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Extraction and characterization of groundnut oil: effect of different storage conditions on its lipid oxidation and physicochemical properties

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ABSTRACT

The effect of sunlight on the quality of groundnut oil in comparison with the same oil samples stored in the dark at room temperature was aimed to study. The oil samples were exposed to sunlight (34-40°C) and ambient storage (~24°C in the dark) for 90 days. The samples were collected every 30 days for the analysis of their physicochemical properties including peroxide value (PV), p-anisidine (p-AnV), total oxidation (TOTOX), thiobarbituric acid (TBA), free fatty acid (FFA), color, induction period on Rancimat and fatty acid composition. At the beginning of the experiment, the PV, p-AnV, TOTOX, TBA and FFA values of groundnut oil were 10.31 meq O_2/kg, 4.39, 25.01, 0.99 and 1.49% respectively; color 1.40R, 22.00Y and induction time (IT) 5.33 h. After 90 days storage at room temperature, the PV, p-An-V, TOTOX, TBA and FFA values were respectively ranged between 10.31-21.32 meq O_2/kg, 4.39-7.36, 25.01-50.12, 0.99-2.42 ppm and 1.49-2.64%; the color in the red and yellow unit 1.40-1.20R and 22.00-19.00Y respectively; and the induction time 5.33-3.77 h. However, the same parameters at the end of exposure to sunlight were ranged in the same order, between 10.31-43.47 meq O_2/kg, 4.39-22.24, 25.01-109.18, 0.99-3.25 ppm and 1.49-5.83% respectively; the color in the red and yellow units 1.40-0.50R and 22.00-1.30Y respectively; and the induction time 5.33-0.03 h. Results also showed that the amount of linoleic and gadoleic acids significantly dropped under sunlight compared to dark at room temperature. Sunlight significantly reduced the quality of groundnut oil. This oil should be stored in the dark at room temperature.

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1. Introduction

Vegetable oils and fats represent one of the major ingredients of people diet. Amongst these lipids, those containing a good proportion of polyunsaturated fatty acids are gradually recommended due to their beneficial effects on human health, as they have been proven to be efficient in the prevention of cardiovascular diseases. This activity is commonly attributed to the presence of Omega (W3) and omega (W6) fatty acids (1) among which some are essential. In addition to their health benefits, lipids are good sources of energy and fat soluble vitamins. Amongst the sources of polyunsaturated oil, there is groundnut oil which contains 78% of unsaturated fatty acids amongst which 32% of them are polyunsaturated (2). However, oils containing a good proportion of polyunsaturated acids are chemically unstable due to their sensitivity to oxidation reactions (3). Lipid oxidation is the principal alteration of oils and fats. It generally results in the reduction of the nutritional value of food as well as the

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changes in color, texture, taste, flavor and other physiological properties (4). It also leads in food to the production of free radicals and reactive oxygen species which have been demonstrated as implicated in several disorders such as cancers, mutations, and cardiovascular diseases (5). These alterations lead to the unacceptability of the food by the consumer and industries suffering from economic losses (6). Three different mechanisms of lipid oxidation are known: auto-oxidation, photo-oxidation and enzymatic oxidation. Amongst these, auto-oxidation has been deeply explored while others are still neglected. Photo-oxidation reactions are the most dangerous of them due to the fact that it is a very fast reaction; which is at least 1000-1500 times faster than auto and enzymatic oxidations, and which leads to the formation of hydro-peroxides without passing through the formation of radicals (7). In local markets in developing countries, fats and oils packed in translucent polyethylene bottles are intentionally stored outside by shop keepers and whole-sellers until they are sold. The objective of keeping the oil outside for the whole day is generally for advertising purposes or because of the lack of space in the shop. Such lack of control in practice for storage and shipping of vegetable oils and fats facilitates the photo-oxidation. The repetition of this practice can lead to a total destruction of the oil, which now represents a danger for consumers (8). Similar studies have already been conducted before, and mostly on refined oils. Very few data are available on the effect of sunlight on crude unsaturated oils, as in rural area crude oils are extracted and sent directly to the market for commercialization. The quality of such oils can significantly be affected by the storage conditions especially under sunlight. The objective of this study was to follow the physicochemical changes in groundnut oil stored in the dark at room temperature and under sunlight.

