

Investigating the Oxidative Inhibition Potentials of Citrus Seed Extract on Palm Oil at Different Storage Temperature

¹Nwogbu, P. I.; ²Egbuna, S. O. and ³Nwogbu, C. C.

^{1&2}Depatment of Chemical Engineering Enugu State University of Science and Technology, Enugu, Nigeria

³Department of Metallurgical and Materials Engineering Enugu State University of Science and Technology, Enugu, Nigeria

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ABSTRACT

This study aims at showing the antioxidant potential of orange (Citrus sinesis) seed extract in the stabilisation of palm oil against oxidation at different temperature of storage. The essential oil of Citrus sinesis seed was extracted by complete extraction using the Soxhlet extractor. The level of alkaloid, tannin, flavonoid, phenolic content and DPPH radical scavenging activity of the extract were 98.2mg/kg, 757.33mg/kg, 147.3mg/kg, 1118.63mgGAE/kg and 52.6% respectively. The extract was added to fresh palm oil at concentration 100ppm, 200ppm and 300pppm and was stored at temperature of 30°, 50° and 70° respectively for 60 days. The sample treated with 300ppm of extract and stored at 30° was the lowest in PV, p-AV and Totox value of 3.36meq/kg, 2.23meq/kg and 9.96 respectively, compared with the untreated sample with 12.04meq/kg, 6.41meq/kg and 30.49. However, higher value was recorded at higher temperature and lower concentration. Citrus sinesis seed extract significantly stabilised palm oil at different storage conditions and prevented it from becoming rancid at the end of storage time. This may be exploited for use as preservatives in food products

Keywords: Palm oil, Citrus sinesis seed extract, Storage duration, Antioxidant, Temperature

1. Introduction

Oil palm (Elaeis guineensis) is one of the most important economic oil crops in Nigeria. According to World Rainforest Movement, oil palm is indigenous to the Nigerian coastal plain though it has migrated inland as a staple crop (Thomas et al 2011). The oil palm is one of the most economically valuable oilseed plants due to its high oil yield per bunch, reaching up to 6,000 kg ha-1 for certain genotypes. Palm oil is of high quality and is widely used in the food, medicine, and cosmetic industries (Padau et al, 2013). Palm oil like other vegetable oils can be used to produce biodiesel through mixing with petro-diesel or through trans-esterification (Padau et al, 2013). In recent years, the global demand for palm oil/refined vegetable oil has been on the increase which may be as a result of increasing population, change in diet pattern, the need for it usage in renewable energy, or its application in cosmetic, lubricant and bakery industries (Egbuna et al, 2007). However, the maximization of production and storage of palm oil has been limited by some constraints, among which is oxidation.

Oil oxidation is an undesirable series of chemical reactions involving oxygen that degrades the quality of the oil. Oxidative deterioration eventually produces rancidity in oil, with accompanying off-flavors and smells (Miller, 2013). Oxidation progresses at different rates depending on factors such as temperature, light, availability of oxygen, the presence of moisture and metals (such as iron) as well as the type of oil (Miller, 2013). Lipid oxidation is one of the major causes of quality deterioration in natural and processed foods and this is a large economic concern in the food industry because it affects many quality characteristics such as flavor (rancidity), color, texture, and the nutritive value of foods (Chayast et al, 2007). Thus lipid oxidation is one of the major processes that limit the shelf life of foods. In addition, the oxidative instability of polyunsaturated fatty acids often limits their use as nutritionally beneficial lipids in functional foods (Chayast et al, 2007).

Besides alteration in fatty acid content, there are only a limited number of approaches that can be used to control lipid oxidation in foods. Exclusion of oxygen from products, while effective, is often not practical during processing and storage. Efforts to reduce oxidation have increased in recent years and most often, the best strategy is the addition of antioxidants (Owoeye & Akinoso, 2016).

