# EFFECTS OF ANTIOXIDANT ON THE PHYSIOCHEMICAL PROPERTIES OF Al-JAZEERA SUNFLOWER OIL.

**ABDELHADI A. MOHAMMED[1], MUTAZ M. A. ELSHIEKH[2] AND AYMAN A. JACKNOON[3]**

[1&2]Department of Chemistry, ACST, Ahfad University for Women, Sudan.
[3]Department of Chemistry, Faculty of Science and Technology, Alneelain University, Sudan.
Corresponding author; Abdelhadi A. Mohammed

**ABSTRACT: The main objective of this research work is to study the effect of antioxidant on preventing the oxidative spoilage of sunflower oil. A sample of sunflower oil was produced from seeds brought from Sudanese agricultural area, the Al-Jazeera project area was investigated separately properties, the physical properties such as color, density and some chemical properties such as acid value and peroxide value upon storing the oil sample in day light zone following the same industrial storage condition. For a period of time ranged from 8 to 16 weeks. Antioxidant such as vitamin C and vitamin E were treated with the stored oil. The change in the physiochemical properties of oil samples were determined.**

**The normal routine analysis of the oil such as GC-MS was done to oils under tests. Concerning GC-MS the major components of the oils were found to be palmitic acid, palmitolic acid, steric acid, Oleic acid and linoleic acid.**

**The average acid value for Al-Jazeera sunflower oil was found to be (3.29, mg KOH/g), prior to the storage period and decreased to (0.31 and 0.50 mg KOH/g), when storing the oil for 8 and 16 weeks respectively.**

**Also the peroxide value of sunflower oil sample was found to be (7.28 meq $O_2$/kg), prior to the storage period and (2.73 and 5.66 meq $O_2$/kg) when storing the oil for 8 and 16 weeks respectively.**

**The initial density 0.9026 g/ml was increase with added antioxidants vitamins, the density values was calculated after storage time and observing in the ranges recommended by standard for edible vegetable oils. Like the density, the color of oils was not change because the oil is rich by the color pigments. However, the vitamins added of fresh sunflower oils increase the oxidation stability. Changes in acid value and peroxide value obtained shows that the oxidative deterioration levels of oils were different between storage conditions. The results of the present study show that light acts as a major catalyst in accelerating the development of rancidity in oils. Also, the addition of vitamins to oil can increase the oxidation stability of oils during storage.**

***Key words. Al-JAZEERA sunflower, oil, shelf life, oxidation stability***

## INTRODUCTION:

Sunflower seed contain a high amount of oil (40% to 50%) which is an important source of polyunsaturated fatty acid of potential health benefits (Monotti, 2004). Oil is a very important resource, much in demand everywhere in the world and is used in a variety of ways (Gizachew, 2020).

The physicochemical properties of oils are effect by the degree of unsaturation, the length of the carbon chain and the type, quantity, distribution on the triglycerol and isomeric form of FA. The sunflower oils are rich of unsaturated fatty acids, rendering the oils susceptible to oxidative rancidity this rancidity occurs after prolonged storage and is accelerated when the oils are stored under inappropriate conditions. The oxidative rancidity affects the quality of sunflower seed oil. (Meng, *et al.;* 2019). The use of antioxidants as inhibitors of free radical autoxidation is of major importance in preserving polyunsaturated oils from oxidative deterioration (Frankel, 2005). An antioxidant can be classified as any substance that significantly delays or inhibits oxidation of a substrate.

Vitamin C or ascorbic acid is vitamin which function as a cofactor in many reaction due to its function as a reducing agent, this property of vitamin C makes it an important antioxidant, vitamin C is especially important as it also function to regenerate other antioxidants including alpha-tocopherol or vitamin E. (Sarkar *et al.;* 2016). Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. “Vitamin E” is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities. That have varying levels of biological activity. (Traber *et al.;* 2006). Alpha- (or α-) tocopherol is the only form that is recognized to meet human requirements. (Traber, 2007).

