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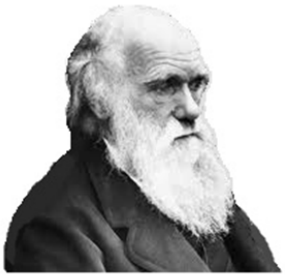

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Comparatives study of thermal stability in different coocking oil

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ABSTRACT

This aim of this research is to investigate thermal stability of coconut oil (CNO) which was heated at 190 °C upon 40 days storage as compared to peanut oil (PNO). The changes in fatty acids composition through (GC), Fourier Transform Infrared (FTIR) spectra, iodine value (IV) and total phenolic content were determined throughout the period of study. Results from GC showed that there was significant changes ($P<0.05$) in the percentage of linolenic acid (from 0.74% to 0.54%) and palmitic acid (from 12.31% to 13.44%) in the PNO upon storage. Higher stability was found in CNO as it contains higher saturated fatty acids. FTIR spectra showed both oils had undergone oxidation and produced hydroperoxides upon 40 days storage but with less oxidation in CNO. Meanwhile, IV and total phenolic content decreased significantly ($P<0.05$) in both oils with percentage reduction of IV in CNO greater than that in EVOO (70.60% vs. 11.90%), contrary to percentage loss in total phenolic content (50.77% vs. 68.19%). As a conclusion, CNO can be considered as good frying oil as it has relatively high oxidative stability as PNO.

***Keywords*:** Coconut oil, Peanut oil, Fatty acid, Phenolic, *Cocos nucifera*

1. INTRODUCTION

Vegetable oils and fats are important part of our diet as they provide energy, fat, soluble vitamins and essential fatty acids required for growth and development of the body. Oils and fats, apart from providing nutrition, are known to play function contributing to the palatability of processed food. Vegetable oils are safe, non-toxic, readily biodegradable products because

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they are natural. They are therefore potential applications for the basis oils of lubricating oil that are environmentally friendly. Biolubricants have good viscosity, which allow ease of flow into contacting surface in addition to friction reduction. Additional crucial attributes of biolubricating fluids encompass cost effectiveness, source availability, environmental compatibility, and thermal stability.

Coconut (*Cocos nucifera*) oil is an edible fats usually appearing as a white creamy solid, derived from the fruit of coconut tree. Coconut is a tree in palm (Arecaceae) family found in moist, tropical climates such as Eastern and Northern part of Nigeria. The traditional used of coconut oil is a dietary oil in the tropics has been challenged hypercholesterolemia (Chowdhury K, et al. 2007).

Several organizations like World Health Organization, National Agencies for Food and Drug Administration Control (NAFDAC), United States Food and Drug Administrations, United States Department of Health and Human Services etc. recommend against the consumption of significant amounts of coconut oil due to its high levels of saturated fat content. But, coconut oil contain 31-47 per cent of lairic acid, a saturated fat that can increase the high density lipoprotein (HDL) cholesterol in the blood through it is unclear if coconut oil may promote atherosclerosis through other pathways (Bouaid et al, 2007; Boskou et al, 2003). However, virgin coconut oil is composed mainly of medium – chair triglycerides; which may not carry the same risks as other saturated fats (Besbes et al, 2005).

Peanut oil derived from groundnut (Arachis hypogaea), with its unique aroma and taste and high smoke point. It is often used in Chinese and southeast Asian cuisine, and some part of West African especially Northern part of Nigeria. Its major component fatty acids are oleic acid (46.8%), linoleic acid (33.4%) and palmitic acid (10.0%). The oil also contains some stearic acid, arachidic acid, arachidonic acid, behenic acid, lignoceric acid and other fatty acids (USA National nutrient Data Base).

This researched study solved some challenges accrued using mineral oil as cutting fluid and suggest alternative to suitable, eco-friendly, non-toxic and bio-degradable solution using vegetable oil. To investigate their optimal use for industrial applications, this study test base oils thermal-physical properties stability [9-13].

2. MATERIALS AND METHODS

Coconut oil (CNO) and peanut oil (PNO) were extracted from coconut and groundnut respectively and were purchased from local market at Ekwulumili, Nnewi-South L.G.A of Anambra State, Eastern Nigeria.

2. 1. The procedure of extraction of the oil's

Coconut oil; the first is a process known as wet milling. Virgin coconut oil is extracted from fresh coconut milk obtained from the mature coconut. The oil was extracted from fresh coconut meat without the intermediate drying processed.