Antioxidants are compounds or systems that delay autoxidation by inhibiting the formation of free radicals or by interrupting the propagation of the free radical (Owoeye & Akinoso, 2016). Some of the most effective free radical scavengers and metal chelators are synthetic compounds that are often perceived negatively by consumers and for the sake of safety, plant materials are used as natural antioxidants for food, cosmetics, and other applications (Fayisoro, 2001).

The processing of citrus fruit results in the formation of waste materials in the form of peels and seeds in high amounts (Malacrida *et al.*, 2012; Siles *et al.*, 2010). The accumulations of these wastes with high organic matter and moisture contents pose a problem to the environment as they become toxic and hazardous environmental pollutants due to spoilage (Garcia *et al.*, 2005; Perazzini *et al.*, 2016). However, these wastes in the form of seeds contain important phytochemicals with antimicrobial properties (Aref *et al.*, 2010). The bitter taste of citrus seeds can be attributed to the presence of flavonoids (Okwu and Emenike, 2006). Flavonoid and phenol are known to exhibit antioxidant properties and hence, make citrus seed extract a potential source of anti-oxidant. Hence, the citrus seed extract is a potential source of variable oil that might be utilized for edible and other industrial applications (Adeniyi *et al.*, 2018) which include an application as a natural antioxidant. Hence, the oil in the citrus seed can be extracted for antioxidant purposes.

2. Materials and Method

Materials

Palm oil was procured from Afor-Amurri, while Orange (*Citrus sinesis*) was sourced from Nkwo-Amoli. Afor-Amurri and Nkwo-Amoli are located in Enugu state Nigeria.

Extraction of Orange (Citrus sinesis) Seed Extract

The orange was juiced to extract the seeds after which the seeds were sun-dried for 2 days. The dried seeds were then milled to powdery form. The seeds extract was obtained by complete extraction using the Soxhlet extractor (Konte, USA) as described by Abubakar et al, (2014). About 10g of powdered seed sample was wrapped in cheesecloth and put into a porous thimble of a Soxhlet extractor, using 150cm³ of n-hexane (with a boiling point of 60°C) as extracting solvent for 6 hours. The oily extract was obtained after the solvent was removed under reduced temperature and pressure and refluxing at 70°C to remove the excess solvent from the extracted oil. The extracted oil was stored for subsequent analyses.

Determination of Alkaloid

5.0g of the sample will be weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol will be added and allowed to stand for 4hours, this will be filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide will be added dropwise to the extract until the precipitation was completed. The whole solution will be allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which will be dried and weighed (Uzoekwe and Hamilton-Amachree, 2016)

 $Alkaloid(\%) = \frac{W_{3} - W_{2}}{W_{1}}$

Where:

W₁ =initial weight of the sample,

W₂ =weight of the extract and

W₃ = final weight of the residue

Test for Tannin

0.2g of the sample will be weighed into a 50 ml sample bottle. 10ml of 70% aqueous acetone will be added and properly covered. The bottle will be put in an ice bath shaker and shaken for 2 hrs at 300°C. The solution will then be centrifuged and the supernatant stored in ice, 0.2 ml of the solution will be pipette into the test tube and 0.8 ml of distilled water added to it. Standard tannin acid solution will be prepared from 0.5mg/ml of the stock and the solution will be made up to 1ml with distilled water. 0.5ml of Folinciocateau reagent will be added to the sample and the standard followed by 2.5 ml of 20% Na₂CO₃. The solution will then be vortexed and allowed to incubate for 40 min at room temperature; its absorbance will be red at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve prepared (Dewole et al. 2013).

Determination of Total Flavanoid Content

The total flavonoid content was determined using the method described by Quettier-Deleu et al., (2000). 4 ml of distilled water was added to 1ml of the extract in a 10-ml volumetric flask, followed by 1 ml of 5% sodium nitrate. 1 ml of 10% aluminum chloride was added after 5 min. This was left to settle for 5 min where 2 ml of sodium hydroxide was then added and topped up to the mark with distilled water. The absorbance readings were taken at 510 nm against a blank (water). The flavonoid content was determined using a standard curve with quercetin (10-180 mg/ml) and the value is expressed as milligrams of quercetin equivalents (QE)/kg of extract.