## OBJECTIVES:

Sunflower oil is considered as a concentrated source of energy for human beings and carriers of oil soluble Vitamins which supply the essential fatty acids that are required for a wide range of biological and physiological functions. The oxidative changes during storage and domestic use make oils unsuitable for consumption. Oxidative stability is an important indicator of oil quality and shelf-life. This study is expected to increase awareness among the population regarding the influence of storage conditions and added

vitamin E and C on the oxidation stability of domestically produced sunflower oil by adapting different oxidation detection methods. Furthermore, the study is important to see the quality and shelf life of domestically produced sunflower oils.

## MATERIALS AND METHODS:

### Materials:

All chemicals and reagents used in physicochemical analysis were analytical grade and were used with no further purification n-hexane, Ethanol, phenolphthalein, potassium hydroxide (KOH), glacial acetic acid, potassium iodide (KI), sodium thiosulfate (Na2S2O3), potassium bromide, starch were all obtained from Merck and Sigma Co. Chloroform was obtained from WINLAB LIMITED, United Kingdom. Vitamin E and C, was purchased from the SHUANGFENG Industrial China. Distilled water was used throughout the work.

Plants Collection:

Fresh Sunflower seed (FSFS), obtained from Aljazeera state area in Sudan.

Equipment:

The equipment's used in this work are electronic balance (OHAUS, Switzerland), soxthlet apparatus, pycnometer, micro pipettes, burettes, conical flasks, thermometer, stirrer, stands and heating device.

Instruments:

GC-MS spectrophotometry technique model (GC/MS –QP2010-Ultra) from japans (Shimadzu Company) with serial number 020525101565SA, The GC operating conditions were: column Rtx…..length (30m)….Diameter (0.25mn), Detector mass spectrometer and carrier gas Helium with flow rate 1.61 ml/min,

Methods:

General Methodology:

The sample of sunflower seed was obtained from agricultural area in Sudan. Al-Jazeera state. Oil from the seed was obtained separately using solvent extraction method. Pure oil was separated using rotary evaporation. An oil sample from extract was investigated chemically and physically, the physical tests were density and color. And the chemical tests were acid value, peroxide value, iodine value. The oil sample was also subjected to GC-MS. Oil from sample was divided into four portions labelled A, B, C and D. portion A was the control sample, portion B was mixed with vitamin C, portion C was mixed with vitamin E and portion D was mixed with an equal amount of vitamin C and vitamin E. the same physical and chemical tests above were replicate to all portion at an interval of three times, average results were obtained. The same procedure was repeated to an oil samples stored in normal day light and temperature for eight and sixteen weeks separately. Results were recorded and conclusion were obtained.

Extraction of Sunflower Oil:

The sample of sunflower seed was extracted by soxthlet method. A total of 50 g sunflower seed sample was weighed and extracted with n-hexane in a Soxthlet Apparatus at a condensation rate of 5 or 6 points per second for 4 hours with 300 ml of hexane at a temperature of 70˚C. The solvent was evaporated to dryness using a rotary evaporator at 40˚C. (AOAC, 2005).

Physiochemical Analysis of Sunflower Oil

Determination density

Determined by the standard method of (AOAC, 2000).

Determination of Color

Color was determined according to standard method of (AOAC, 2000).

Determination Acid Value

The AV is the number of mg of KOH necessary to neutralize the free acid in l g of sample. Acid value was determined according to (Okpuzor, *et al.;* 2009).

The acidity is frequently expressed as free fatty acid for which calculation shall be.

Free fatty acids as oleic acid = 28.2 VN

Per cent by weight = W

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

Determination Peroxide Value

Peroxide value (PV) was evaluated according to (AOCS, 2003).

Preparation of Methyl Ester of Fatty Acid

The fatty acid methyl esters were prepared as described in the International Olive Council (IOOC, 2009).

Fatty Acid Profile

Fatty acid profile was analyzed by GC according to the method described by (IOOC, 2009).

Determination of Fatty Acid Methyl Easters

Gas chromatography has been used for the qualitative and quantitative analysis of the fatty acids reported in the relative area percentage, the GC/MS technique model (GC/MS –QP2010-Ultra) from japans (Shimadzu Company) with serial number 020525101565SA and capillary column (Rtx -5ms -30m x 0.25mm x 0.25µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60◦C with rate 10◦C /min to 300◦C as final temperature degree with 6 minutes hold time, the injection port temperature was 300◦C the ion source temperature was 200

◦C and the interface temperature was 250 ◦C. the sample was analyzed by using scan mode in the range of 40 to 500 m/z charge to ratio and the total run time was 30 min. Identification of components for the samples was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST). The results were recorded.