Coconut milk is pressed from the meat and the oil is separated from the water through mechanic centrifuge method. Peanut oil; was extracted from groundnut using pressing method, the base steps involved in the extracted ground oil were; cleaning , dehulling, cooking, pressing and lastly filtration.

2. 2. Heat treatment and storage studies

The oils were heated to speed up the onset of degradative reactions such as hydrolysis, oxidation and polymerization in the oils used in this study. Frying was conducted in a Philips deep fryer (Cucina DH 6151, China) equipped with thermostat. First, 500 ml of oil sample was preheated to a temperature of 190 °C. After 15 min, 30 g of raw French fries was added. The French fries were fried for 5 min and then removed. Following this procedure, the oil temperature was allowed to return to 190 °C within 5 min before subsequent frying. Each of the oil samples completed six frying operations of French fries, equivalent to a period of 75 min. The oil was allowed to cool down to room temperature before analysis was carried out. Oil samples after completion of 6 frying operations were filled into test tubes with screw cap. The entry of oxygen was further prevented by wrapping the tubes with parafilm. Additionally, the tubes were covered with a layer of aluminium foil to exclude the light completely and then stored at room temperature. Samples were taken out for analysis every tenth day for a total storage period of 40 days.

2. 3. Determination of FAMEs composition by Gas Chromatography

Perkin Elmer Gas Chromatograph equipped with Flame Ionization Detector (Elmer Instruments Autosystem XL) was used to determine the FAMEs composition of the tested oils. The column used was fused silica capillary (SP™ 2380, Supelco; 30 m × 0.25 mm × 0.25 μm film thickness). Flow rates of compressed air and hydrogen were at 450ml/min and 25 ml/min respectively while the nitrogen gas was at the pressure of 180 psi. The split ratio used was 50:1. Initial oven temperature was 140 ºC, held for 5 min then programmed to 240 ºC at 4 ºC per min, and finally held for 10 min at this temperature. The temperatures of injector and FID were both set at 250 ºC. Total runtime was 40 min. The oil was converted to fatty acid methyl esters (FAMEs) according to the modified method described by Boskou (2003). First, 0.5 g of oil was dissolved in 25 ml n-hexane. Then, 5mL of the mixture was transferred into a test tube and mixed with 250 μl 0.5M sodium methoxide in methanol. The mixture was then shaken vigorously in a vortex mixer for 60 seconds. Five millilitres of saturated sodium chloride solution was then added and the mixture was again shaken vigorously for 15 seconds. After 10 min, 3 ml of hexane layer containing FAME (the top layer) was transferred into a vial followed by addition of small amount of anhydrous sodium sulphate. Finally, approximately 1 μl of sample was injected manually into the gas chromatograph. The above procedures were performed in duplicate in fresh oil samples as well as after cooling down (0 day) and in the end of experimentation (stored for 40 days) in heated oil samples. The chromatographic data was recorded and analyzed in a personal computer (Optiplex GX 160, Dell) using Total Chromo Navigator Software, Perkin Elmer, USA. Individual fatty acids were identified by comparing their retention time with standard FAME mix GLC-10 and Standard Supelco-37 components FAMEs. The percentage of each component was calculated by dividing the individual peak area with total peak area.

2. 4. Collection of FTIR spectra

Potassium bromide (KBr) powder was ground by using the grinder specialized for FTIR spectrometer. Then the KBr powder was heated in an oven at 100 °C for 30 min to reduce the moisture content. By using the presser specialized for FTIR spectrometer, the KBr powder was pressed strongly to form a transparent KBr disk. The KBr disk was then placed in the standard

holder specialized for FTIR spectrometer. A background spectrum was first obtained by analysing the KBr disk. Next, the KBr disk was taken out and a small drop of oil was placed on KBr disk to form a thin capillary film. The disk was placed in the holder again for analysis. FTIR spectra of the oil samples were collected through FTIR spectrometer Thermo Nicolet-Avatar 320. The infrared spectra were recorded in the range 4000 cm^{-1} to 400 cm^{-1}. All data were recorded and integrated in a personal computer operating under Nicolet Omnic software version 6.