Determination of Total Phenolic Content

The total phenolic content of the samples will be determined using the Folin- Ciocalteau reagent as described by Correia Da Silva *et al.* (2010) and Gulcin et al. (2004). A portion of the samples, dissolved in ethanol, will be mixed with Folin-Ciocalteau reagent (100µl) and distilled water (3ml) and mixed for 1 min. Sodium carbonate (300µl, 15%) will be added to the mix. The solution volume will be adjusted to 5ml using distilled water. After 2 hrs, absorbance will be measured at 760 nm. A standard curve will be prepared using gallic acid with a concentration range from 0.5 to 25 µg/ml. Total phenolic content will be expressed as g gallic acid equivalents (GAE)/kg of extract.

Determination of Free Radical Scavenging Ability

The free radical scavenging ability of the samples against 1, 1-diphenyl-2picrylhydrazyl (DPPH) free radical will be evaluated using the method described by Owoeye & Akinoso (2016). An aliquot (0.2ml) of 0.5g of the samples will be homogenized in 20 ml methanol. The mixture will be mixed with 7.6ml of 0.4mM methanolic solution containing 1, 1-DPPH radical; the mixture will be left in the dark for 30min before measuring the absorbance at 516nm. The percentage of scavenging effect will be determined by comparing the absorbance solution containing the test sample to that of the blank sample as indicated in the equation below.

%DPPH Scavengng activity =
$$\frac{A_0 - A_1}{A_0} \times 100$$

Where:

 A_0 is the measurement of the blank

 A_1 is the measurement of the sample.

Sample Treatment

The palm oil samples will be treated with orange seed extract, ascorbic acid, and citric acid at various concentrations of 100ppm, 200ppm, and 300ppm. The treated palm oil together with the control (untreated) was stored in a transparent container at room temperature (30°C), 50°C, and 70°C for 60 days. Chemical analysis, such as peroxide value and anisidine value as well as the TOTOX value and antioxidant effectiveness of the stored palm oil were determined at an interval of 15 days.

Determination of Peroxide Value

30ml of acetic acid chloroform solution was measured into a flask containing 2g of the oil sample. 0.5ml saturated solution of potassium iodide was also added, followed closely by the addition of 30ml of distilled water. The flask content was then titrated against 0.1M sodium thiosulphate (Na₂S₂O₃) until the yellow color almost disappeared. 0.5ml starch indicator was added and the titration continued until the end-point (where the blue-black color just disappeared). A blank titration was also performed. Where, S and B represent sample and blank titrations respectively (Abdalaziz et al, 2017)

 $Peroxide \ value = \frac{A \times N \times 1000}{Weight \ of \ oil}$

Where, A= ml Na₂S₂O₃ (Test-Blank)

N= Normality of Na₂S₂O₃ solution

Determination of *P*-Anisidine Value

1 g sample of oil was dissolved in 25 ml of isooctane, (test solution (a)). Then 5 ml of test solution (a) was mixed with 1 ml p-anisidine (0.25% in glacial acetic acid), (test solution (b)). To prepare the reference solution, 1.0 ml of a 2.5 g/l solution of p-anisidine in glacial acetic acid was added to 5.0 ml of isooctane, and the solution was then shaken (Nigel Saunders, 2003 as described by Tadesse et al 2017). Then the absorbance of both test solutions and reference solution were measured at 350 nm. The p-anisidine value was calculated as

P-anisidine value= <u>25(1.2A1-A2)</u> m

Calculate the anisidine value from the expression:

A1 = absorbance of test solution (b) at 350 nm,

A2 = absorbance of test solution (a) at 350 nm,

m = mass of the substance to be examined in test solution (a), in grams.

