Oxidative Stability of Refined Red Palm Olein under two Malaysian Storage Conditions

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Abstract: Refined red palm olein (RPOO) is the first cooking oil that is a pro-Vitamin A source due to its high carotenoid concentration. The quality specifications from the manufacturers are usually applied to freshly produced oil. However, there is currently no information regarding the oxidative stability and phytonutrient content (Vitamin E and Carotene) for RPOO after prolonged storage time. The objective then is to study the effect of two local storage conditions and storage period(s) on the oxidative stability of RPOO. In this study, peroxide value (PV), $p$-anisidine value (AnV), induction period (IP), free fatty acid (FFA), and Vitamin E content were determined periodically for twelve months under local storage conditions (supermarket and kitchen). Carotene content, however, was determined only at initial and at the 12th month of storage time periods. It was found that there was an overall progressive but slow increase in PV and $p$-AnV. For PV, the storage effects were inconsistent. However, the effects were significant ($p < 0.01$) on the AnV throughout storage. At the end of the 12-months, for both storage conditions, the PV $< 10$ meq O$_2$ g$^{-1}$, the AnV $< 10$, the FFA $< 0.2$ % (palmitic acid), with a 30% drop in the total Vitamin E, and carotenoids content showed no significant drop ($p < 0.01$). The PV and AnV were also within Codex Alimentarius’ recommended limits. Finally, the oxidative parameters showed that RPOO remains stable after year storage under the two simulated local storage conditions (the aforementioned supermarket and kitchen).

Key words: oxidation, edible oil, palm oil, Vitamin E, carotenoids, storage

1 Introduction

In Malaysia, there are currently 53 refineries processing crude palm oil (CPO), a majority of which produce refined bleached and deodorized olein (rbdPOO) which contains Vitamin E but does not contain carotenoids. The carotenoids in rbdPOO are mostly destroyed during the bleaching stage of the refining process. The rbdPOO is a yellow, light-colored oil. There are only two producers of refined red palm olein in Malaysia, produced by different refining technologies and marketed under different brand names these are Carotino and Nutrolein. Currently, only Carotino red palm olein is available at the hypermarket and supermarket in Malaysia.

Red palm olein (RPOO), although marketed as cooking oil, is unlike rbdPOO. It is a specialty nutritious edible oil containing important phytonutrients that are maintained at high levels, e.g. Vitamin E, carotenoids, sterols, and ubiquinone as compared to rbdPOO. The color of this oil is a deep red due to the presence of a high concentration of carotenoids. The technology for production of the RPOO used in this study was developed in the Malaysian Palm Oil Board (MPOB) and transferred exclusively to a local company.

RPOO has been produced and marketed throughout Malaysia and in certain other countries since the late 1990s. The RPOO was produced in a two-stage process. The first stage still used the conventional method where CPO was pre-treated with phosphoric acid, followed by bleaching earth and filtration. However, the next stage of deodorization and deacidification was carried out in a milder manner using molecular distillation. Low temperature ($<170^\circ$C) and pressure (5-30 mTorr) were used during this stage. The milder process resulted in not only retained palm carotenoids, but it was found that other nutritional phytonutrients e.g. Vitamin E, sterols, and ubiquinone-10 were also preserved.

To date, there has been no study examining the oxidative and phytonutrient stability of RPOO in its commercial packaging during long-term storage. Periodic surveillance of lipid oxidation and its nutritional content is important. The reasoning behind this is to ensure that the oil is stable after a period of storage within the generally accepted
shelf-life period. It is also intended to monitor whether the nutritional content, especially palm carotenoids and Vitamin E (which may be highly susceptible to oxidation), are maintained at useful levels. RPOo has been used as a potentially rich source of provitamin A in developing countries due to its high carotenoid content.

The main threats to the quality of edible oil during storage are oxidative and hydrolytic deterioration, this will then eventually lead to rancidity. The oxidation products formed are the cause of off-flavors of edible oil. Two types of oxidative deterioration may occur during storage which is auto-oxidation and photo-oxidation. Hydrolysis causes the liberation of free fatty acids. Oxidative, hydrolytic deterioration and loss of phytonutrients can be influenced by external conditions for e.g. exposure to light, oxygen, moisture, and heat. In addition, fatty acid composition and the presence of other components such as free fatty acids, mono-, diacylglycerols, and metals presence such as an iron will also influence the extent of oil deterioration.