2. Materials and methods

Fresh groundnut seeds (Arachis hypogaea) were purchased at Muea central market, Buea South-West Region, Cameroon, in March 2018. All chemicals, solvents used in this study were of analytical grade and were purchased from Sd. Fine Chemicals, HiMedia Laboratories Pvt. Ltd, Mumbai, India and Sigma-Aldrich, St. Louis. Standard antioxidants and fatty acid methyl esters (C4–C24) were procured from Sigma-Aldrich.

2.1 Oil Extraction

This method was used for oil extraction from dried groundnut seeds as described by Womeni et al. (2016) (9). Groundnuts were ground and 800 g of sample was macerated in 2000 ml of hexane at room temperature during 48h, while shaking constantly. Then, the mixture was filtered using the whatman paper N°1, and the filtrate was concentrated in a rotatory evaporator at 45°C. The extracted oils were stored in the refrigerator at 4°C for further analysis.

2.2. Sample preparation and storage

Groundnut oil was respectively introduced into eight identical translucent glass bottles of 60 ml volume. The first set of three bottles was stored in the dark at room temperature for a period of 90 days, while the second set (three bottles) was exposed to sunlight for the same period (8 h exposure/day). The last set of two bottles served for initial characterization of the oil. The oil samples were exposed under sunlight from March 21st 2018 to June 21st 2018. The mean values for the average temperature (°C) taken during these months was 30.4±5.51. At the monthly intervals, one bottle of each set was collected, and the oil directly analyzed by measuring changes in quality indexes, induction time and color.

2.3. Physicochemical analysis of oil samples

2.3.1. Color Measurement

The change in color of groundnut oil samples during the storage was measured using a 1- inch cell on a Lovibond Tintometer according to the AOCS Cc 13e-92 (2003).

2.3.2. Measurement of oxidation parameters

Peroxide value was measured spectrophotometrically by IDF standard method, 74A: 1991 (IDF, 1991). Free fatty acid values and P-anisidine assays were conducted according to AOCS Official Method CD 18-90 and CD 1-25 respectively (AOCS, 2003). Total oxidation (TOTOX) values were calculated by TOTOX=2PV+AnV according to Shahidi and Wanasundara (10). Thiobarbituric acid value was evaluated as described by Draper and Hadley (11).

2.3.3. Changes in the induction time during the storage

Induction times of palm olein samples were determined by an automated Metrohm Rancimat instrument (Model 892). Each oil sample (≥ 5 g) was

separately weighed in a Rancimat test tube. After reaching the temperature of the instrument to 110°C, the measuring vessels were filled with 60 ml deionized water and connected to the instrument via electrodes. The reaction tubes were individually placed in their respective heating blocks and the reaction started after starting the gas flow (20 l/h). Induction time, the time elapsed from the beginning until the oil starts to become rancid, was automatically recorded by the instrument.

2.3.4. Changes in fatty acid composition

2.3.4.1. Fatty acid methyl esters preparation

Fatty acid methyl esters (FAMEs) of samples were prepared by transesterification using 2% sulfuric acid in methanol described by Christie, 1993. The FAMEs were extracted into ethyl acetate and thoroughly washed with water to make them free of acid, and dried over anhydrous sodium sulfate. The dried esters were analyzed in a gas-chromatograph using a flame ionization detector (GC/FID).

2.3.4.2. Gas chromatography

The analysis of the fatty acid methyl esters was conducted on an Agilent gas chromatograph (Agilent Technologies, Palo Alto, CA, USA, N° of series 7890A), using a flame ionization detector, and a DB-225 capillary column (30 m x 0.25 µm of film thickness). Initially, the column temperature was maintained at 160°C for 2 min. Then, it increased to 220°C (5°C/min) and was finally maintained at 220°C for 10 min. The mobile phase was nitrogen, and its flow rate was 1.5 ml/min. The temperature of the detector and injector were 250 and 230°C respectively. The fatty acids were identified by comparing their retention times to that of standards fatty acid methyl esters.

2.4. Statistical analysis

Analysis was done in triplicate. Data were subjected to one-way analysis of variance (ANOVA) with Dunnet and Student-Newman-Keuls tests using Graphpad-InStat version 3.05, to evaluate the statistical significance of the data. A probability value at $p<0.05$ was statistically significant.