2. 5. Iodine value

Iodine value was determined the AOAC Official Methods of Analysis (1984), with minor modifications. Approximately 0.3 g of sample was weighed into a 250 ml conical flask followed by addition of 10 ml of carbon tetrachloride. Twenty five millilitres of Wijs reagent was added into the flask. The contents were mixed thoroughly by swirling and stored in a dark place at room temperature. At the end of 30 min, 20ml of 10% potassium iodide solution and 100 ml of distilled water were added to the sample solution. The mixture was titrated immediately with standard 0.25M sodium thiosulfate solution until the yellow colour almost disappears. Three millilitres of starch indicator solution was added and titration was carried on drop wise with vigorous swirling until disappearance of the blue starch-iodine colour. The blank was prepared by adding 10 ml of carbon tetrachloride into an additional flask without the oil. The blank was then titrated in the same manner as described previously. The iodine value was then calculated by using the formula as described by Chowdhury *et al*. (2007). IV test was performed in duplicate for each of the oil samples.

2. 6. Total phenolic content

The phenolic content of oil was extracted according to the method described by Rotondi *et al*. (2004) with some modifications. Approximately 15 g of oil was weighed into a 50 ml Falcon tube. Ten millilitres of n-hexane was mixed with the oil. The mixture was extracted with 10 ml of methanol: water (60:40). The mixtures were shaken for 5 min and then centrifuged at 5500 rpm for 5 min. The hydroalcoholic phase was collected and the hexane phase was re-extracted twice with 10 ml of methanol: water (60:40) each time. The combined hydroalcoholic fractions from three extractions were subjected to final washing with 10 ml of n-hexane to remove residual oil in a separatory funnel. The excess solvent was evaporated under vacuum at 40 ºC until dryness in a rotary evaporator. The residue was reconstituted in 2 ml methanol: water (60:40) for CNO sample and 20 ml methanol: water (60:40) for PNO sample. The difference in amount of methanol:water (60:40) used was due to large difference in amount of phenolic compounds present in the samples.

The total phenolic content was determined by the Folin-Ciocalteu reagent assay (Lim *et al*. 2007). First, 0.5 ml of the extract obtained was mixed with 1.5 ml of Folin–Ciocalteu reagent previously diluted with distilled water (1:10). After standing at room temperature for 3 min, 1.2 ml of 15% sodium carbonate solution was added. The mixture was placed in dark room for 60 min. After that, absorbance was measured at 765 nm against the blank using a spectrophotometer (Secomam UVi light XTD). The calibration curve was obtained by repeating the above procedures with known concentrations of gallic acid solutions. The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 gram of oil (mg GAE per 100 g of oil).

Since the assay quantifies all phenolic compounds, the selection of gallic acid as a standard is based on the availability of a stable and pure compound. In addition, gallic acid is cheaper than other options. Analyses were performed in duplicate for each of the extract.

2. 7. Statistical analysis

Since all analyses were done in duplicate, the presented results are the mean of the obtained values. Minitab 15 statistical software; was used for statistical analyses of fatty acids profile, IV and total phenolic content. Analysis of variance (ANOVA) and Fisher LSD mean comparison test were applied. Significant differences were detected at ($P < 0.05$).

3. RESULTS AND DISCUSSIONS

3. 1. Changes in fatty acids composition upon storage

Vegetable oils undergo changes in terms of chemical and physical properties when they interact with the food or atmosphere. Types of reaction that are known to lead to degradation of vegetable oils include polymerization, oxidation and hydrolysis. The changes in fatty acids composition of PNO and CNO are shown in Table 1. In PNO, the fatty acid composition suffered slight changes upon heating and storage. There was a significant decrease ($P<0.05$) in percentage of linolenic acid after heat treatment while that of palmitic acid increased significantly ($P<0.05$) with storage time. This trend was due to lipid oxidation in the oil and was in good agreement with that reported by Besbes *et al* (2005) and Chowdhury K. *et al*. (2007).

The significant decrease in percentage of highly unsaturated fatty acids (in this case, linolenic acid) is due to the fact that they are more susceptible to oxidation than other fatty acids. The other fatty acids experienced no significant changes in terms of their percentage. The changes in percentage of individual fatty acids in PNO do not only account for chemical reactions that occur during frying and storage, but also due to the leaching of the oils from French fries to PNO during frying. On the other hand, the fatty acids composition of CNO remained fairly constant throughout the experiment.

In CNO, the relative content of unsaturated fatty acids was small to produce any detectable changes caused by high temperature and storage, and thus did not alter the proportion significantly. High percentage of saturated fatty acids in CNO caused this oil to have higher stability as compared to PNO which has lower percentage of saturated fatty acids but higher unsaturated fatty acids. However, it should be noted that a constant fatty acid composition presented in terms of percentage does not necessary indicate that the concentration of particular fatty acids remain unchanged.