Since its introduction to the local and international market, RPOo has not been evaluated for its oxidative and nutritional stability during a prolonged storage period. The objective of this study was to evaluate the effects of storage conditions and storage period prevalent in Malaysia e.g. supermarket and kitchen condition on the stability of RPOo in its actual commercial packaging. This study will be limited to unopened bottles of RPOo during long term storage. For the kitchen condition, this study focused on a situation where some consumers who may purchase then keep excess bottles of oil in storage for future use. Some of these oils may be then stored for up to a year in the consumer’s typical kitchen condition.

2 Materials and Methods
2.1 Materials

Refined red palm oleins (RPOo) were purchased from a local supermarket. The solvents used for the High-Performance Liquid Chromatography (HPLC analyses) were HPLC grade acetonitrile, methanol, methylene chloride, and hexane. For the quality parameters, reagent grade tetrahydrofuran, and reagent grade chloroform were used. All these solvents were obtained from Merck (Darmstadt, Germany). The tocotrienols standards were obtained from Davos Life Sciences (Singapore). Sodium thiosulphate-pentahydrate, p-anisidine (99%), glacial acetic acid (>99.85%) and reagent grade isopropanol (>99.5%) were obtained from Sigma Aldrich (St. Louis, Missouri, USA). Potassium hydroxide was purchased from Merck (Darmstadt, Germany).

2.2 Chemical methods
2.2.1 Oil sample treatment

The RPOo under investigation was freshly produced, and they were then sent to the retailers within a week. The oils in this study were from the same production batch. They were then purchased from a local retailer upon their receipt. The purchased oils were then stored under the two controlled storage conditions within a day. The RPOo samples (500 g) were stored in their original packaging, which is transparent polyethylene terephthalate (PET) bottles. Their oxidative and nutrient stability were then monitored under the two storage conditions most prevalent in Malaysia. These are supermarket conditions (air-conditioned atmosphere at 20–22°C) with exposure to fluorescent light (commercial cool-white fluorescent tubes (4 feet), 40 watts with light intensity of 2400 lux) for 9 hours per day. For the local kitchen conditions, samples were kept in the dark (27–33°C) in a cupboard. For each storage condition, 18 bottles were stored. The storage period (s) were up to a year. The peroxide value (PV) and anisidine value (AnV) were measured at the following intervals: 1, 2, 4, 6, 9 and 12 months. Other parameters were measured for 3 induction hours, 4 (FFA) and 5 times (Vitamin E) at various intervals throughout the storage period.

It was assumed that all unopened oil samples had the same oxidative quality and nutrient content at the beginning of the storage period. The headspace in each of the commercial bottles was also assumed to be the same. At every designated month due for analyses, two random bottles of the oil were removed periodically from each of the storage areas for analysis. There was a spare unused bottle of rbdPOo stored (in each area) in case of occurrence of an accident e.g. spillage of content during handling. The contents of the two bottles were then mixed together in a container before an oxidative and quality parameter analyses were performed. The number of replicates for the various analyses were from the mixed oils. For each of the parameters analyzed, these sample replicates were then analysed simultaneously. The oxidative and acid value analyses were performed as soon as possible, which is within 30 minutes to prevent further oxidative and hydrolytic deterioration. The leftover samples were stored in a freezer at about −18 and −20°C and analyzed within a day or two for Vitamin E and carotene content.

The contents of the two bottles were mixed together in a container before the oxidative and quality parameter analyses were performed. The number of replicates for the various analyses were derived from the mixed oils. For PV and FFA, the samples replicates were analysed simultaneously within 30 minutes to prevent further decomposition of the oil. For the other parameters the samples were also analysed within a day, however, in these instances, the oil samples were placed in a freezer prior to analyses.
2.2.2 Peroxide value, anisidine value, free fatty acids, fatty acid compositions and carotene content