Finally, the totox value was calculated and which was equal to the sum of the p-anisidine value plus twice the peroxide value

Total Oxidation Value (TOTOX)

PV and AV will change over time as hydro-peroxides are produced and decomposed. Total oxidation value (TOTOX) gives a more complete picture of the oxidative status of oil. This value combines the history of the oil (AV) with the present status (PV). TOTOX considers both primary and secondary oxidation products and is calculated based on PV and AV. One PV-equivalent is believed to give rise to two AV-equivalents. TOTOX is calculated by the following formula:

Totox value = 2PV + AV

Anti-oxidant Effectiveness of the Inhibitors

The anti-oxidant effects of the anti-oxidants were determined through percentage oxidative inhibition. This was calculated by comparing the peroxide value of the oil at different duration in the present and absence of the different types of antioxidants. The percentage oxidative inhibition was calculated using the equation below

$$\%I = \frac{P_{vo} - P_v}{P_{vo}} \times 100$$

Where:

 $P_{vo} = peroxide value of uninhibited oil$

 $P_v = peroxide value of the inhibited$

3. Results and Discussions

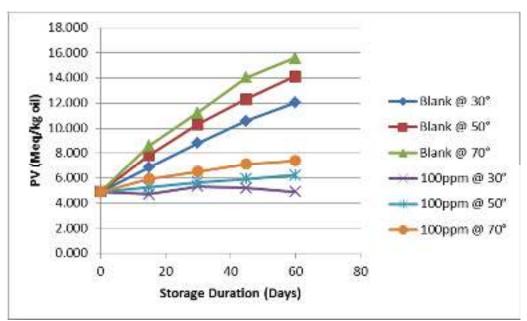
Table 3.1: Phytochemical Constituents and Radical scavenging activities of the citrus seed extract

Parameters	
Alkaloid (mg/kg)	98.2
Tannin (mg/kg)	757.33
Flavonoid (mg/kg)	147.33
Total Phenolic content(mgGAE/kg)	1118.63
DPPH Scavenging Activity (%)	52.6

The level of alkaloid, tannin, flavonoid, and phenolic content of the extract was 98.2mg/kg, 757.33mg/kg, and 147.3mg/kg, and 1118.63mgGAE/kg respectively, this indicates that the extract could be a potential antioxidant. Flavonoids are plant secondary metabolites with very strong antioxidant and free radical scavenging activities (Hashemi, et al, 2016). A similar value of total phenolic content of 1152.88mgGAE/kg was recorded by Malacrida et al. (2012) in their work "Phytochemicals and Antioxidant Activity of Citrus Seed Oils". Phenolic constitutes one of the major groups of compounds acting as primary antioxidants or free radical terminators. Phenolic compounds are

considered to be primary or chain-breaking antioxidants in free radical chain reactions, converting oil radicals to more stable products, thus extending the shelf life of vegetable oils (Schaich, 2005). Phenols contain hydroxyls that are responsible for the radical scavenging effect (Zhenshen et al., 1999 as cited in Ansari et al. 2013). In this study, the presence of these phytochemicals in the extract explains its free radical scavenging and metal chelating potentials.

The DPPH scavenging activity of the extract was 52.6% as represented in Table 1. This indicates that the citrus extract reacts with the hydrogen donors to reduce the radicals to the corresponding hydrazine (Jang et al.2010). A similar value of 54.20% was recorded by Malacrida et al. (2012) while Jorge et al. (2015) in their study reported 58.9, 56.0, 70.2, and 58.9 for four varieties of oranges. Phenolic compounds are known to have antioxidant activity and the scavenging activity of the extract was likely due to these compounds (Abdelmegid, 2014; Owoeye and Akinoso, 2016). Part of the antioxidant activity observed in Citrus extract could be the synergic effect of more than two compounds present in it (Owoeye and Akinoso, 2016).