The chromatograms of PNO and CNO at different stages during the study are shown in Figure 1 and 2 respectively. There were noticeable decreases in the peak height and peak area of each component in both oil samples, which may indicates that the oils were degrading and broken down.

This proved that the concentration of each fatty acid was actually decreasing despite the fact the exact reduction was not calculated quantitatively due to certain limitation in this preliminary study. The degradations resulted from oxidation and other reactions such as polymerization that take place during heat treatment and storage.

Table 1. Changes in fatty acids composition in CNO and PNO

Rete-ntion Time (min)	Fatty acids	CNO (%)			PNO (%)		
		Before heat treatment (fresh)	After heat treatment (day 0)	After heat treatment (day 40)	Before heat treatment (fresh)	After heat treatment (day 0)	After heat treatment (day 40)
1.41	Caprylic acid (C8:0)	9.00	±0.76a	10.18	±1.67a	10.55	±1.29a
2.09	Capric acid (10:0)	5.66	±1.35a	7.05	±0.74a	6.23	±0.85a
3.81	Lauric acid (C12:0)	51.31	±2.94a	59.79	±1.99a	48.63	±2.21a
7.44	Myristic acid (C14:0)	18.50	±0.16a	16.69	±2.54a	17.54	±0.38a
11.99	Palmitic acid (C16:0)	7.76 12.31	±0.34a ±0.28a	8.12 13.34	±1.01a ±0.24ab	9.02 13.44	±0.01a ±0.20b
12.55	Palmitoleic acid (C16:1)	0.85	±0.02a	0.08a	±0.06a	0.86	±0.01a
16.60	Stearic acid (C18:0)	2.71 3.61	±0.24a ±0.18a	2.64 3.75	±0.41a ±0.21a	3.03 4.30	±0.14a ±0.06a
17.00	Oleic acid (C18:1)	4.31 75.21	±0.37a ±0.70a	4.09 74.63	±0.43a ±0.72a	4.69 74.12	±0.09a ±0.26a
17.97	Linoleic acid (C18.2)	0.77 7.30	±0.15a ±0.24a	0.42 6.78	±0.00a 0.12a	0.31 6.76	±0.09a ±0.06a
19.37	Linolenic acid (C18:3)	0.74	±0.02a	0.58	±0.02b	0.54	±0.04b

*Each value from the table represents the mean ± standard deviation of duplicate results.
Means within each row for each oil with different superscripts are significantly different (P<0.05).

3. 2. FTIR spectra for fresh oils

FTIR shows a few advantages over classical parameters in the measurements of oil stability. With only a small drop of oil and a couple of minutes to prepare the sample, the infrared spectrum collected can give information on different functional groups present. Also, minimal errors in the determination of frequency and absorbance can be expected since it is fully computerized (Bouaid *et al*, 2007). In the characterization of vegetable oils, the bands of interest are absorption peaks at 3600-2800 and 1800-700 cm^{-1}. The band assignments and their respective mode of vibration are shown in Table 2 and Figure 3.

As clearly indicated in Figure 3, there were visible peaks near 3006 and 1655 cm^{-1} in spectrum of PNO but not in that of CNO. The band near 3006 cm^{-1} was associated to the stretching vibration of *cis* double bonds CH groups while the latter denoted the stretching vibration of carbon-carbon double bonds. The *cis* double bonds CH groups and carbon-carbon double bonds are found widely in unsaturated fatty acids such as oleic acids, linoleic acids and linolenic acids. This correlated well with initial fatty acid profiles (Table 1) which show that PNO contains more unsaturated double bonds than CNO. In fact, there was only approximately

5% of fatty acids in CNO are made of unsaturated double bonds compared to over 80% of that in PNO.

Table 2. FTIR absorbance bands and their characteristic functional groups

Number	Wave numbers (cm^{-1})	Characteristic group and mode of vibration
1	3470	Overtone of –C=O (ester)
2	3006	Stretching of =C–H (cis)
3	2925	Asymmetric stretching of –CH (CH_2)
4	2854	Symmetric stretching of –CH (CH_2)
5	1746	Stretching of –C=O (ester)
6	1655	Stretching of -C-C- (cis)
7	1465	Bending (scissoring) of –C–H (CH_2 and CH_3)
8	1377	Symmetric bending of –C–H (CH_3)
9	1237	Stretching, bending of –C–O, –CH_2
10	1163	Stretching, bending of –C–O, –CH_2 –
11	1099	Stretching of – C–O
12	723	Bending (rocking) of –(CH_2) n–, HC=CH– (cis)

Source: Guillén and Cabo (1997)

3. 3. FTIR spectra for oxidized oils

In PNO, the band near 3006 cm^{-1} experienced a progressive decrease in intensity as well as wave number throughout the period of study (Figure 4). As discussed before, this band is corresponding to the *cis* double-bond (=CH) stretching vibration therefore its intensity is affected by the number of cis double bonds present.