3. Results

3.1. Changes in peroxide value

The changes in peroxide value of groundnut oil samples stored under sunlight and in the dark at room temperature are presented in figure 1. It is clearly observed that oil samples exposed to sunlight have exhibited significantly higher ($p<0.001$) peroxide values (10.31 to 43.47 meq O_2/kg from day 0 to 90 respectively) compared to the same samples stored in the dark at room temperature (10.31 to 21.32 meq O_2/kg from days 0 to 90 respectively).

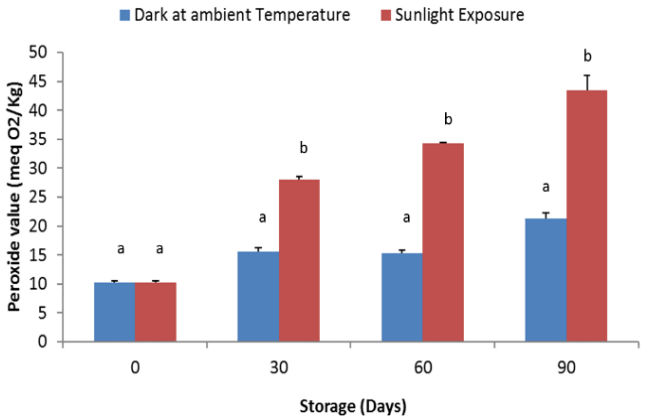

Figure 1. Changes in peroxide values of groundnut oil samples stored in the dark at room temperature and exposed under sunlight.
[a-b] for a specific day, values with different superscripts differ significantly at $p<0.05$.

3.2. P-Anisidine value

Figure 2 shows the changes in p-Anisidine values of groundnut oil samples under different storage conditions. As previously observed with peroxide value oil samples exposed to sunlight have exhibited significantly higher ($p<0.001$) p-Anisidine values (4.49 to 22.24 from day 0 to 90 respectively) compared to the samples stored in the dark at room temperature (4.49 to 7.36 from day 0 to 90 respectively).

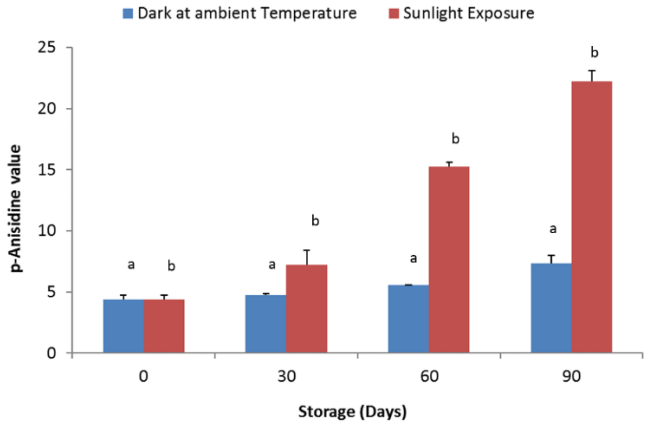

Figure 2. Changes in p-Anisidine values of groundnut oil samples stored in the dark at room temperature and exposed under sunlight.
[a-b] for a specific day, values with different superscripts differ significantly at $p<0.05$.

3.3. Total oxidation value

The evolution of the total oxidation value of groundnut oil samples during storage in the dark at room temperature and under sunlight is illustrated in figure 3. Oil samples exposed to sunlight have exhibited the highest ($p<0.001$) TOTOX value (25.01 to 109.18 from day 0 to 90 respectively) compared to those stored in the dark at room temperature (25.01 to 50.12 from day 0 to 90 respectively) during the entire storage period.