Effect of Antioxidant Treatment of Palm Oil on the Peroxide Value

Figure 1: Peroxide values of treated and untreated palm oil at 30°C, at 50°C, and, at 70°C for 60 days (100ppm).

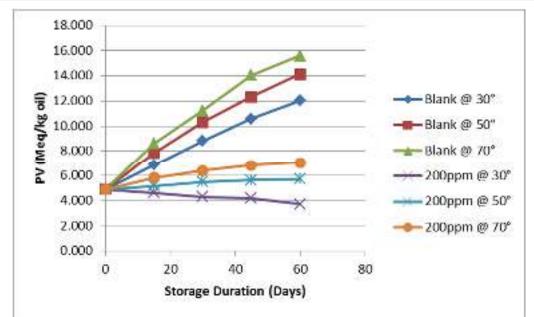


Figure 2: Peroxide values of treated and untreated palm oil at 30°C, at 50°C, and, at 70°C for 60 days (200ppm).

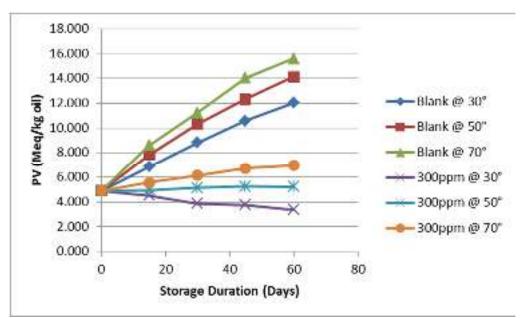


Figure 3: Peroxide values of treated and untreated palm oil at 30°C, at 50°C, and, at 70°C for 60 days (300ppm).

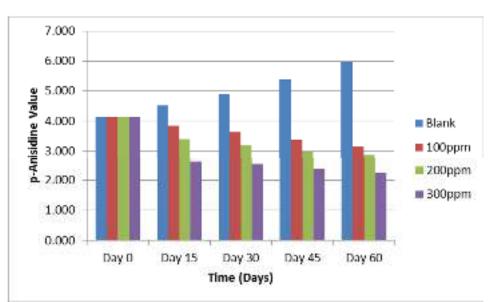
Within the 60 days storage time at 30°c,50°c, and 70°c, the effect of the addition of citrus extract is shown in Figures 1,2, and 3 for 100ppm, 200ppm, 1nd 300ppm respectively. The initial peroxide value of the pam oil (blank) was 4.889meq/kg which was within the accepted range of 10meq./kg as recommended by (FAO/WHO, 2009); it also implies that the oil is considerably stable. For the sample without the addition of an antioxidant, the peroxide value significantly increased from 4.889meq/kg to 12.038Meq/kg on the 60th day of storage for the sample stored at 30°c. This is an indication that the peroxide value of the sample increased significantly over storage time (Zaeroomali et al., 2014; Goudoum et al., 2015). A significant reduction in peroxide value was noticed within the first 15 days for

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sample-treated citrus extract. The reduction in peroxide value was attributed to a lag in the rate of formation of peroxides at first during an induction period that varies with time. This indicates that the antioxidants started to prevent the oil samples from the formation of hydroperoxides in the early stage (Owoeye and Akinoso, 2016). Throughout the experiment, the treated samples stored at room temperature showed PV less than 5.0meq./kg which is below the PV of 10 meq./kg acceptable for oil (Ambindei et al., 2020). The decrease in PV upon the addition of citrus extract in this study was in agreement with the findings of Ambindei et al. (2020) who worked on the Stabilisation potentials of the essential oils of *Thymus vulgaris* L., *Cinnamomum zeylanicum* B. and *Mentha piperita* L. on palm olein at accelerated storage. Likewise, Owoeye and Akinoso (2016) used Ginger and Basil extract as a natural antioxidant at concentrations of 100, 200, and 300 ppm to prevent deterioration of palm oil. Also, Dabire et al. (2012) successfully prevented the deterioration of cottonseed oil using the essential oil of *Ocimum basilicum* as a natural antioxidant.

