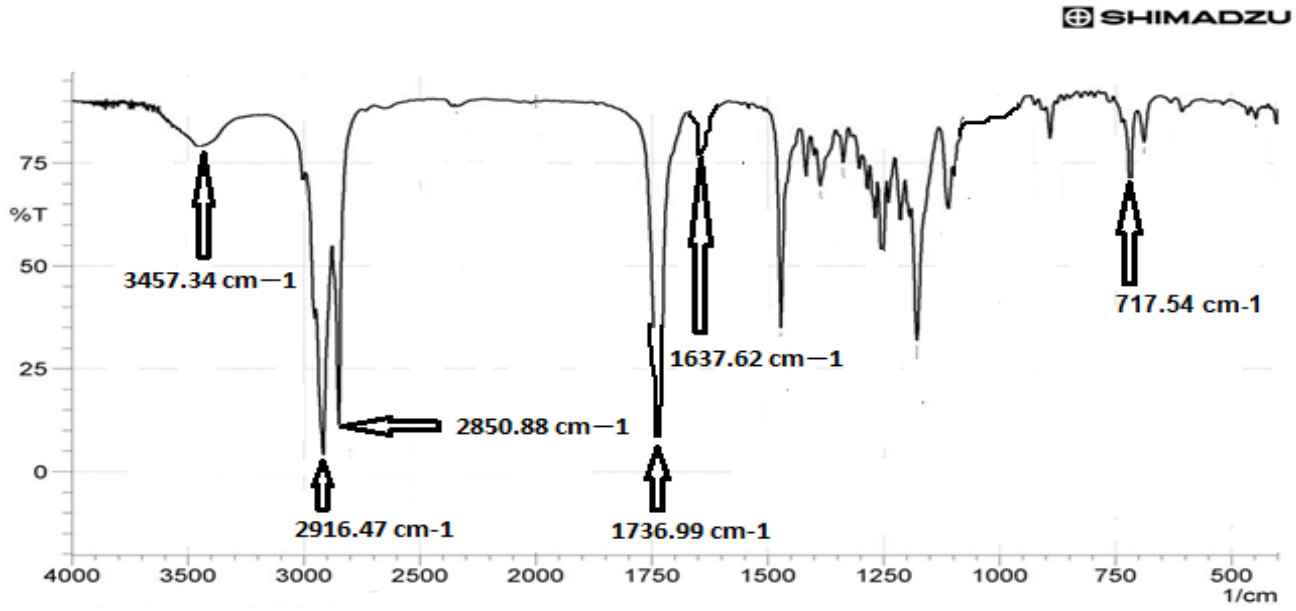

Fig. 3b - FTIR for stored oil sample after 6 months

Fig. 3b revealed that the peak intensities at 717.54 cm^{-1} showed decreased in intensity that could be caused by the loss of cis double bonds. The gradual decrease in the peak intensity after 6 month is an indication of possible transformation of unsaturation in the stored oil due to likely oxygen cleavage at the double bond positions. To further confirm the possibility that oxidation has been initiated, increase peak intensity can be seen in regions of 1736.99 and 1637.62 cm^{-1}, which is due to the formation of carboxylic compounds. Two peaks at 2916.47 cm^{-1} and 2850.88 cm^{-1} shows no difference in intensity as observed from the fresh sample in Figure 3a. Slight absorbance increase was observed around 3457.34 cm^{-1}, corresponding to the formation of hydroxyl group (-OH) formed as a result of possible oxidation.

3.3 GC-MS

The results from this finding is also a confirmation that the formation of an unstable primary hydroxyperoxide either from the process of extraction, transportation, etc, could initiate other multiple reaction, thereby generating series of results as captured in Figure 4 and Table 3. These results denote a possible transformation of the primary oxidation products into secondary products. However, their low percentage composition as highlighted in Table 3 further confirmed a slow oxidation process in agreement with the assertion by Fatima et al., [3].

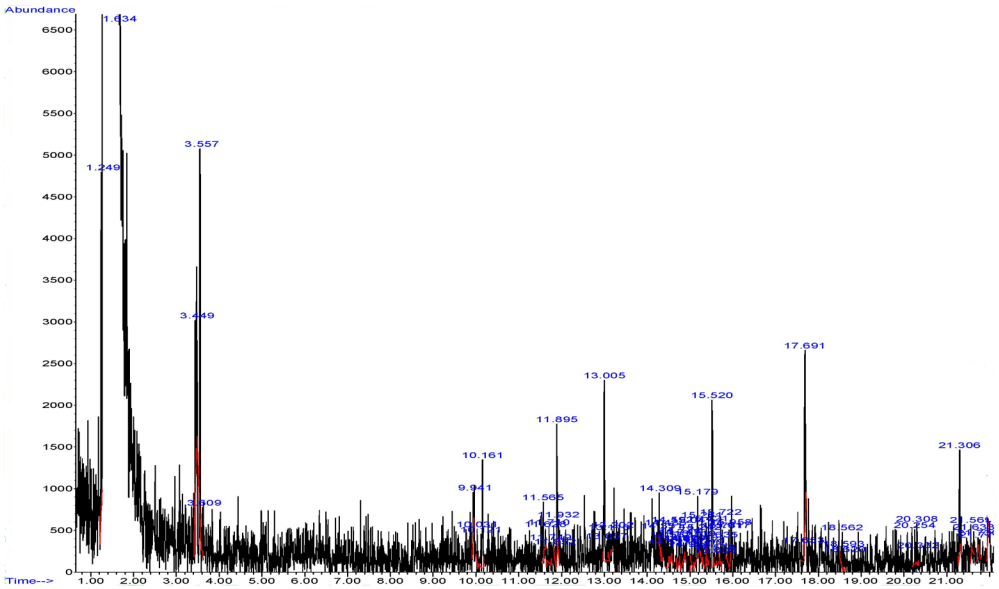

Fig. 4 - GCMS Chromatogram for PSO stored for 6 months

Table 3 - Mass spectra for possible secondary products of oxidation

Peak No	Compounds	Precursor ion (m/z)	R. T (min)	Peak area	% Composition
4	Dodecanoic acid, 3-hydroxy	207.0	3.557	12430	1.67
7	4,5-Decanediol, 6-ethyl-	202.0	10.161	12100	1.51
11	3-(2-Thienyl)-4,5-dihydro-5-isoxazolemethanol	183.0	11.895	40147	0.83
15	3,4-Pentadienal, 2,2-dimethyl-	109.0	15.179	24787	0.22
17	2,6-Dimethyl-8-oxoocta-2,6-dienoic acid, methyl ester	196.0	18.582	13356	1.32
19	Dodecyl isobutyl ether	199.0	21.562	10528	1.09

Conclusion

In conclusion, the results showed the influence of storage and oil composition on crude PSO oxidation. The rate of oxidation shows a slow process after 6 months despite properly sealed in a brown amber bottle. This could be attributed to the presence of some heavy metals most especially the presence of Cu^{2+} that could initiate the process of oxidation if trace of water molecules is present in the oil. The relatively low oxidation could be attributed to the low temperature of below 4^{o}C of storage. The results from this finding is also a confirmation that the formation of an unstable primary hydroxyperoxide could be either from the process of extraction, transportation, etc, that further initiate other multiple reaction, thereby generating series of secondary products. Due to the multiple factors that could trigger the process of oxidation in oil, more parameters that will provide enough information in order to evaluate different stages of oxidation should be envisaged.

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