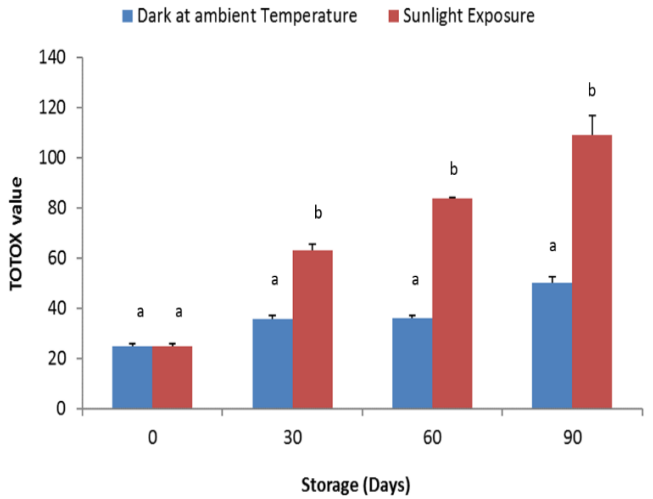

Figure 3. Changes in total oxidation value of groundnut oil samples stored in the dark at room temperature and exposed under sunlight. [a-b] for a specific day, values with different superscripts differ significantly at $p<0.05$.

3.4. Thiobarbituric acid value

The changes in TBA value of groundnut oil samples under different storage conditions are illustrated in figure 4. As previously observed with other parameters, the TBA value of oil samples exposed to sunlight was significantly higher ($p< 0.001$) than that of the same oil kept in the dark at room temperature.

3.5. Free fatty acid content

The variation the free fatty acid content of groundnut oil samples under different storage conditions is presented in figure 5. Groundnut oil of decrement was significantly higher ($p<0.001$) in oil samples exposed to sunlight. It is important to note that on the 90th day, oil samples stored under sunlight had an induction time of almost 0.

3.6. Induction time

The changes in induction time of groundnut oil samples exposed to sunlight in comparison to samples stored in the dark at room temperature are presented in figure 6. A significant decrease ($p<0.05$) in induction time was recorded in all oil samples. However, the rate

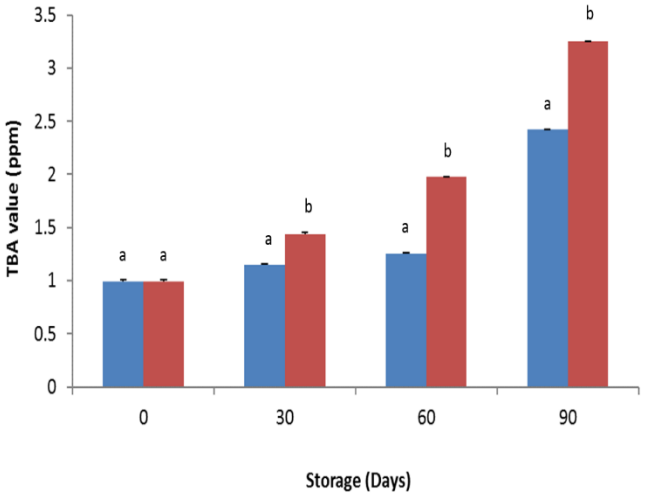

Figure 4. Changes in TBA values of groundnut oil samples stored in the dark at room temperature and exposed under sunlight. [a-b] for a specific day, values with different superscripts differ significantly at $p<0.05$.

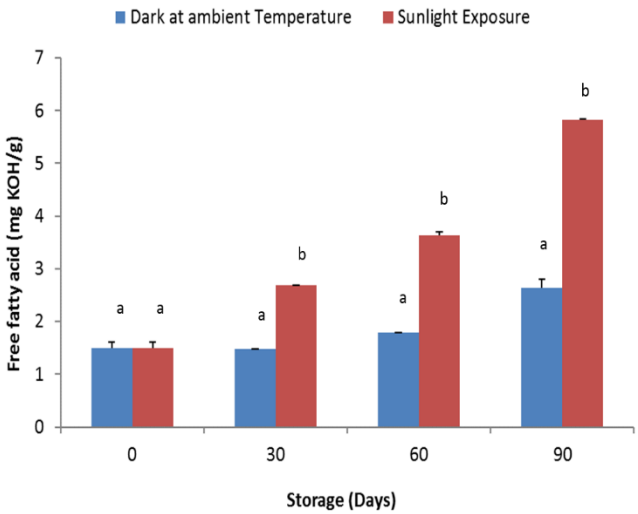

Figure 5. Changes in free fatty acid values of groundnut oil samples stored in the dark at room temperature and exposed under sunlight. [a-b] for a specific day, values with different superscripts differ significantly at $p<0.05$.