The peroxide value (PV), p-anisidine value (AnV), free fatty acids (FFA) were determined according to the MPOB Test Method p2.3, MPOB Test Method p2.4, MPOB Test Method p2.5, and MPOB Test Method p2.6 (Malaysian Palm Oil Board, 2005) respectively. The fatty acid composition was determined according to the American Oil Chemist’s Society methods AOCS Ce 1-62 and Ce 2-66. For the PV method, the liberated iodine was titrated with a standard solution of sodium thiosulphate. The FFA method involved titration of the oil dissolved in isopropanol and titrated with a solution of sodium hydroxide. PV and FFA are expressed as milliequivalent of active oxygen per kilogram (meq O₂ kg⁻¹), and % palmitic acid, respectively. The AnV method determines the number of aldehydes (principally 2 alkenals) in oils and fats. It also involved a spectrophotometric measurement of the absorbance at 350 nm resulting from the reaction of the aldehydic compounds in an oil and AnV in the presence of acetic acid.

Carotene content was measured using a UV-spectrophotometer at 446 nm in iso-octane. The carotene content of palm oil is expressed in mg kg⁻¹ and calculated as β-carotene. For each of the mentioned experimental parameters, the mixed oils from two different bottles withdrawn from their respective storage places were analyzed in several replicates. The average values were then reported.

2.3 Determination of oxidative stability

The accelerated oxidative stability test was carried out using the Rancimat test (AOCS Official method Cd 12b-92 1997) on a 679 Rancimat apparatus (Methrom, Herisau, Switzerland). The oil (2.5 g) was heated at a constant temperature of 120°C with an airflow of 20 L h⁻¹ and passed through a conductivity flask filled with distilled water. The induction periods in hours were recorded.

2.4 Vitamin E analysis

Palm oil Vitamin E content, expressed in parts per million (ppm), refers to the sum of the individual isomers which are alpha-tocopherol, alpha-, gamma- and delta-tocotrienols. The method was obtained from Tay et al., (2000)². The HPLC system (1100 series Agilent Technologies) was used and equipped with a quaternary pump, auto-injector system, and a thermostated column compartment. Detection was performed via an Agilent fluorescence detector set at an excitation wavelength of 295 nm and an emission of 323 nm. The analyses were performed on a Licrosorp (Merck, Darmstadt, Germany) normal phase silica column (25 × 0.46 cm, 5 μm) protected by a guard column (1.5 × 0.46 cm, 10 μm). The mobile phase mixtures of hexane: tetrahydrofuran: isopropyl alcohol (94:5:1 v/v/v) were used. The flow rate was set at 1 mL min⁻¹. The Vitamin E isomers were detected by a fluorescence detector set at 295 and 323 nm. The individual vitamin E isomers were identified and quantified by calibrating with the isomer’s standards.

2.5 Statistical analysis

The data was analyzed statistically and the means of the oxidative parameters and phytonutrients under the two storage conditions were compared by Student’s t-test for equal variances using a two-paired t-test with an alpha level of 0.01 using the data analysis package in Microsoft Excel 2013.

3 Results and Discussion

RPO in their respective commercial PET containers and sealed with a screw cap were stored under local kitchen and supermarket conditions. The pilot plant scale physical specification of red palm olein produced fresh was 0.04% FFA, PV of 0.1 meq O₂ kg⁻¹, moisture with impurities of 0.02% and no traces of iron. The properties of the RPO used in this experiment was 0.05% FFA, iodine value of 51.5, moisture and impurities of 0.04% and 37°C slip melting point. Table 1 showed the fatty acid composition of red palm olein used in this study. As indicated by many other storage studies, all types of edible oils will show a progressive deterioration in oxidative stability. External factors such as packaging material used, the storage conditions and the presence of nutrients with antioxidant properties will determine the extent of deterioration.