Statistical Analysis:
The statistical analysis was performed with the SPSS package software, version 20 (SPSS). Results were presented as means ± standard deviation of the two triplicates of each experiment. Analysis of variance (ANOVA) was performed. Significant differences among the means ($p < 0.05$) were determined by Duncan's multiple tests.

**RESULTS AND DISCUSSION:**
Characterization Analysis of Sunflower Oils:
The chemical and Physical parameters are usually used for the identification of oils. Normally more than one character is determined so that the identification can be made with more assurance since the oils vary in their properties. The composition is not constant it depends upon certain factors such as climatic conditions, nature of soil, type of plant and variety of edible oil.

Physiochemical Characterizations Analysis:
Physicochemical properties of oils are determined to know the quality, purity and identification. Characteristic properties are properties that depend on the nature of the oil. These are used to characterize oil, irrespective of location or sources of origin .The initial determined of Physiochemical characterization for sunflower oil samples have been made before the storage and antioxidants vitamin added Table 1. Shows the results of some characteristics of sunflower oil such as density, color, AV and PV.

**Table. 1**: Physiochemical Characterization of Sunflower Oil.

| **Sample** | Density at 20°C (g/cm-$^3$) | Color | Acid value (mg KOH/g oil) | Peroxide value (meq O2/kg oil ) |
|---|---|---|---|---|
| **SFO** | 0.9005± (0.001) | 12.2 ± (0.07) | 3.29 ± (0.02) | 7.28 ± (0.04) |

The above results show that density of sunflower oil are always greater than the refined oil. Therefore oil may provide more protection for human health than refined oil.

Changes in density:
Density is considered as a good index of purity of oils, the increase in chain length of fatty acid present in oil tends to increase the density of oils. (Table 1) Shown the density value of oils samples were within the FAO/WHO standard for edible vegetable oils. The density of Al-Jazeera sunflower oil shows that the oil is less dense than water because the impurities are not present in oil.

Changes in Color:
Change in color indicates the deterioration of oil due to oxidation. Color of oils depends upon the nature of coloring material like chlorophyll and carotene present in oil. Sunflower oil samples have pale yellow color indicating the presence of color pigments, so the color of oils was not change because the oil is rich by the color pigments, according the color of Al-Jazeera sunflower oils became stable.

Changes in Acid Value (AV):
AV is a measure of the FFAs in oil. Normally, FAs are found in the TAG form, however; during processing, the FAs may be hydrolyzed into FFA. Production of FFA is the best predictor of fat deterioration and the presence of FFA could be used to monitor the extent of oils abused (Atta *et al.*, 2008). Table 1. Shows the lower AV found, the lower the level of FFA which results in increased oil quality. The initial AV of SFO presented in this study show a lower value than Codex Standard for Named Vegetable Oils (CODEX-STAN210-1999) (4.0 mg KOH/g). The observation of low initial AV for Al-Jazeera sunflower oil (3.29 ± 0.02 mg KOH/g of oil) indicates a low formation of FFAs. Probably, this is due to oil seed without moisture content. The acceptability level of virgin sunflower oils is below 4.0 mg KOH/g (measured in potassium hydroxide per gram). (Alimentarius, 1999).

Changes in Peroxide Value (PV):
Determination of peroxide value can give an idea about the early stages of oil oxidation. The PV indicates the level of oxidation during production and storage. One of the most important parameters that influence lipid oxidation is the degree of unsaturation of its FAs. When double bonds of unsaturated fats are oxidized, peroxides are among the oxidation products formed. The peroxide values for the fresh oil was very low which indicate the high quality of the SFO used in this work. Change in PV without storage and antioxidant vitamins were shown in Table. 1.

