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Effect of storage conditions on the degradation of roselle (*Hibiscus sabdariffa*) seeds oil

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ABSTRACT

Degradation of edible oil toward rancidity is wasteful and/or health challenging. In this study, oil extracted from Roselle seeds was analyzed for elemental and microbial content. The oil under different conditions of storage (lightened L, dark air-free AF and dark air-tight/covered ATD conditions) were progressively studied for 16 weeks to investigate the changes in the physicochemical properties signaling deterioration. Fourier transformed infrared (FTIR) analysis before and after the storage period were carried out to investigate the changes in the functionality of the oils. Elemental analysis presented Fe, Ca. Zn, K and Na in 159.615 mg/l, 370.10 mg/l, 27.918 mg/l 600 mg/l and 170 mg/l respectively; phytochemical analysis presented saponins only while microbial analysis indicated the presence of bacteria and fungi. The changes in physicochemical properties and the FTIR spectra over the storage period revealed different rates and fashions of deterioration. From the study, the conditional degradation of Roselle seed oil was least severe in ATD compared with other typical storage conditions. The oil will therefore be expected to have the longest shelf-life in ATD.

Keywords: Roselle seed, oil, storage conditions, degradation/deterioration.

INTRODUCTION

Roselle (*Hibiscus sabdariffa L.*) is a plant that is indigenous to the tropics. It belongs to the Malvaceae family and has various local names as documented in a work reported by Schippers (2000). In Nigeria, it is known as Isapa pupa in the South Western part of the country, Zobo in the Northern part, and it's majorly used in brewing (Adejumo, 2003). The seed has been found to be a source of highly valued vegetable oil with properties similar to that of crude olive oil (Atta and Imaizumi, 2002). There is an early report of the extraction and use of Roselle seed oil in China, in Sudan, the oil is consumed and also used in the production of poultry feeds (Al-Wandawi et al., 1984). In many part of the world including Nigeria, Roselle seeds are being wasted as there is no such value addition as the established or large scale extraction of its oil. The major importance of vegetable oils lies in their nutritional value. In recent years vegetable sources have accounted for about three fifths of the world's oil consumption, while the rest are obtained from animal fats and marine oils (Nkafamiya et al., 2007a). Edible oils are consumed in various ways, in cooking, as spread, and the main

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demand for them comes from the margarine industries (Nkafamiya et al., 2007b).

Lipids are nonpolar compounds. They are mostly esters of long chain fatty acids, alcohols and closely related derivatives. Fats and oils are formed by the reaction between glycerol and fatty acid and the unreacted fatty acids in the medium are referred to as free fatty acid (FFA) (Nkafamiya et al., 2007b). In the metabolism of lipids, it is broken down to glycerol and fatty acids and energy is generated from the fatty acids. Other chemical compounds are increasingly being identified in oil, and these compounds have been scientifically proved to be responsible for important bioactivities (Akinpelu et al., 2016), this has therefore widen the purpose of its consumption beyond food.

To explore the potential economic development of traditional oils, it is important to study their physical and chemical properties and obtain information providing preliminary insight on the qualities, resistance to rancidity, and prospective industrial characteristics (Boahen et al., 2013). Several factors affect the stability of oil and these include the nature of the oil, storage and processing condition among others. During the period of storage or/and usage under several conditions especially in food processing, complex series of reactions that generates a wide spectrum of new components that may have important physiological effects are produced (Soriguer et al., 2003).

Recommending the direct or indirect consumption of the oil from our locally obtained Roselle seed therefore necessitates the investigations into the oil's deterioration while stored under some typical conditions. This is expected to suggest the appropriate storage condition and time with which the oil can still be satisfactorily safe for consumption.

MATERIALS AND METHODS

Collection and preparation of samples

Dried seeds Roselle seeds were purchased from Jimeta modern market, Yola Adamawa State of Nigeria. They were cleaned to remove dirt, sun-dried for three days, grounded and sieved to obtain uniform particle size.

Extraction of oil

The grinded sample (40 g) was weighed and rapped in a filter paper. It was then placed in the thimble of the soxhlet chamber. The soxhlet chamber was filled with 250 ml of diethylether and the set up was heated at 60 ºC for 4 h to extract the oil. After the extraction, the trace of diethylether in the oil sample was evaporated (Inuwa et al., 2011). The oil samples were then collected in different containers (bijou bottles) and stored under different conditions and analyzed after every two weeks.