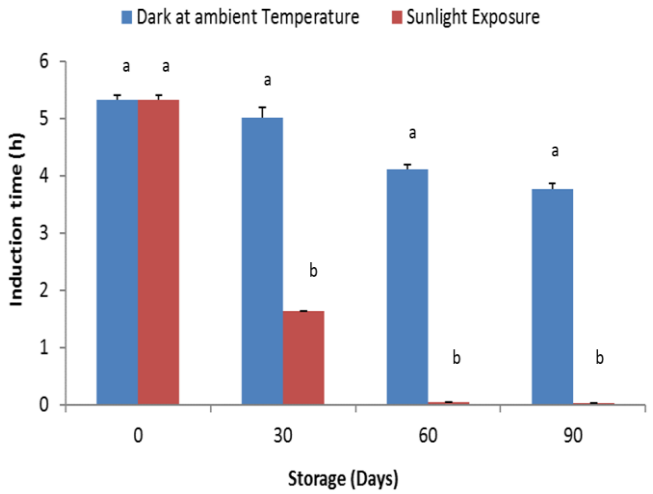

Figure 6. Changes in induction time of groundnut oil samples stored in the dark at room temperature and exposed under sunlight.
[a-b] for a specific day, values with different superscripts differ significantly at $p<0.05$.

3.7. Changes in color

Table 1 shows the changes in color of groundnut oil samples in the red and yellow units during the storage under sunlight and in the dark at room temperature. Initially, the oil color was 1.40±0.00 and 22.00±0.00 in the red and yellow units respectively. After 90 days of storage, these values decreased to 1.20±0.00 and 19.00±0.00 for the red and yellow units respectively in oil samples kept in the dark at room temperature. However, on the same day the color in the red and yellow units for oil samples stored under sunlight were 0.50±0.00 and 1.30±0.00 respectively.

Table 1. Changes in color of oil samples during storages

Storage time(day)	Ambient (dark)		Sunlight	
	Red unit	Yellow unit	Red unit	Yellow unit
0	$1.40\pm0.00^{a}{}_{A}$	$22.00\pm0.00^{a}{}_{A}$	$1.40\pm0.00^{a}{}_{A}$	$22.00\pm0.00^{a}{}_{A}$
30	$1.50\pm0.00^{a}{}_{B}$	$20.00\pm0.00^{a}{}_{AB}$	$0.90\pm0.00^{b}{}_{B}$	$4.60\pm0.00^{b}{}_{B}$
60	$1.20\pm0.00^{a}{}_{C}$	$19.00\pm0.00^{a}{}_{B}$	$0.60\pm0.00^{b}{}_{C}$	$1.70\pm0.00^{b}{}_{C}$
90	$1.20\pm0.00^{a}{}_{C}$	$19.00\pm0.00^{a}{}_{B}$	$0.50\pm0.00^{b}{}_{D}$	$1.30\pm0.00^{b}{}_{D}$

Data are presented as mean (±SD) (n=3). (a-b) Values of the same row and color with different superscripts are significantly different ($p<0.05$). (A-D) Values of the same column with different superscripts are significantly ($p<0.05$) different.

3.8. Changes in fatty acid composition

The chromatogram showing the fatty acid composition of fresh groundnut oil is presented in figure 7. The following fatty acids were detected in that oil: Palmitic acid (11.98%); Stearic acid (4.31%); Oleic acid (45.63%); Linoleic acid (31.13%); Arachidic acid (1.50%); Gadoleic acid (1.18%); Behenic acid (3.11%) and Lignoceric acid (1.10%). The effect of different storage conditions on the fatty acid composition of groundnut oil is presented in Table 2. It is clearly observed that, the amount of Stearic acid (18:0), Linoleic acid (18:2) and Gadoleic acid (C22:1) have significantly decreased ($p<0.05$) during storages, and the high rate of reduction was registered in the sample stored under sunlight. At the beginning, their percentages of occurrence were respectively 4.31, 31.13 and 1.18%. After 90 days of storage, these values dropped to 3.84, 31.03 and 1.06% respectively in the dark at room temperature; and to 3.85, 29.75 and 1.04% respectively under sunlight.