Peroxides are responsible for the taste and odor of rancid fats, their concentration as represented by the PV is often useful in assessing the extent to which the rancidity has advanced. Rancid taste often begins to be noticeable when the PV is above 20 meq O2/kg (Food Adulteration, 1954). At the beginning of the experiment, the PV of SFO was (7.28 ± 0.035 meq O2/kg. Which is less than 10 meqO2/Kg, and therefore within the acceptable value range for fresh oil. This value within the range considered as

satisfactory and in agreement with the maximum Codex standard PV (15 meq O2/Kg). For virgin vegetable oil. (Alimentarius, 1999).

Characterization and Identification of SFO by using GC-MS

The oil from Al-Jazeera sunflower was analyzed. Figure. 1. and Table 2. Reflect the profile of fatty acids presence in oil.

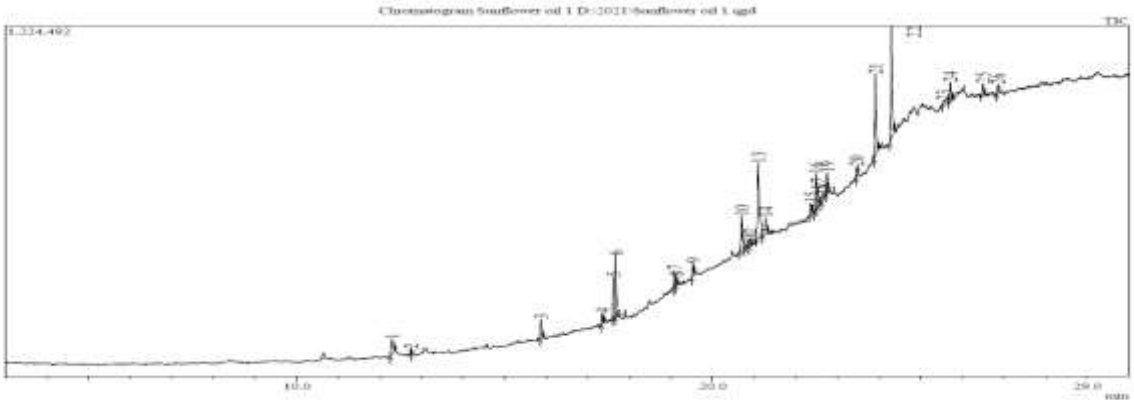

Figure 1. GC/MS Chromatogram of Al-Jazeera sunflower oil.

The GC-MS analysis of Al-Jazeera sunflower oil revealed some fatty acid with high concentrations, Table.2.

Table 2: The major fatty acids of Al-Jazeera sunflower oil.

| IUPAC Name | Common name | Formula | Content (%) |
|---|---|---|---|
| Hexadecanoic acid | palmitic acid | C15H30O2 | 7.73 |
| 9- Hexadecenoic acid | palmitoleic acid | $C_{16}H_{30}O_2$ | 4.81 |
| Octadecanoic acid, | steric acid | $C_{18}H_{36}O_2$ | 7.00 |
| 9-Octadecenoic acid, | Oleic acid | $C_{18}H_{34}O_2$ | 42.31 |
| 9-12, Octadecenoic acid, | linoleic acid | $C_{18}H_{32}O_2$ | 19.12 |