However, at a higher temperature of storage, more increase in PV was recorded. This is an indication that oil deteriorates faster at high temperatures. At the highest temperature of storage (70°c), the citrus extract was able to reduce the PV of the sample to 6.95, 7.04, and 7.40meq/kg for treatment with 300ppm,200ppm and 100ppm respectively which is within the upper limit of 15meq/kg established by Codex 210(Codex Alimentarius, 2013); this is against the blank value of 15.59meq/kg. The above statement is also an indication that PV also decreases with an increased concentration of antioxidants. This concord with the findings of Owoeye and Akinoso (2016) in their work titled "Prevention of Oxidation in Palm Oil Using Plant Extract". It was also observed that PV increased rapidly up to the 45th day, then a reduction in the rate of increase was noticed between the 45th and on the 60th day. The slow increasing rate in PV may probably be due to an increase in the rate of secondary oxidation. Peroxide radicals, which are products of primary oxidation, are further oxidized to secondary oxidation products such as aldehydes, ketones, acids among others, thereby leading to a drop in PV as reported by Gordon (2001).

In all, treated samples recorded lower PV than the untreated sample. This may be attributed to the presence of antioxidants which have retarded the peroxide formation in oil by giving their hydrogen atoms (Womeni *et al*, 2016).



Anisidine Value

Figure 4: p-Anisidine values palm oil at 30°C for 60 days treated with citrus extract compared to untreated oil.

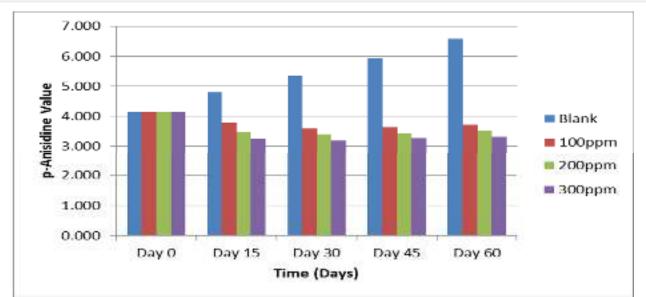


Figure 5: p-Anisidine values palm oil at 50°C for 60 days treated with citrus extract compared to untreated oil.

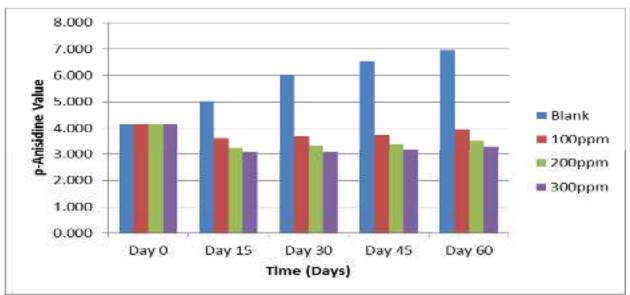
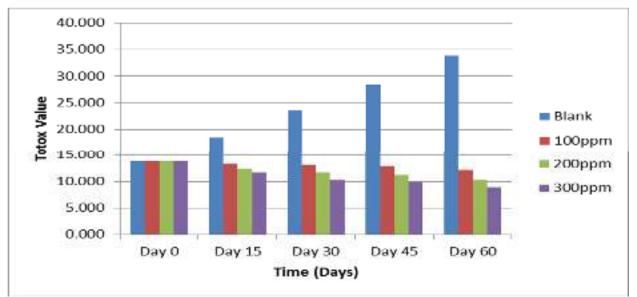


Figure 6: p-Anisidine values palm oil at 70°C for 60 days treated with citrus extract compared to untreated oil.