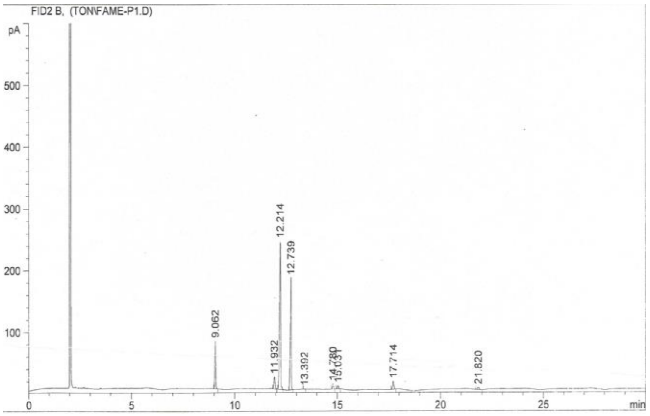

Figure 7: Gas chromatography-flame ionization detection of the fatty acid composition of fresh Groundnut oil [RT (retention time)=9.062: Palmitic acid (C16:0) (11.98%); RT=11.932: Stearic acid (C18:0) (4.31%); RT=12.214: Oleic acid (C18:1) (45.63%); RT=12.739: Linoleic acid (18.2) (31.13%); RT=13.392: Arachidic acid (20:0) (1.50%); RT=14.780: Gadoleic acid (C22:1) (1.18%); RT= 17.714: Behenic acid (C22:0) (3.11%) and RT= 21.820: Lignoceric acid (C24:0) (1.10%)].

Table 2. Changes in fatty acid composition of groundnut oil during storage in the dark at room temperature and under sunlight

		Initial	Ambient	Sunlight
Symbol	Common name	Day 0	Day 90	Day 90
C16:0	Palmitic acid	11.98 ± 0.04^{a}	11.57 ± 0.22^{b}	12.18 ± 0.11^{a}
C18:0	Stearic acid	4.31 ± 0.00^{a}	3.84 ± 0.03^{b}	3.85 ± 0.00^{b}
C18:1	Oleic acid	45.63 ± 0.00^{a}	45.76 ± 0.04^{b}	45.65 ± 0.02^{a}
C18:2	Linoleic acid	31.13 ± 0.04^{a}	31.03 ± 0.02^{b}	29.75 ± 0.01^{c}
C20:0	Arachidic acid	1.50 ± 0.08^{a}	1.58 ± 0.03^{a}	1.64 ± 0.03^{a}
C20:1	Gadoleic acid	1.18 ± 0.06^{a}	1.06 ± 0.03^{b}	1.04 ± 0.02^{b}
C22:0	Behenic acid	3.11 ± 0.02^{a}	3.43 ± 0.03^{b}	3.69 ± 0.06^{c}
C24:0	Lignoceric acid	1.10 ± 0.06^{a}	1.54 ± 0.02^{b}	1.60 ± 0.03^{b}

The values are presented as mean±standard deviation. Values of the same row with different superscripts are significantly different ($p<0.05$).

4. Discussion

The results of oil characterization showed that groundnut oil samples exposed under sunlight have exhibited significantly peroxide, p-Anisidine, TOTOX and TBA values. Enhance in peroxide value observed in all the samples during the storage mark the formation of primary oxidation products (hydroperoxides). The highest peroxide value of oil samples exposed under sunlight after 90 days compared to those kept at room temperature can be attributed to the higher rate of formation of hydroperoxides. It has been demonstrated that, the exposure of oil samples to sunlight accelerate photosensitizers excitation. This leads to the formation of free radicals and singlet oxygen (12). The singlet oxygen directly reacts with the highest electron density of double bonds producing both conjugated and non-conjugated hydro peroxides (13,14). This is surely the reason why the peroxide value of groundnut samples exposed under sunlight was significantly higher from that of the same oil stored in the dark at room temperature. These results are in accordance with those of Raza et al. (2009) who obtained similar results with sunflower oil during 7 weeks storage under sunlight and in the dark at ambient temperature (15).

The variation of the p-Anisidine value during storage also showed that groundnut oil samples under sunlight was the most affected. This is the outcome of the higher decomposition rate of hydroperoxides into secondary oxidation products, mainly 2-alkenal and 2.4-Dienal (9). In fact hydroperoxides easily decompose into secondary oxidation products when the temperature is high. The room temperature used in this study was about 25°C while the average temperature under sunlight was more than 30°C. This can justify the higher decomposition rate of groundnut oil exposed under sunlight in comparison with the same oil stored at room temperature. These results are in agreement with those reported by Anwar et al. (2007) who showed that the p-Anisidine value of soybean oil was significantly higher after exposure to sunlight compared to the dark at room temperature. Similar observations were also reported by Raza et al. (2009) with sunflower oil.