3.1 Peroxide value

The PV measures the extent of primary oxidation of oils. The primary oxidation products are lipid hydroperoxides. There was a sharp increase in PV from its first reception from 0.12 meq O₂ kg⁻¹ to 2.88 and 3.03 meq O₂ kg⁻¹ for the

| Table 1 | Fatty acid composition of red palm olein used in this study. |
|---------|------------------------------------------------------------|
|         | Fatty Acid  | Content (%) |
|         |             | n = 4       |
|         | C12:0       | 0.3 ± 0.0   |
|         | C14:0       | 1.0 ± 0.0   |
|         | C16:0       | 37.1 ± 0.1  |
|         | C16:1       | 0.2 ± 0.0   |
|         | C18:0       | 4.0 ± 0.0   |
|         | C18:1       | 44.4 ± 0.1  |
|         | C18:2       | 11.3 ± 0.0  |
|         | C18:3       | 0.4 ± 0.0   |
|         | C20:0       | 0.3 ± 0.0   |

J. Oleo Sci.
maximum acceptable limit from the Codex Alimentarius was a progressive increase in the PV in both storage conditions in this study as compared to the refined palm dative stability of RPOo was better when stored at kitchen conditions. This may be attributed to factors such as oxygen, heat, and light exposure which trigger lipid oxidation. It was observed that the storage condition’s effects on the PV were not significant (\( p > 0.01 \)) except for the 6th and 12th months (\( p < 0.01 \)).

These results showed that the effect of the storage conditions on the PV was not obvious and was inconsistent. This inconsistency seems to indicate that the storage conditions do not have a major effect on the PV. The possible reason may be attributed to a breakdown of hydroperoxides to secondary products over the intervening months. This decomposition can take place faster than the formation of new hydroperoxides depending on oxidation factors e.g. light, heat, trace metals. The speed of hydroperoxide decomposition can, therefore, give false low levels. It is difficult then to deduce the effect of storage conditions on the PVs. Overall, it was posited that progressive PVs increase for each storage conditions was then largely due to the storage time (s).

The PVs for both storage conditions after 12 months of storage were found to remain below 10 meq O\(_2\) kg\(^{-1}\). The maximum acceptable limit from the Codex Alimentarius (2013)\(^{20}\) for oleaginous seed was 10 meq O\(_2\) kg\(^{-1}\). The oxidative stability of RPOo was better when stored at kitchen conditions in this study as compared to the refined palm olein stored at 20-25\(^\circ\mathrm{C}\) in the dark\(^{30}\). The PV exceeded 5 meq O\(_2\) kg\(^{-1}\) after 6 months and above 10 meq O\(_2\) kg\(^{-1}\) at the 12th month of storage for refined palm olein\(^{30}\). Conversely in this study, although the kitchen conditions have a higher temperature range of 27-33\(^\circ\mathrm{C}\), the PV was 5.06 ± 0.38 meq O\(_2\) kg\(^{-1}\) after 12 months of storage. Edible oil should have a PV of below 7.5 meq O\(_2\) kg\(^{-1}\) to be considered sensory acceptable\(^{31}\). In conclusion, based on these PV values, RPOo is considered to be oxidatively stable after 12 months period of storage.

### 3.2 Anisidine value

The secondary oxidation products formed were measured in terms of \( p \)-anisidine value (AnV). AnV is an unspecific measure of saturated and unsaturated carbonyl compounds. As was found with PV, AnV showed a similar progressive increase starting initially at 1.8 at the reception to 4.1 (kitchen) and 5.07 (supermarket) after 12 months of storage (Table 3). The reported initial AnV for the same brand of red palm olein was 1.58 – 1.62\(^{32}\). The AnV for palm olein marketed in Malaysia from a survey usually falls in the range of 1.04 – 1.78\(^{32}\). The low value of the AnV showed that the processing technology was able to remove existing secondary products in crude palm oil. The subsequent increase of AnV with period time is attributed to secondary products from the decomposition of hydroperoxides during storage.

It was observed that the AnVs were significantly higher (\( p < 0.01 \)) for the supermarket conditions as compared to kitchen conditions throughout the storage period. Unlike the PVs, there was a significant effect of the storage conditions on the AnV. Autoxidation occurs in the presence of oxygen and is catalyzed by heat, metal catalysts, and