Thus, the changes observed indicate a decrease in the number of cis double-bond, therefore a decrease in degree of unsaturation as a consequence of oxidation process. In contrast, in VCO (Figure 5), the intensity of its band near 3006 cm^{-1} remained fairly constant as only flat lines, not bands were observed throughout the study. However, this did not indicate that no oxidation occurred in VCO. As oxidation proceeds, the concentration of hydroperoxides

increases and this gave a broad band which overlapped with that of the glyceride ester carbonyl groups (Gullén and Cabo, 2004).

The increase in concentration of hydroperoxides can be reflected by increase band absorbance at 3470 cm^{-1}. Anyway, FTIR cannot provide sufficient quantitative data to show oxidative stability of oils. Thus, chemical analysis such as Iodine Value, Peroxide Value, Phenolic content etc need to be carried out to further investigate thermal stability of oils.

3. 4. Iodine Value

IV is a measure of the degree of unsaturation in oil. The initial IV of PNO and CNO was 81.87 ± 0.81 and 11.09 ± 0.69 respectively (Table 1). The values are similar to those differences in initial IV between PNO and CNO are due to the different fatty acids composition. The lower IV in CNO compared to PNO indicates that CNO is far more saturated than CNO. This was in good agreement with the results obtained from GC-FID as well as FTIR spectra at the band near 3006 cm^{-1}. As revealed by statistical analysis of the data, both IVs of PNO and CNO were decreased significantly ($P<0.05$) after 40 days of storage to 72.13 ± 0.77 and 3.26 ± 0.11 respectively.

This reduction in IVs suggests a decrease in number of double bond in the oils. The maximum reductions in IVs of both oils were achieved by the heat treatment as deep frying accelerates the oxidation in the oils (Naz *et al*. 2005). In term of percentage loss, the decrease of IV in PNO was much smaller compared to CNO.

This is because of the higher total phenolic content in PNO. IV does not take into account the structural differences present in different fatty acids such as the nature, quantity and position of the unsaturated bonds in the chain available for oxidation (Bouaid *et al*. 2007). Therefore, IV is not considered as the best way to evaluate the oxidative stability of oil. Other tests must be present to support the results and conclusions that drawn from IV.

3. 5. Total phenolic content

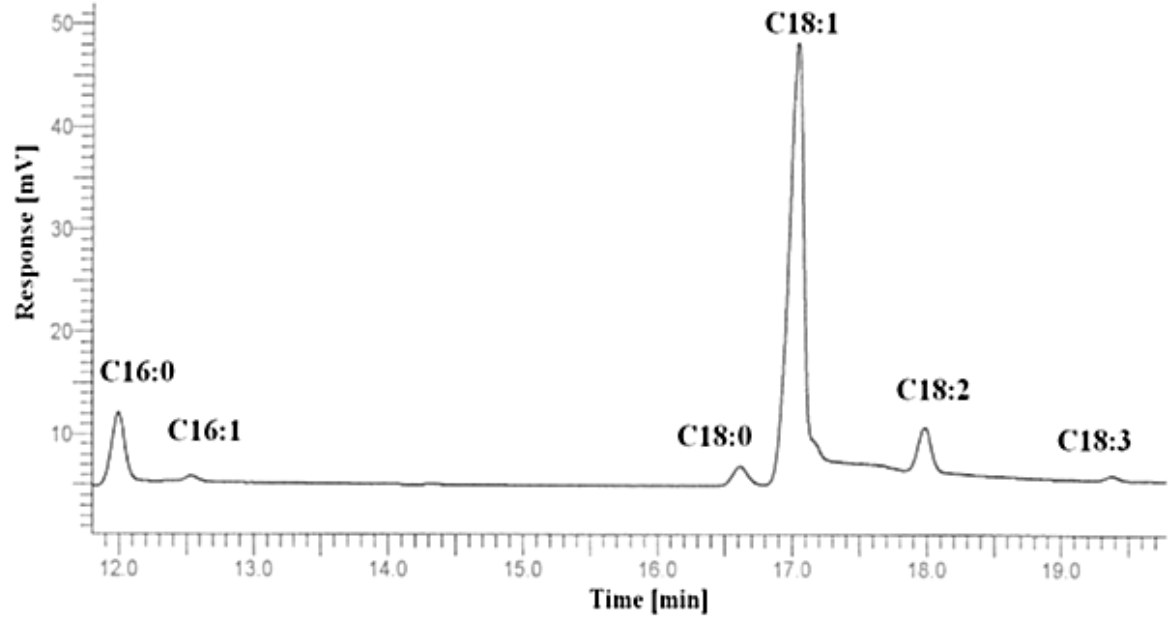
Phenolic compounds are the main factor rendering nutritional importance to cold-pressed oil. The changes in total phenolic content of PNO and CNO are recorded in Table 1. The initial total phenolic content of PNO was 12.95 mg GAE per 100 g of oil. On the other hand, CNO was found to contain 0.65 mg GAE per 100 g of oil. The total phenolic content of PNO decreased significantly from 12.95 in fresh sample to 6.94 in heated sample (0 days) and finally to 4.12 mg GAE per 100 g in the end of the experimentation (40 days after heat treatment).