Determination of mineral composition

Oil sample (2 g) was mixed with 20 ml of nitric/per chloric acid. The mixture was allowed to stand overnight and then heated at 80 ^{o}C on a hot plate for approximately 2-3h until a clear solution was obtained. The clear solution was heated to dryness and reconstituted with deionized water. The concentration of iron, zinc and calcium in the solution obtained was determined using atomic absorption spectrophotometer, while sodium and potassium was determined by flame emission technique (AOAC, 1995).

Physicochemical properties of oil

The standard method by the America Oil Chemist Society (AOCS) as described by Nkafamiya et al. (2007a) was adopted in the determination of peroxide value (PV), iodine value (IV), free fatty acids (FFA), saponification value (SV) and refractive index (RI). These analyses were carried out every two weeks for the oil samples left under different conditions for sixteen weeks. These conditions include; exposure to day light (lightened L), shielded (dark air-free AF) and covered (dark air-tight/covered ATD). Averages of triple determinations were recorded.

Test for saponins

Oil sample (10 ml) was mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil. The formation of emulsion indicates the presence of saponins (Mir et al., 2013).

Test for phenols

To the oil sample, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds (Kumburawa et al., 2007).

Test for flavonoids

A few drop of 1% NH_3 solution was added to each oil sample in a test tube. A yellow coloration indicates the presence of flavonoids compound (Mir et al., 2013).

Test for alkaloids

To 3 ml of oil sample in a test tube, 1 ml of 1% HCl was added. The mixture was heated for 20 minutes cooled and filtered. About 2 drops of Mayer's reagent was added to 1 ml of the filtrate. A creamy precipitate indicates the presence of alkaloids (Idris et al., 2015).

Microbial count

The microbial content was analyzed according to Verla et al. (2014). The oil sample was serially diluted and 1.0 ml of 1 x 10^3 dilutions was used for inoculation in triplicate. Bacterial count was determined using nutrient agar (NA) and MacConey agar (MCA) while potato dextrose agar (PDA) was used for fungal count. All NA and MCA plates were incubated at 37 ^oC for 24 hrs while PDA plates were incubated at room temperature for 2-5 days. Plate count was carried out using; Gallenkamp digital colony chamber of microbial load as colony forming units per ml (cfu/ml) of the sample was calculated.

Statistical analysis

Statistical packages for social sciences (SPSS) were used to arrive at the mean, standard deviation and correlation.

RESULTS

The mineral composition of the oil is presented in Table 1. The oil contains calcium (Ca), iron (Fe), zinc (Zn) phosphorus (P), potassium (K) and sodium (Na). Of these potassium is highest (600 mg/l), while zinc is lowest (27 mg/l). The phytochemical composition of the Roselle seed oil is presented in Table 2. Saponins alone were identified to be present in the oil. The microbial count for the oil samples before and after the storage is presented in Table 3. The data showed that the oil samples contain lower density of bacterial and fungal when fresh. The data also further shows an increase in the number of count with storage time.

The changes and the rate of changes in the physicochemical properties of oil will

present a reliable study on its degradation. The iodine value for the oil under study at different conditions over the period of sixteen (16) weeks was presented in Figure 1. The IV for the oil decrease with the time of storage under all conditions. However, the decrease is slightly more rapid for the oil in L than AF and its relatively stable in ATD. The results for the refractive index for the oils under this study were presented in Figure 2. The RI value for the oils stored under light (L) is the highest and value kept increasing with the storage time. Similar phenomenon was reported by Arya et al. (1969) and Abdellah and Ishag (2012). The results of peroxide value with the period of storage for the oils under study are presented in Figure 3. The results under ATD are the lowest and most stable with the storage time. For the sample under light (L) and the Shielded (AF), result showed that the rate of deterioration of the oil may be very rapid starting from the 6^{th} week and may reach its peak 10 – 12 week, after which an equilibrium may be attained. The free fatty acid of the oil is presented in Figure 4. The FFA of the oil increases with the period of storage. The increase in FFA suggested that the reactions leading to the formation of FFA are progressive (Nkafamiya et al., 2007a). These reactions are typically favored in the presence of oxygen (in air) and/or the ultraviolet rays in the ambient light (Ngassapa et al., 2016), hence faster rate shown for the oil under L and AF. The variation of saponification values of the oil with period of storage is presented in Figure 5. Oil sample under ATD still show the lowest saponification values and the best stability over the storage period. Under the lightened (L) and shielded (AF) conditions, the SV goes through a sharp rise starting from the 2^{nd} till the 8^{th} week of storage followed by equilibrium and a steady rise respectively. This illustrates the fashion and rate at which the average molecular weight of the fatty acids from the triglyceride changes.