### Table 2

| Storage Period (months) | PV (meq O\(_2\) kg\(^{-1}\)) \( n = 4 \) | 
| --- | --- | 
| supermarket (21-23\(^\circ\mathrm{C}\)) | kitchen (27-33\(^\circ\mathrm{C}\)) | 
| 0 | 0.12 ± 0.06 | 0.12 ± 0.06 | 
| 1 | 2.88 ± 0.16\(^{a}\) | 3.03 ± 0.19\(^{a}\) | 
| 2 | 3.03 ± 0.10\(^{a}\) | 3.41 ± 0.39\(^{a}\) | 
| 4 | 3.57 ± 0.28\(^{a}\) | 3.38 ± 0.32\(^{a}\) | 
| 6 | 4.41 ± 0.20\(^{a}\) | 3.49 ± 0.19\(^{a}\) | 
| 9 | 4.43 ± 0.30\(^{a}\) | 4.78 ± 0.34\(^{a}\) | 
| 12 | 5.56 ± 0.25\(^{a}\) | 5.06 ± 0.38\(^{a}\) |

Data are presented as mean ± standard deviation. \( n \) = number of replicates. The values in each row with the same superscript are not significantly different (\( p > 0.01 \), t-test).

### Table 3

| Storage Period (months) | AnV (unit) | 
| --- | --- | 
| supermarket (21-23\(^\circ\mathrm{C}\)) | kitchen (27-33\(^\circ\mathrm{C}\)) | 
| 0 | 1.80 ± 0.07 | 1.80 ± 0.07 | 
| 1 | 2.39 ± 0.03\(^{a}\) | 2.32 ± 0.02\(^{b}\) | 
| 2 | 3.32 ± 0.12\(^{a}\) | 2.52 ± 0.10\(^{b}\) | 
| 4 | 4.10 ± 0.11\(^{a}\) | 3.72 ± 0.12\(^{b}\) | 
| 6 | 4.48 ± 0.06\(^{a}\) | 3.81 ± 0.02\(^{b}\) | 
| 9 | 4.65 ± 0.42\(^{a}\) | 3.69 ± 0.08\(^{b}\) | 
| 12 | 5.07 ± 0.07\(^{a}\) | 4.10 ± 0.08\(^{b}\) |

Data are presented as mean ± standard deviation. \( n \) = number of replicates. The values in each row with the same superscript are not significantly different (\( p > 0.01 \), t-test).
visible light which accelerate the free radical formation of fatty acids. These free radicals will react freely with atmospheric oxygen leading to free radical chain reaction\(^{38}\). In the case of kitchen condition, as samples were stored in the dark, only autoxidation will occur. Autoxidation of oils and the decomposition of hydroperoxides increase as the temperature increases\(^{38}\). The temperature at the kitchen condition although slightly higher than the supermarket condition can be considered mild. It is not likely to cause a fast increase in autoxidation and hydroperoxide decomposition.

The absence of visible light and trace iron\(^{39}\) also may explain the slower formation of hydroperoxides and decomposition. This could explain the lower AnV as compared to the supermarket conditions. Exposure to light can cause photo-oxidation\(^{12, 34, 35}\). Photo-oxidation is induced by exposure to fluorescent lighting for 9 hours per day simulating actual local conditions in the supermarket. The transparency of the PET packaging allowed penetration of fluorescent light\(^{35, 36}\). For the supermarket condition, photo-oxidation and autoxidation may occur at the same time and this may result in more hydroperoxides forming and decomposition to secondary oxidation products. Furthermore, photo-oxidation usually occurs at a faster rate than auto-oxidation. It follows then that the zero-order kinetic produces more hydroperoxides. Light also promotes faster decomposition to secondary oxidation products\(^{40}\). AnV for both storage conditions also showed a progressive increase within the storage period. The AnV after 12 months for all storage conditions were still found to be below 10. According to Rossell\((1989)\), well-refined oils with an AnV below the 10 threshold are still acceptable\(^{37}\).

### 3.3 Induction period

The resistance of the samples towards oxidative rancidity were measured by performing an accelerated test using an automated Rancimat apparatus. The initial induction period (IP) was 10.75 hours (hrs). The initial IP was similar as reported by Wu et al., 2018\(^{38}\). It was observed that the storage condition effects did not significantly affect the IP (Table 4). This is because no significant differences\((p > 0.01)\) were observed when comparing the IP from the two storage conditions as measured over the 12 month time period. At the end of the twelve-month storage, the IP was shortened to 9.07 hrs (supermarket) and 9.27 hrs (kitchen), but these values were not significantly different. There was an overall decrease in the oxidative stability of the oil for both storage conditions at the end of the twelve-month period. This was indicated by the shortened IP. It was however only reduced by less than 15% for both storage conditions (Table 4).