There were significant changes observed in CNO as well, from initial level of 0.65 to 0.51 after heat treatment (0 day) to a final value of 0.32 mg GAE per 100 g in day 40. The reduction indicates degradation of phenolic compounds in the oil samples and is related to the decrease of oxidative stability since phenolic compounds enhance the total antioxidant activity. In this study, the total phenolic content of fresh EVOO was approximately 20 times higher than that in fresh CNO (12.95 vs. 0.65 mg GAE per 100g of oil).

In the end of the experimentation, the concentration of phenolic compounds in PNO was still approximately 13-fold of that in CNO (4.12 vs. 0.32 mg GAE per 10g of oil). The higher total phenolic content in PNO at all-time throughout the study explains its' better stability than CNO as revealed by relatively lower concentration of oxidative and degradation products as well as smaller percentage loss in IV at the end of experimentation. However, the percentage loss in total phenolic content of PNO was higher than CNO (68.19% vs. 50.77%).

The better retention of phenolic compounds in CNO could be explained by involvement of other antioxidants such as tocopherols and tocotrienols in counteracting oxidation process (Chowhury K. *et al*., 2007), which are not necessary from the family of phenolic compounds. From Iodine Value and Total Phenolic Content results, we found that both oils are stable to certain extent. Thus, we can conclude that CNO is relatively stable as compared to PNO.

A



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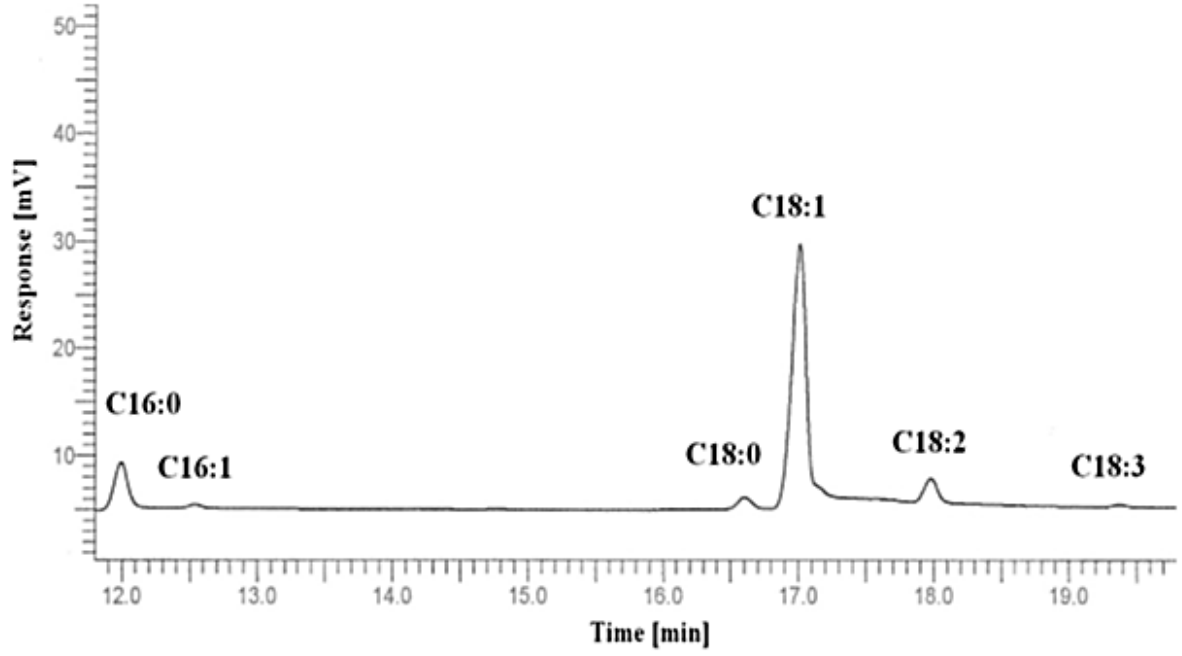