The FTIR spectra of the Roselle seed *(Hibiscus sabfarifa)* oil before storage is presented in Figure 6. The spectra showed peaks in the region 3300-3400 cm^{-1} suggesting hydroxyl group stretching, 2850-3000 cm^{-1} suggesting the presence of =C-H stretching and (1640-1670 cm^{-1}) suggesting the presence of C=C stretching. The spectra of Roselle seeds (*Hibiscus sabdarifa*) oil after storage under the three conditions is presented in Figure 7. The spectra of all the oil samples stored under the different conditions show the new peaks within the region 1740-1755 cm^{-1} suggesting the presence of carbonyl group C=O stretching (ketones) which are products of the oxidation process in the oil. The spectra of the oil sample under ATD shows the narrowest peak within this region; hence, the oil under ATD is with the lowest amount of the oxidation product.

Table 1: Mineral composition of the oil samples.

(mg/l)	Roselle seed oil
Fe	159.62 ± 0.17
Ca	370.10 ± 0.11
Zn	27.92 ± 0.13
K	600.00 ± 0.25
Na	170.00 ± 0.03

Table 2: Phytochemicals content of the oil samples.

Phytochemicals	Roselle Oil
Alkaloids	-
Phenols	-
Resins	-
Saponin	+
Flavonoid	-

Table 3: Microbial counts (cfu/ml) of the oil samples with time of storage.

	Roselle seeds oil		
Time (weeks)	0	5	10
TBC (Nutrient agar)	0.14×10^3	0.46×10^3	0.96×10^3
TCC (MacConkey agar)	0.24×10^3	0.52×10^3	1.14×10^3
TFC (Potato dextrose agar)	0.22×10^3	0.54×10^3	1.18×10^3

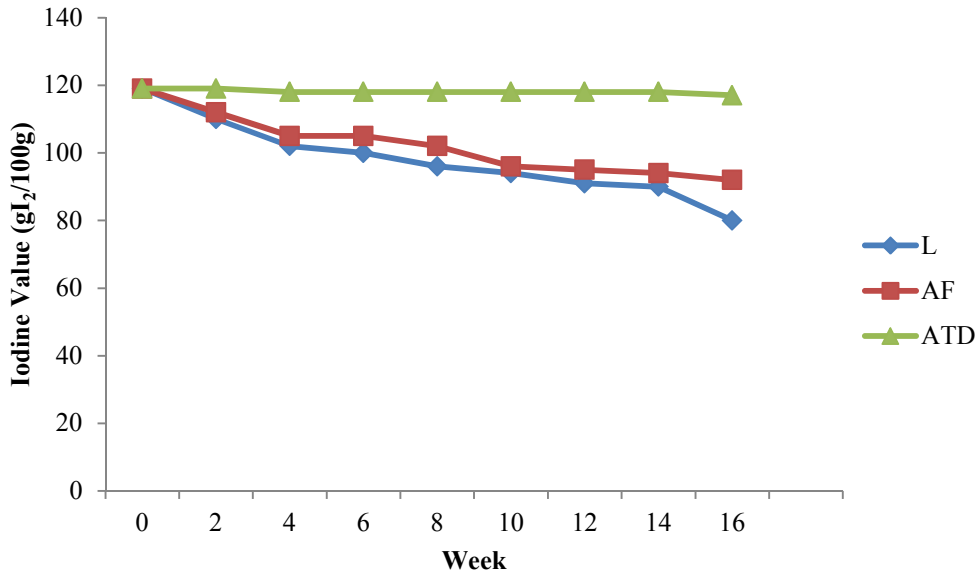

Figure 1: Variation of the iodine value of the oil samples with time of storage.