In another storage study, it was found that a drastic reduction of about 36.7% from the initial value for refined palm olein stored under similar conditions, such as the kitchen conditions in this study (storage at 20-25°C in the dark) after a year of storage\(^{39}\). RPOo resistance to oxidation as compared to rbdPOo may be attributed to its antioxidants e.g. Vitamin E, carotenoids, and ubiquinone. The reported vitamin E content for RPOo and rbdPOo was 707 ppm and 561 ppm, respectively\(^{39}\). RPOo contains about 22 ppm ubiquinone-10\(^{2}\). The vitamin E, carotenoids, and ubiquinone in RPOo may be attributed to its higher resistance to oxidation\(^{12}\).

It was observed that the IP value in this study decreased while there was an increase in PV over the same storage period (Table 2). This does, therefore, indicate there may be a relation between these two parameters. This observation was found in another storage stability study of low free fatty acid and freshly extracted CPO\(^{40}\). It was observed that a drastic increase of PV of both of these CPO stored at 60°C for 77 days was also followed by a major reduction of the IP for e.g. for low FFA-CPO PV initial values ranged from 18.8 to 24.3 h. The IP was shortened to 1.1 hr at the end of 77 days. In the case of ambient temperature storage (28°C), the PV showed a progressive but slow increase after 21 days while the corresponding IP showed only a slight drop. The same was observed for another storage study for refined palm oil, refined palm olein, and palm stearin by Almeida et al., 2019\(^{39}\). It was observed that for storage conditions studied, 26-32°C and 20-25°C, the PVs for each of these samples increased while the IP decreased with storage time. However, for those stored at cool temperatures (4-8°C), this resulted in a slower increase in PV, the reduction of IP was also found to be slower.

### 3.4 Free fatty acids

The free fatty acid (FFA) or acid value is the number of milligrams of potassium hydroxide necessary to neutralise the free acids in 1 g of the oil sample. FFA is expressed as a percentage of palmitic acid for palm oil and fractions. The FFA for the oil samples was found to increase progres-

| Storage Period (months) | IP (h) | n = 4 |
|-------------------------|--------|-------|
|                         | supermarket (21-23°C) | kitchen (27-33°C) |
| 0 | 10.75 ± 0.13 | 10.75 ± 0.13 |
| 6 | 9.85 ± 0.53\(^{*}\) | 9.74 ± 0.29\(^{*}\) |
| 9 | 9.23 ± 0.10\(^{a}\) | 9.34 ± 0.17\(^{a}\) |
| 12 | 9.07 ± 0.18\(^{a}\) | 9.27 ± 0.25\(^{a}\) |

Data are presented as mean ± standard deviation. n= number of replicates. The values in each row with the same superscript are not significantly different (\(p > 0.01\), t-test)
pronounced at later storage periods, however. Tagoe hydrolysis. storage condition did not significantly influence the rate of FFA leads to an increase in hydrolysis and therefore higher content and microbial load as storage time increases, this 2012 found palm oils tend to have a higher moisture condition showed significantly higher FFA in the 9th month. This showed that for the first six months the storage condition did not significantly influence the rate of hydrolysis. The storage condition effects on the FFA become more significantly for both storage conditions (Table 5). The initial FFA was 0.03% at the point of inception and within Palm Oil Refiners Association of Malaysia (PORAM) standard specification of a maximum of 0.1% for freshly produced refined palm olein. FFA was still below 0.1 for the supermarket condition at the 9th month. The storage effect showed no significant differences \((p > 0.01)\) for FFA during the 2nd and 6th months. This showed that for the first six months the storage condition did not significantly influence the rate of hydrolysis.