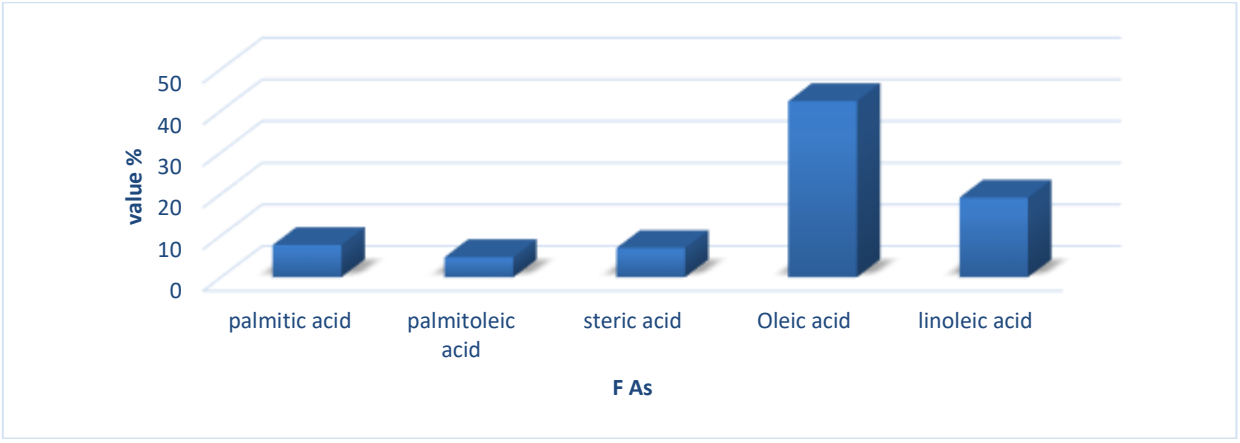


Figure 2: Fatty acid contents of Aljazeera sunflower oil.

The major fatty acids presence in Al-Jazeera sunflower oil were oleic (C18:1), linoleic (C18:2), Stearic (C18:0), palmitoleic (C16:1) and palmitic (C16:0). And acids which together composed about 80.97% of the total fatty acids and 16.96% of other components. For Al-Jazeera sunflower oil, saturated fatty acids represent 14.73% of the total fatty acids while the unsaturated fatty acid represents 66.24%.

The oil extracted from Al-Jazeera sunflower was analyzed using GC-MS, the result obtained showed high content of fatty acids, rich in oleic acid 42.31%, followed by linoleic acid which was 19.12%. For palmitoleic acid the result showed that the content was 4.81%. Al-Jazeera sunflower oil contain also palmitic acid which was 7.73% and Stearic acid also found in considerable amount which was 7.0%.

**Change of Physiochemical Characteristics during Storage and Antioxidant Vitamins.**

Table.3. Effect of Light on the physiochemical characteristics of SFO1 during storage time upon treatment with antioxidant vitamins.

| Treatment | Storage Time | Indicators | | |
|---|---|---|---|---|
| | | Acid Value ** (mg KOH/g oil) | Peroxide Value** (meq O2/kg oil) | Density (g/cm-3) |
| Control. | 8 Weeks | 0.53 ± (0.01) b | 7.28 ± (0.40) b | 0.925 ± (0.002) |

| | | | | |
|---|---|---|---|---|
| VIT. C | | 0.42 ± (0.07) cd | 3.4 ± (0.13) df | 0.921± (0.001) |
| VIT. E | | 0.45 ± (0.07) c | 3.48 ±(0.20) f | 0.920 ± (0.001) |
| MIX VIT. | | 0.31 ± (0.02) d | 2.73 ± (0.18) df | 0.918 ± (0.001) |
| Control. | 16 Weeks | 0.96 ± (0.15) a | 9.69 ± (0.61) a | 0.9265± (0.004) |
| VIT. C | | 0.59 ± (0.06) b | 7.53 ± (1.01) be | 0.9247±(0.007) |
| VIT. E | | 0.59 ± (0.07) b | 6.63 ± (1.49) b | 0.9269 ±(0.004) |
| MIX VIT. | | 0.50 ± (0.03) bc | 5.66 ± (0.31) bc | 0.9223 ±(0.001) |

* = Means are significantly different at p≤0.05,
** = Means are significantly different at p≤0.01
Means followed by the same superscript are statistically similar according to DMRT.