C

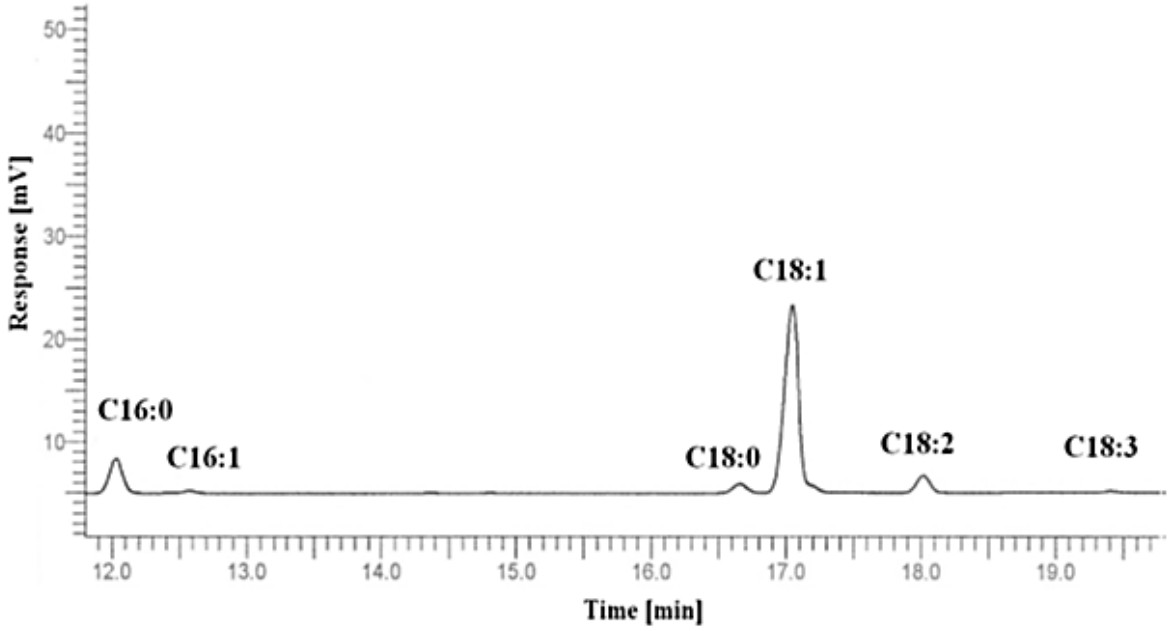

Figure 1, 2 and 3. Chromatograms of PNO (**A**) Fresh; (**B**) After heat treatment at day 0; and (**C**) After heat treatment at day 40.