The storage condition effects on the FFA become more pronounced at later storage periods, however. Tagoe et al., 2012 found palm oils tend to have a higher moisture content and microbial load as storage time increases, this leads to an increase in hydrolysis and therefore higher FFA. It was observed that the oil stored under kitchen condition showed significantly higher FFA in the 9th and 12th months, as compared to supermarket conditions. This may be attributed to the occurrence of hydrolysis as a result of the higher temperatures found for the kitchen condition. At the end of 12th months, the % FFA (acid value) was 0.13\% (0.28 mg KOH g\(^{-1}\)) and 0.19\% (0.42 mg KOH g\(^{-1}\)) for the supermarket and kitchen conditions respectively. Codex Stan 21—1999 set a non-regulatory guideline for application by commercial partners. It was considered that a refined oil may be considered free from foreign and rancid odor taste when the acid value is less than 0.6 mg KOH g\(^{-1}\). This meant that after a year’s storage, RPOo was still within acceptable limits agreed by commercial standards.

### Table 5 Free Fatty Acid of red palm olein (RPOo) during storage time and under different conditions.

| Storage Period (months) | AVE Free Fatty acid (% palmitic acid) |
|-------------------------|--------------------------------------|
|                         | supermarket (21-23°C) | kitchen (27-33°C) |
| 0                       | 0.03 ± 0.00          | 0.03 ± 0.00          |
| 2                       | 0.04 ± 0.01\(a\)     | 0.05 ± 0.01\(a\)     |
| 6                       | 0.06 ± 0.01\(a\)     | 0.08 ± 0.01\(a\)     |
| 9                       | 0.09 ± 0.01\(b\)     | 0.11 ± 0.01\(b\)     |
| 12*                     | 0.13 ± 0.02\(a\)     | 0.19 ± 0.01\(b\)     |

Data are presented as mean ± standard deviation. \(n=4\) is number of replicates. The values in each row with the same superscript are not significantly different \((p > 0.01, t\)-test\).

The open drop in total vitamin E content was about 30-70% for sunflower oils stored in transparent 5-L PET bottles and exposed to daylight at an ambient temperature of 25°C during storage and under different conditions. The drop in Vitamin E may be due to its role as antioxidant offering protection against lipid peroxidation. The PV, AnV, and induction period all point to the fact that the oil was still stable and lipid oxidation had not advanced rapidly throughout the time in storage. A study carried out for sunflower oils stored in transparent 5-L PET bottles and exposed to daylight at an ambient temperature of 25°C supports the present findings. This study found that there opened bottled samples was 776 ppm. In another study the initial tocol as found in Table 6, was observed that the total Vitamin E value did not show an obvious drop in the 1st month of storage for all conditions. In the fourth month, it was observed there was a drop in the Vitamin E content to about 600 ppm levels for all storage conditions. The drop in total vitamin E content was about 30% for both supermarket and kitchen conditions in the 12th month. It was found that from the 1st to 9th months of storage the drop in vitamin E levels was not significant \((p > 0.01)\) for both storage conditions. This showed that the differences in oxidation factors in both these conditions showed no marked increase in the depletion of Vitamin E in RPOo. This may be because the oxidation factors e.g. exposure to light (supermarket) and heat (kitchen) caused a similar oil oxidation speed with the same rate of Vitamin E uptake.

It was observed that all the four Vitamin E homologs were still detected with similar percentage composition as the initial ones (Table 7). This showed that the degradation of the Vitamin E homologs was at a similar rate. This is unlike the findings by Kamaruzaman (2015) for red palm olein in blended chicken nuggets and Yi et al., for palm olein/fish oils where they found faster degradation of \(\alpha\)-tocopherol and \(\alpha\)-tocotrienols. This seems to show that Vitamin E homologs degradation may be different in its original form than when it is processed with other food/oil components.

The drop in Vitamin E may be due to its role as antioxidant offering protection against lipid peroxidation. The PV, AnV, and induction period all point to the fact that the oil was still stable and lipid oxidation had not advanced rapidly throughout the time in storage. A study carried out for sunflower oils stored in transparent 5-L PET bottles and exposed to daylight at an ambient temperature of 25°C supports the present findings. This study found that there