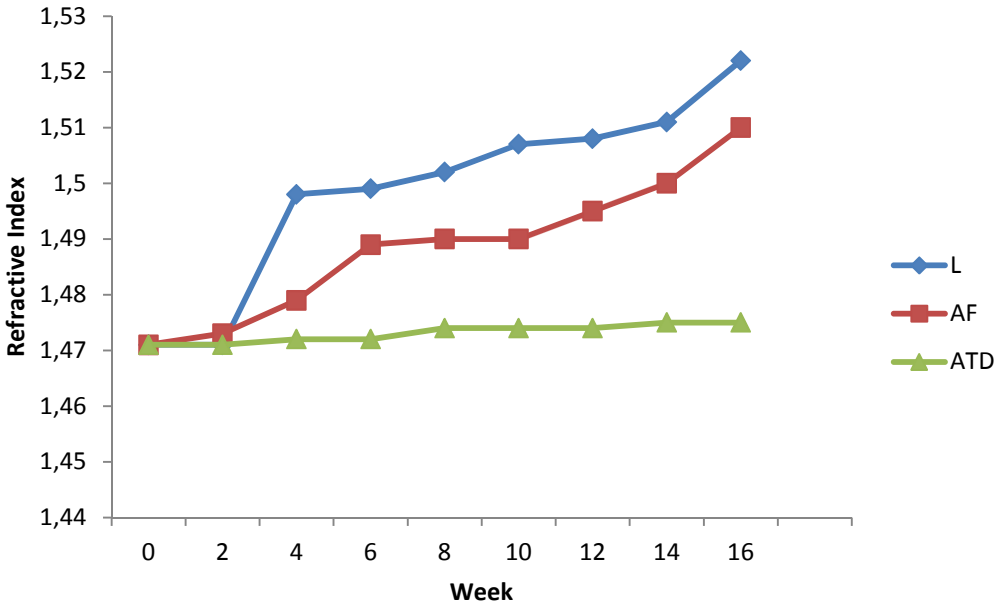

Figure 2: Variation of refractive index of the oil samples with time of storage.

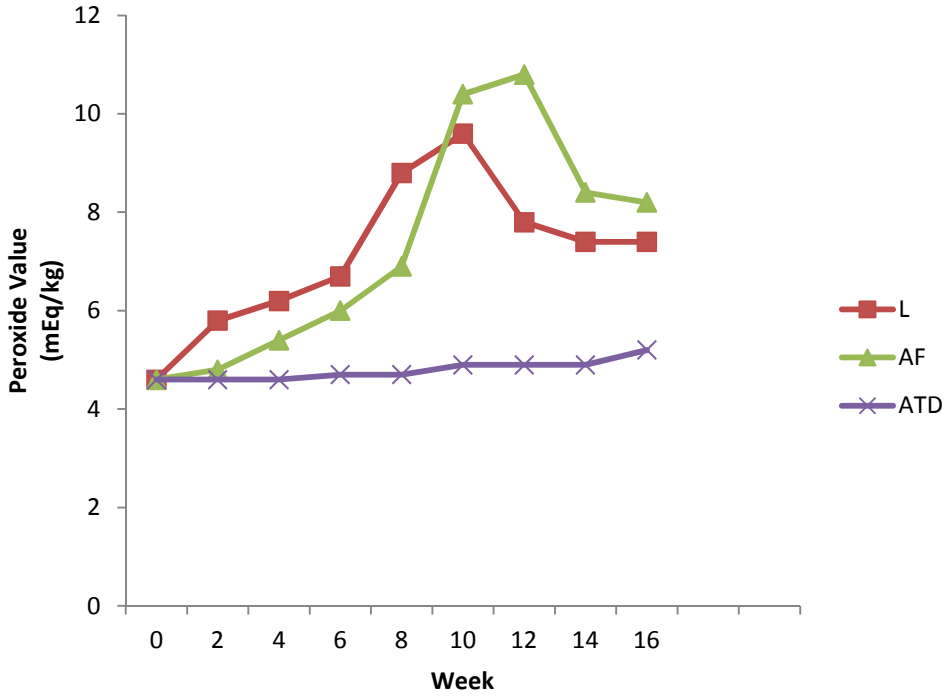

Figure 3: Variation peroxide values of the oil samples with storage time.