p-Anisidine Value (*p*-AV) is a reliable measurement of the amount of secondary oxidation products of oils and fats, it determines the aldehydes content in the oil samples as a result of decomposing hydro-peroxides (Ambindei et al., 2020). The results of *p*-AV for samples within the test period are shown in Figures 4, 5and 6. *P-AV* of samples increases with an increase in temperature and storage duration but decreases with an increase in the concentration of antioxidants. During the storage at room temperature (30°), it was noticed that the *p*-anisidine value in the sample without antioxidants was significantly increasing compared to those stabilized with the citrus extract. This could be attributed to the action of additives as an antioxidant, as it has been proven that by limiting peroxide formation in oils, their decomposition will also be delayed, so, secondary oxidation products formation will be less (Womeni *et al.*, 2016). It was also observed that samples stored at higher temperatures (Ben-Ali et al. 2014). The finding shows that the decomposition rate was faster at high temperatures; hence, we can say that *p*-AV is a function of temperature. Palm oil becomes more rancid at high temperature as a result of unsaturated aldehydes responsible for the off-flavors of oils (Shahidi and Wanasundara 2002) Since aldehydes are more heat-stable than the hydro-

peroxides, the p-anisidine value (p-AV) is considered as the most reliable test for estimation of advanced oxidative rancidity in oils.

In this study, there was a significant increase in the *p*-AV throughout the storage time for the uninhibited samples. The *p*-AV of palm oil samples stored at different temperatures (30, 50, and 70°C) increased with an increase in storage duration but reduced for the inhibited with increasing concentration of the antioxidants. All samples treated with antioxidants had *p*-AV less than 10 by the 60th storage day, implying a strong antioxidant effect of the extract that inhibits the peroxides from decomposing to form Aldehyde. These concords with the finding of Turan (2013) on the stability of canola oil during accelerated storage conditions.



Totox Value

Figure 7: Totox values palm oil at 30°C for 60 days treated with citrus extract compared to untreated oil.

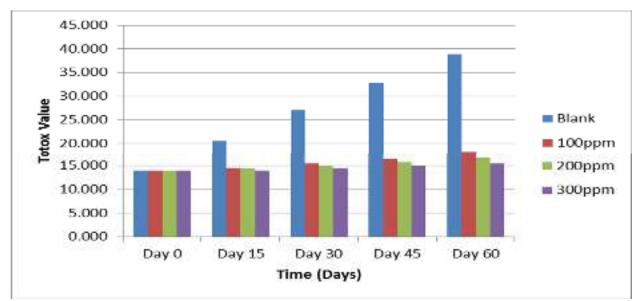


Figure 8: Totox values palm oil at 50°C for 60 days treated with citrus extract compared to untreated oil.

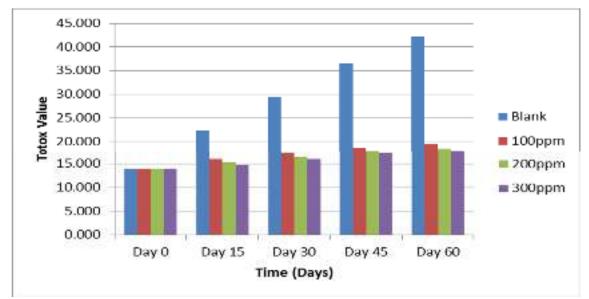
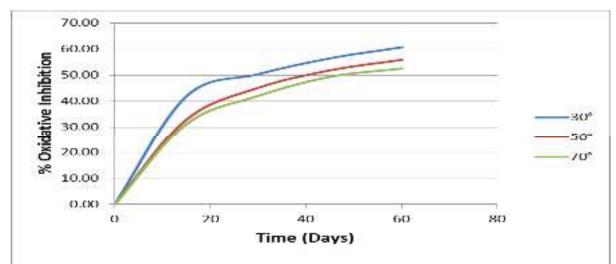


Figure 9: Totox values palm oil at 50°C for 60 days treated with citrus extract compared to untreated oil.