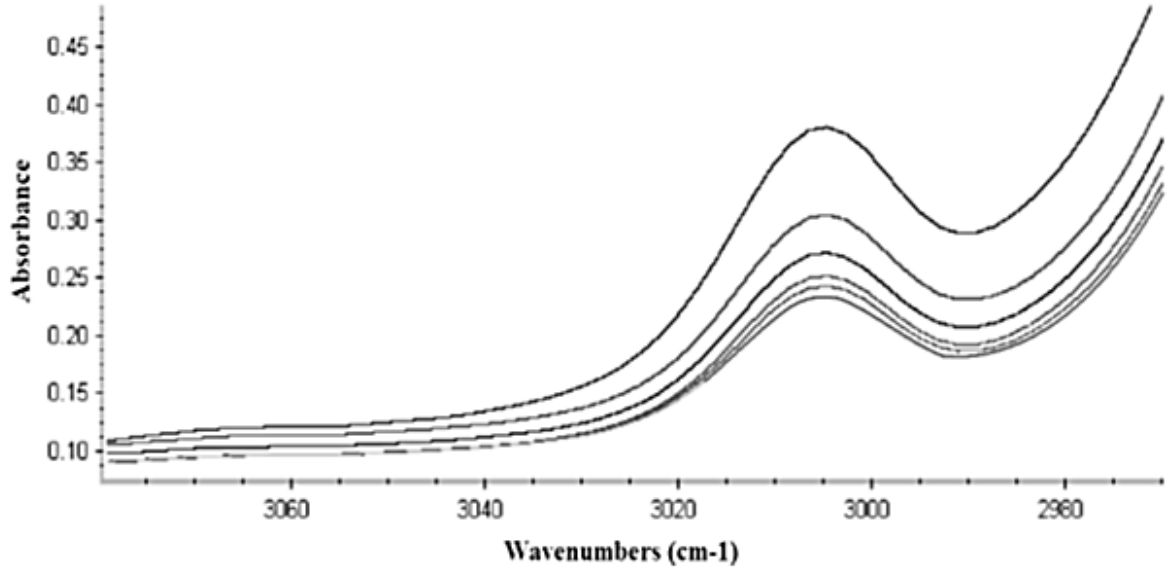

Figure 4. Changes in the region near 3006 cm^{-1} of PNO at different stages of the storage Study. (From top to bottom: raw, storage day 0, 10, 20, 30 and 40)

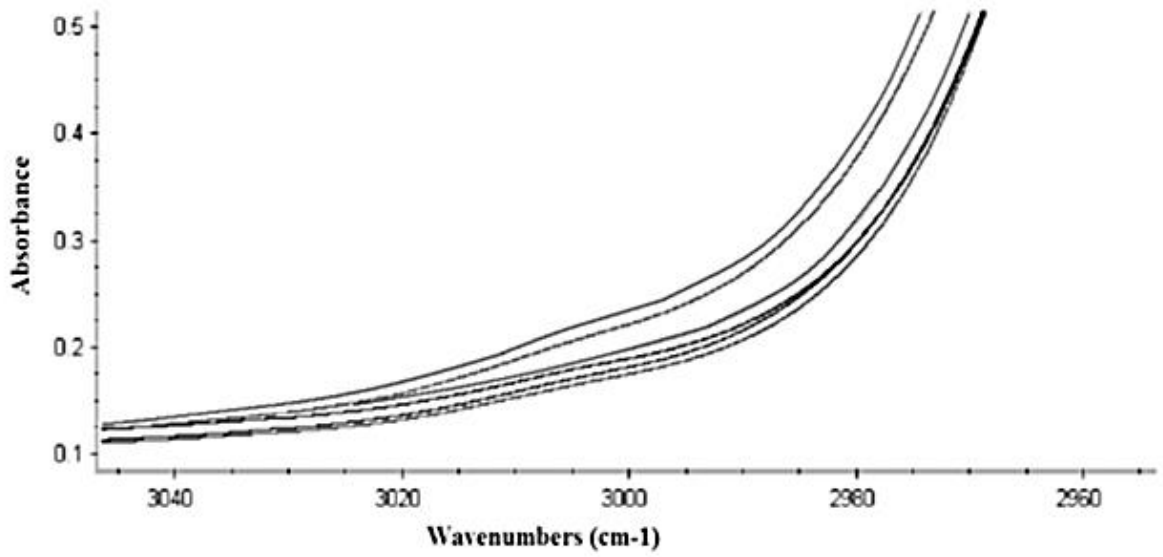

Figure 5. Changes in the region near 3006 cm^{-1} of CNO at different stages of the storage Study.(From top to bottom: raw, storage day 0, 10, 20, 30 and 40).

4. CONCLUSION

Heat has significantly decreased the stability of CNO and PNO. Results from GC showed higher stability in CNO with no significant changes in proportion of each fatty acid was observed throughout the storage. While percentage of palmitic acid and linolenic acid in PNO showed significant decrease upon storage. The occurrence of oils oxidation was proven lower in CNO through FTIR spectra. Besides that, Iodine Value (IV) and Total Phenolic content (TPC) showed reduction of 70.60% in CNO and 11.90% in PNO while reduction of TPC, 50.77% in CNO and 68.19% in PNO. This reduction indicated degradation of phenolic compounds and unsaturated fatty acids in oils after heat treatment and upon storage. Thus, in term of thermal stability, we can conclude that CNO is relatively stable as compared to PNO and is suitable for frying.

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