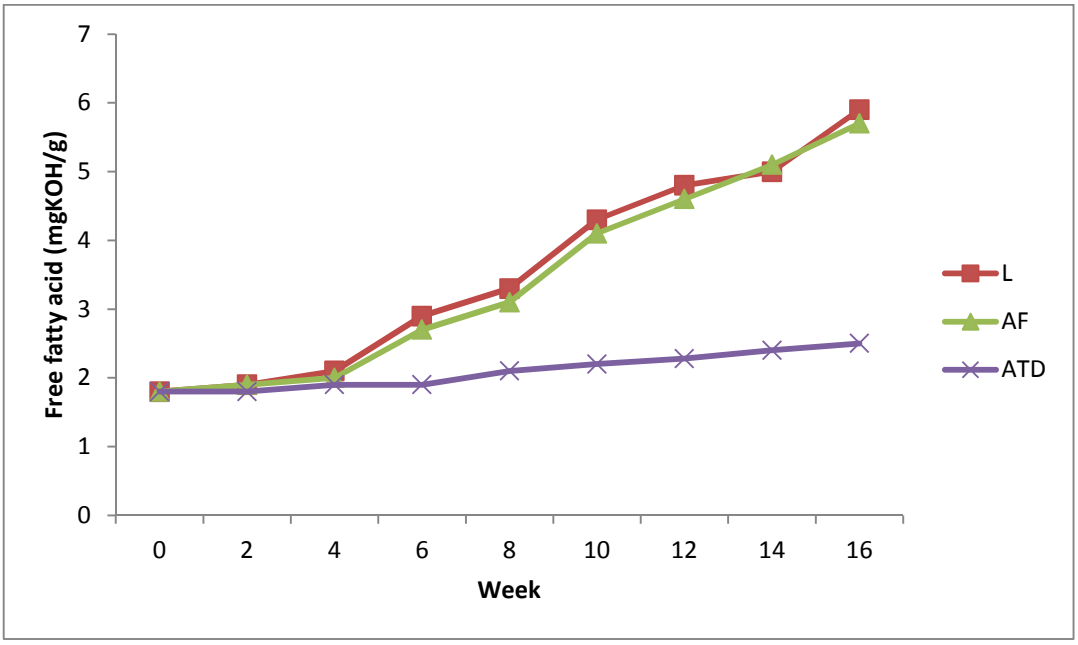

Figure 4: Variation of FFA of the oil samples with storage time.

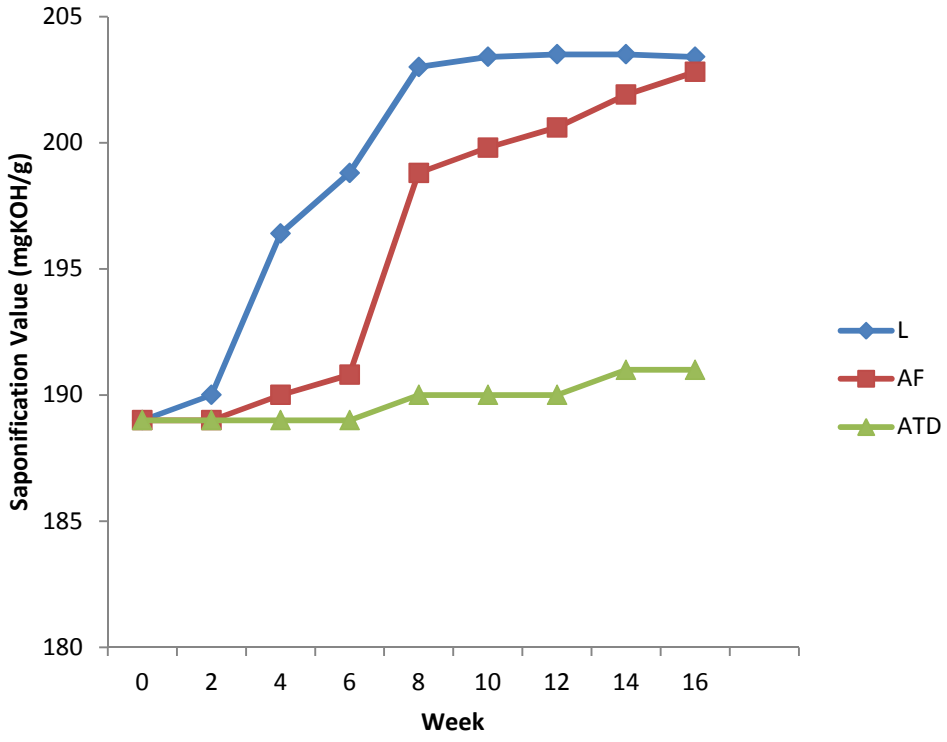

Figure 5: Variation of saponification value of the oil samples with time of storage.