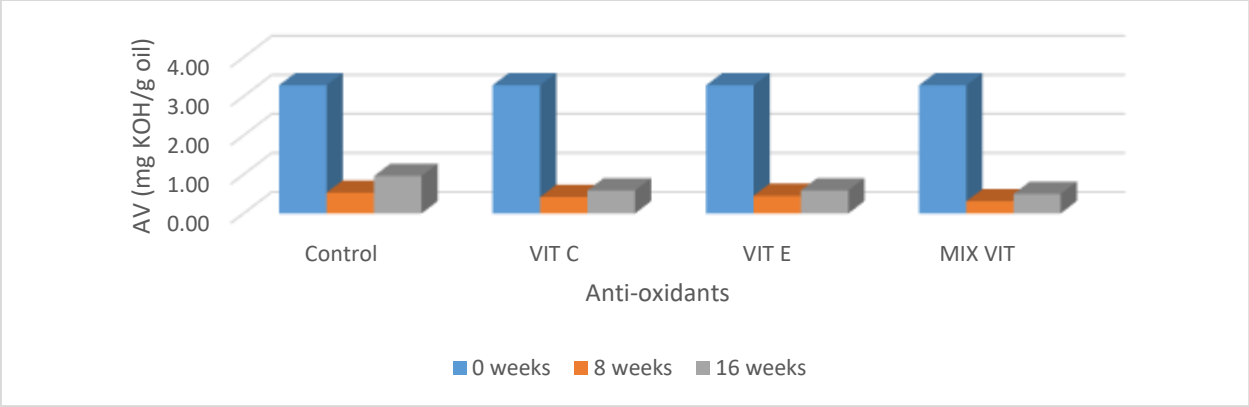


Figure 3. Effect of Light on AV of SFO during storage time upon treatment with different antioxidant vitamins.

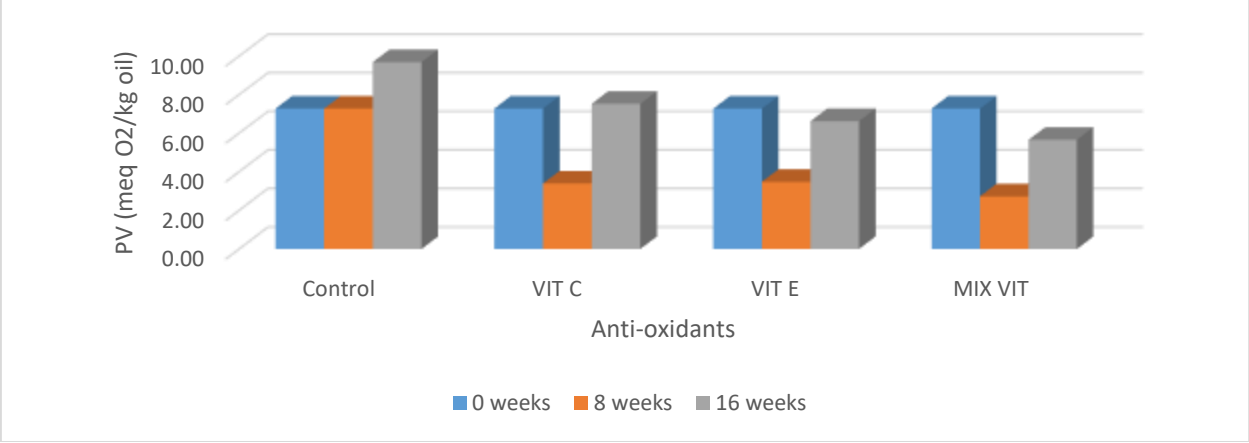


Figure 4. Effect of Light on PV of SFO during storage time upon treatment with different antioxidant vitamins.
Concerning the control group the acid value decreased gradually when storing the oil samples for 8 weeks to a larger extend than when storing the oil sample for 16 weeks. Figure 3. Like the acid value, Figure 4. Shows the peroxide value decrease slowly when the storage time increase, when storing the oil samples for 8weeks to 16 weeks. The Density from first time of storage period were within the range of standard codex recommended of vegetable oil. These values were increased during storage and up to 16 weeks.

**Effects of acid value:**
During the storage time, the AV of Aljazeera sunflower oil samples stored with vitamins decreased slowly and steadily Table.3. The effect of vitamins was high in maintaining the formation of FFA during the eight weeks and sixteen weeks of mixed vitamins for SFO samples (0.31 ± 0.02 and 0.50 ± 0.03) mg KOH/g respectively. And it starts to more capacity as storage time increased. The vitamins E, C and mixed was decreased the AV of Aljazeera sunflower oil samples kept at light by compared to SFO control samples stored in light without added vitamin. Similarly, the AV of control samples were presented in this study. Fig.3. Shows lower value than Codex Standard Oils (CODEX-STAN210-1999). The observation of SFO initial value probably. The acceptability level of virgin sunflower oils is below 4.0 mg KOH/g.