The total oxidation value which gives an idea of the global oxidation state of the oil also showed that the oil samples stored under sunlight was the most altered. These results are in line with those reported by Ayu et al. (2017) who demonstrated that the TOTOX value of red palm oil was significantly increasing with light intensity compared to the dark at ambient temperature (16).

The result of the TBA test also demonstrated that photo-oxidized groundnut oil samples have presented the highest TBA value which is proportional to the concentration of malonaldehydes released into the oil. It is well known that malonaldehydes are secondary oxidation products formed during the oxidation of polyunsaturated fatty acids present in oils such as groundnut, sunflower and soybean oils (17,18). These findings confirm those previously obtained with the p-Anisidine value where the concentration of secondary oxidation products in auto-oxidized groundnut oil was significantly lower than that of the photo-oxidized ones. The free fatty acid value of edible oil informs on the hydrolysis rate of their triglycerides which can be caused by enzymes such as lipase, moisture and high temperature (19). The results of the changes in free fatty acids of groundnut oil samples exposed under sunlight and stored in the dark at room temperature showed that sunlight significantly increases the de-esterification of triglycerides in favor of free fatty acids. These results are in line with those reported by Anwar et al. (2007) and Raza et al. (2009) who showed that sunlight significantly increases the acidity of sunflower and soybean oils than the dark at room temperature.

The determination of the induction time of oils and fats generally informs on their ability to resist towards oxidation reaction. Long induction time indicates higher resistance to oxidation. The test quantifies the formation of organic acids and other volatile and non-volatile products in oils and fats (9). The results of the changes in induction time of groundnut oil samples stored under sunlight and in the dark at room temperature showed that the induction period of groundnut oil samples was significantly decreasing during the storage. The highest rate of decrement was registered in oil samples exposed to sunlight and was almost 0 at the end of the storage. These results confirm those previously obtained where groundnut oil exposed under sunlight has exhibited the highest formation rate of primary and secondary oxidation products.

The changes in color of groundnut oil during storage at room temperature and under sunlight showed that sunlight significantly reduced the color of groundnut oil. This can be attributed to the destruction of oil pigments by solar radiation. It has been demonstrated that sunlight significantly reduces the beta-carotene content of oils and fats. It is also known that the color of oils such as palm oil is attributed to their high beta-carotene content. The mechanism used by sunlight radiation in the destruction of beta-carotene might be the same used in the degradation of other oil

pigments. These results are in agreement with those reported by Anwar et al. (2007) who demonstrated that soybean oil color significantly reduced in both red and yellow units during exposure to sunlight compared to same oil stored in the dark at room temperature. However, the results obtained in this study were not in agreement with those reported by Raza et al. (2009) who showed that the color of sunflower oil was remaining constant in both red and yellow units during photo-oxidation.

The analysis of the fatty acid composition of groundnut oil demonstrated that it is rich in important fatty acids, among which oleic, linoleic and Gadoleic acids, which are unsaturated fatty acids. The benefits of unsaturated fatty acids such as oleic acid and linoleic acid on human health have already been proven. The results also demonstrated that sunlight significantly reduced the amount of Stearic acid and linoleic acid compared to dark at room temperature. The decrease in these fatty acids in oil samples exposed to sunlight compared to the dark at room temperature and at the beginning of the experiment can be attributed to photo-oxidation and auto-oxidation reactions catalyzed by sunlight. These results are in accordance with those reported by Djikeng et al. (2017) who showed that auto-oxidation reactions induced by heat reduced the amount of linoleic acid in palm olein compared to fresh oil significantly.

5. Conclusion

Results showed that sunlight promotes the formation of primary and secondary oxidation products in groundnut oil. It destroys its color, catalyzes the hydrolysis of its triglycerides and significantly reduce its induction time. Oil samples should not be stored under sunlight as it destroys their quality. They should be stored in the dark at room temperature.

Competing interests

Authors have declared that no competing interests exist.

Acknowledgements

None.

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