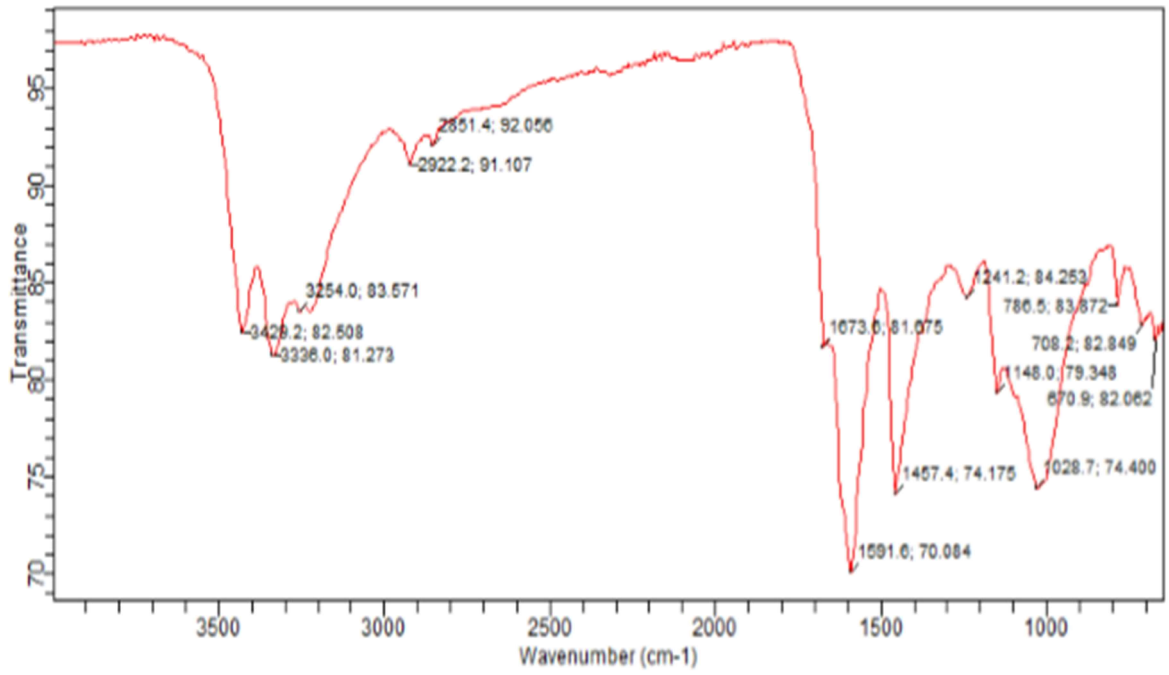

Figure 6: IR spectra of Roselle seeds oil before storage.