The variation in Totox value during the storage duration was represented in figures 7-9. The TOTOX values of the samples were determined using the PV and *p*-AV and it comprises the information for primary and second oxidation analyses, indicating the overall oxidation state of the assessed oils samples (O'Keefe & Pike 2010). The TOTOX values of palm oil used in this study increased regularly over the storage period but decrease with the increasing concentration of citrus extract. After storage for 60 days at 30°, 50° and 70°, the sample treated with 300ppm at 30°c gave the least TOTOX value of 8.96 while the untreated sample at 70°c gave the highest value of 42.14. These findings demonstrated that the experimental temperatures were effective in increasing the formation of both primary and secondary oxidation products and thus hasten the deterioration of the palm oil (Ambindei et al., 2020). It also indicates that the effectiveness of anti-oxidant is dependent on their dosage. In general, the recommended standard TOTOX value is less than or equal to 19.5 meq/kg, which increases linearly with both PV and *p*-AV (De Abreu et al., 2010). In all, the extract was able to prevent rancidity in the oil even at elevated temperatures. The presence of a relatively high percentage of phenolic compounds and flavonoids found in these citrus extracts could explain their strong antioxidant activity.



Anti-Oxidant Effectiveness of the Anti-Oxidants on Palm Oil

Figure 10: Percentage oxidative inhibition at 100ppm dosage

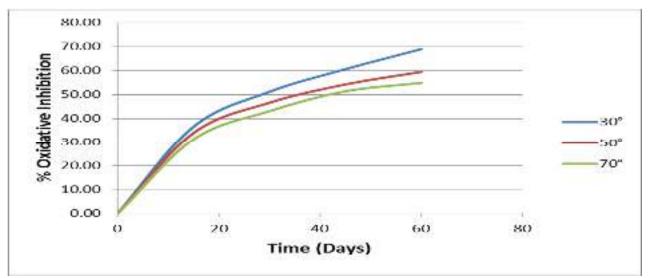


Figure 11: Percentage oxidative inhibition at 200ppm dosage

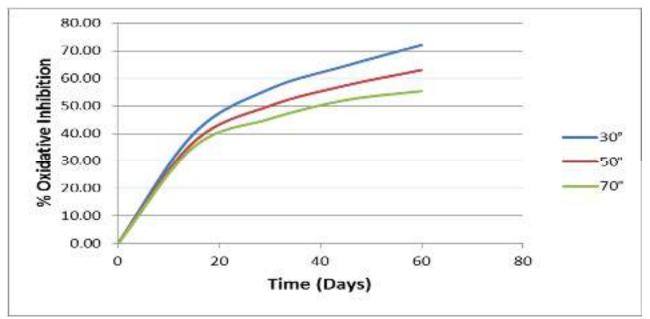


Figure 12: Percentage oxidative inhibition at 300ppm dosage

Figures 10-12 Show the results of the antioxidant effects of the various antioxidants during the specified storage time. The percentage oxidative inhibition of the different antioxidants at 30°, 50°, and 70° were calculated and plotted. It can be seen from the figures that palm oil containing 300ppm citrus extract and stored at 30° c gave 72% oxidative inhibition within the 60 days while samples containing 200ppm and 300ppm gave 69% and 61% respectively within the same condition. This implies that percentage inhibition increase with increasing dosage of extract. The percentage inhibition also reduces with increasing temperature of storage as shown in figures 11 and 12.

4. Conclusion

Extract from Citrus sinesis seed exhibited good radical scavenging ability and remarkably stabilised palm oil against oxidation at different storage conditions. It exhibited an antioxidant effectiveness of 72.08%, 63.01% and 55.41% respectively for 300ppm dosage at temperature of 30°, 50° and 70° respectively. This extract can, therefore, be exploited in food industry as suitable alternatives to synthetic chemicals as food additives and preservatives.

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