**Effects of peroxide value:**
At the beginning of the experiment, the PV of SFO was (7.28 ± 0.04), meq O2/kg. This value falls in the range considered as satisfactory and in agreement with the maximum Codex standard PV (15 meq O2/Kg) (Atta *et al.;* 2008). For virgin vegetable oil. The PV of oil samples stored for 16 weeks registered a progressive increase with the increment of storage period. From Table 3 it was observed that changes in PV of SFO stored under similar conditions with and without added vitamins were significant ($p<0.05$). At the end of 16 weeks of storage. The change in PV of Al-Jazeera SFO kept for 16 weeks at light was significantly ($p<0.05$) higher than the PV value of Al-Jazeera SFO stored for 8 weeks. The change in PV of Al-Jazeera sample probably due to higher content of saturated palmitic acid, which is less prone to oxidation than unsaturated fatty acids, linoleic acid and Oleic acid. (Paul, *et al.,* 1992) The higher PV of control SFO is mostly because Al-Jazeera SFO has an appreciable amount of unsaturated FA to fix oxygen and easily oxidized. These findings are similar to the work of Huang *et al.;* 1981) who reported that high PUFAs, especially linoleic acid (18:2), are prone to oxidation, hydrolysis, and thermal degradation. Figure 4. Shows the PV changes of oils stored under light after the addition of antioxidant vitamin E and C.

A lot of literature states that faster oxidation occurs due to exposure to light (Khan and Shahidi 2002). The absence of light minimized the hydroperoxide formation and also synergistically supported by minor components found in oils which acts as an antioxidant in the dark, (Khan and Shahidi, 2000). Such decreases in PVs had been reported by Neff *et al.;* 1994).

**Effects of Density (g/cm$^{-3}$):**
Density is determined and calculated at temperature 20°C as a ratio of mass in air of a given volume of the oil or fat to that of the same volume of at 20°C (Theodore, 1983). It can reveal the extent of adulteration and may be used as a means of acceptance of oils, during the storage time, the density of Aljazeera sunflower oil samples stored with and without added vitamins increased slowly and steadily with increase storage time in Table.3. Show the initial density (0.9005 g/ml) this value increase with added antioxidants vitamins the density values was calculated after storage time. And observing in the ranges recommended by the (FAO/WHO, 1993-1994). Standard for edible vegetable oils, probably. This is due to the effect of antioxidant vitamins on the oils. These results of density are the acceptability of oil quality.

**Effects antioxidant vitamins:**
The Vitamins functions as an antioxidant by serving as free-radical terminators and scavenging single oxygen molecules. The ascorbic acid and α-tocopherol concentration is an important factor that effects antioxidant activity in oils. Studies in purified TAGs obtained from Aljazeera SFO showed that antioxidant activity α-tocopherol was greater at concentrations (200 ppm) and it loses efficacy at higher concentrations due to its participation inside reactions. Figure 3. Shows the AV changes of oils stored under light after the addition of 200 mg/kg vitamin E and C.

## CONCLUSION:
Oxidative stability is an important indicator of oil quality and shelf-life. The oxidative changes during storage and domestic use make oils unsuitable for consumption. The present investigation is an overview of changes in acid value, peroxide value and Density of sunflower oil from 16 weeks of storage. The results observed during the study show that prolonged storage of sunflower oil at ambient (25-33) °C temperatures can lead to oxidative deterioration of oil samples. Initially, oil samples show acceptable acid value from the recommended value and significantly decreased during storage. Higher change in acid value observed for a sample stored and addition antioxidant vitamins. Also, additions of vitamins decreased the formation of free fatty acids in sunflower oil. The initial peroxide value of both oil.

## RECOMMENDATION:
The investigations presented in this study suggest:

- Better packing and storage conditions can lead to an improvement in the oxidative stability of vegetable oils and other related products containing fats and oils
- Lipid oxidation products make the oil unfit for human health; therefore, to minimize the oxidation phenomenon, some antioxidants should be added to increase the storage and shelf life of oils and oil products
- Producers, shopkeepers, and users should store oils and oil products in dark places protected from light
- The government or any responsible body should follow that local oil producers have put the production and expiry dates of domestically produced oils to safeguard the health of people

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