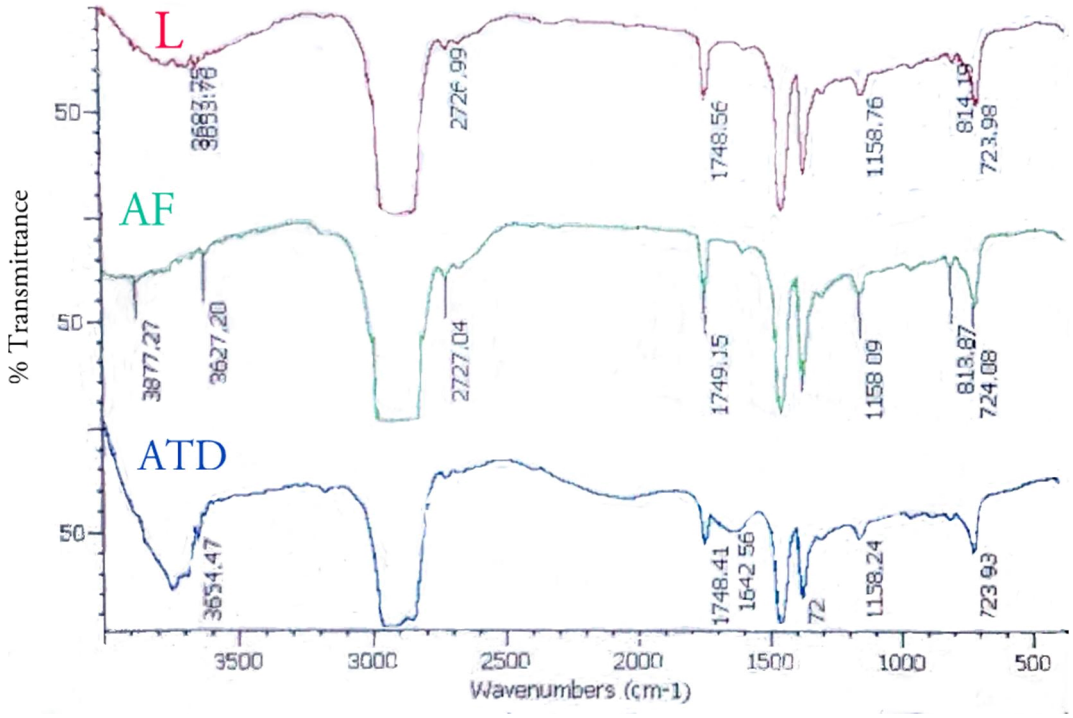

Figure 7: IR spectra of Roselle seeds oil after storage.

DISCUSSION

Potassium (600 mg/l), calcium (370 mg/l) and sodium (170 mg/l) are major elements, while iron (159.62 mg/l) and znic (27.92 mg/l) are essential elements in the human body. The presence and proportion of these elements in Rosselle seeds oil suggests that Roselle seeds oil could contribute in the required intake of these elements (Nkafamiya et al., 2007a). The presence of saponins alone in the oil may select the oil for some pharmaceutical applications, as saponins are well-known for antioxidant, antimicrobial and astringent activities (Nwokonkwo, 2015). The increasing number of count with storage time obtained from the microbial count can be attributed to reproduction of the microorganism. The high numbers of count in the tenth week of storage suggest that microorganism enhances degradation of the oil samples (Verla et al., 2014).

The decrease in iodine value shows that the level of unsaturation in the fatty acid is reducing (Boahen et al., 2013), and this can be associated with lipid oxidation at the points of unsaturation i.e. at the double bonds (Nwabueze and Okocha, 2008; Akinterinwa et al., 2015). Since the decrease is most rapid in oil exposed to light, it added that the ultraviolet ray in the light may be enhancing the suspected oxidation by molecular oxygen in the air in the case of AF. High and increasing refractive index in L may be due to an enhanced photocatalysed oxidation (Wsowicz et al., 2004). The increase in the RI value may also be as a result of production of oxidation product as the oil tends towards rancidity (Nkafamiya et al., 2007b). Peroxides are intermediates in the oxidation reaction leading to the deterioration of lipids; therefore, high peroxide value is an indication of oil's susceptibility to rancidity (Belewu et al., 2010; Akinterinwa et al., 2015). The fashion of the peroxide value may signal the fashion of deterioration. The low and relatively stable values obtained in ATD presented it to be the best storage condition for Roselle seed oil. The increasing formation of free fatty acid signals the auto-breakdown of the triglyceride in lipids thereby destabilizing its structure and hence, degradation. The high and significantly increasing saponification value of the oil samples under L and AF indicates the transitional change in molecular weight and molecular weight distribution in the oil. It also shows hindered hydrolysis reactions among the possible reactions leading to the deterioration of the oil under L and AF (Nkafamiya et al., 2007b; Akinterinwa et al., 2015).

Carbonyl compounds such as aldehydes and ketones are products of the autoxidation of lipids (Wsowicz et al., 2004; Choe and Min, 2009). The emergence of the carbonyl peaks attributable to carbonyl compound (ketones) on the spectra of the oil samples after the 16 weeks storage period confirms the change in the functionality of the oil towards rancidity. The area under infrared spectra peaks is a relative measure of specie concentrations. The smaller area under the carbonyl peaks for ATD oil sample indicates that it contains the least amount of autoxidation products.

Conclusion

Studies on the composition and deterioration process of novel oils are essential to safely predetermine storage condition and time as well as consumption viability. This study has presented interesting data on the elemental, phytochemical and microbial content of Roselle seed oil. Possible effects of these content and environmental factors on the deterioration of the oil towards rancidity over sixteen weeks storage period was also studied under three typical conditions. Roselle seed oil (with appropriate refining) can hereby be presented as a source

of nutritional minerals, and its most appropriate storage can be advised to be under a dark air-tight condition.

COMPETING INTERESTS

There is no competing interest among the authors.

AUTHORS' CONTRIBUTIONS

All authors contribute satisfactorily in the work and in this report.

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