### Table 6 Vitamin E content of red palm olein (RPOo) during storage and under different conditions.

| Storage Period (months) | Total Vitamin E (mg kg\(^{-1}\)) |
|-------------------------|----------------------------------|
|                         | supermarket (21-23°C) | kitchen (27-33°C) |
| 0                       | 776.0 ± 18.4          | 776.0 ± 18.4          |
| 1                       | 754.8 ± 10.2\(a\)    | 745.3 ± 13.0\(a\)    |
| 4                       | 629.8 ± 7.3\(a\)     | 615.3 ± 4.9\(a\)     |
| 6                       | 621.7 ± 5.3\(a\)     | 614.8 ± 2.2\(a\)     |
| 9                       | 624.0 ± 2.8\(a\)     | 619.0 ± 2.2\(a\)     |
| 12                      | 524.3 ± 7.7\(a\)     | 514.0 ± 5.6\(a\)     |

Data are presented as mean ± standard deviation. \(n=4\) is number of replicates. The values in each row with the same superscript are not significantly different \((p > 0.01, t\)-test\).
was a reduction of 63-65% and 52-64% for total Vitamin E measured and β-carotene content, respectively during a 10-month storage period. Furthermore, the studies also found a trend between the tocopherol content and IP determined by the Rancimat Test at 110°C. This means that Vitamin E in RPOo together with other antioxidants in the oil e.g. ubiquinone may be responsible for slowing the oxidation of the oil. Carotenoids, therefore, will not be further degraded to oxidized products. The stability of the carotenoids in RPOo may be attributed to the presence of tocopherol and tocotrienols isomers. Vitamin E is a much stronger antioxidant, it is first attacked by free radicals over the carotenes from forming radicals. Studies have shown that tocopherols can prevent carotenoid radicals from forming from attack due to the protective effect of Vitamin E by the significantly higher FFA for kitchen conditions. The possible reason for carotenoids stability in RPOo is the probable reduction of sensitizers for e.g. chlorophyll due to the new refining process. A storage study for 7 days under different fluorescent lights with varying intensity by Ayu et al. (2017), showed that carotenoids content in refined red palm olein was not degraded. The reason for this is that the refining process was able to reduce sensitizers from 10.0 mg/kg in crude palm oil 8.74 mg/kg in the final product which was red palm olein. Chlorophyll is a known sensitizer which promotes photo-oxidation of carotenoids.

### 4 Conclusion

The low initial quality parameters of RPOo used in this study e.g. PV which was 0.12%, AnV of 1.8 and FFA below 0.1% in this study showed that the oil was a freshly refined oil when it was first acquired at the supermarket. As the storage period progressed, the results of the oxidative parameters showed that there was an onset occurrence of progressive autoxidation. The PV and AnV after a year storage, the storage conditions effect was only observed indicating the stability of the oil. At the end of twelve-month storage, the storage conditions effect was only observed for AnV and free fatty acid.

It was found that storage under fluorescent light caused more lipid oxidation as indicated by higher p-AnV for supermarket condition. Storing at higher temperatures caused more hydrolytic reactions with time as is observed by the significantly higher FFA for kitchen conditions. The Vitamin E content was found to be degraded at 30% from initial values. During this study, the carotene content remained similar to initial values for both storage conditions. Both oxidative and quality parameters showed good oil stability at the end of storage. It should be noted however that storage. These findings however differ from a storage study conducted by Almeida et al., 2019 for crude palm oil. They found that the carotenoids decreased with storage at 26-32°C with exposure to natural light and 20-25°C in the dark. This study indicates that carotenoids in refined oil are more stable compared to that of crude oil. Another possible reason for carotenoids stability in RPOo is the probable reduction of sensitizers for e.g. chlorophyll due to the new refining process. A storage study for 7 days under various fluorescent lights with varying intensity by Ayu et al. (2017), showed that carotenoids content in refined red palm olein was not degraded. The reason for this is that the refining process was able to reduce sensitizers from 10.0 mg/kg in crude palm oil 8.74 mg/kg in the final product which was red palm olein. Chlorophyll is a known sensitizer which promotes photo-oxidation of carotenoids.

| Table 7 | Percentage Area of HPLC Profile of vitamin E homologs at 0 month and at the end of the 12th month. |
|---------|--------------------------------------------------------------------------------------------------|
| Storage method | Storage period (month) | α-T | α-T3 | γ-T3 | δ-T3 |
| Supermarket (21-23°C) | 0 | 18.6 | 29.6 | 40.2 | 11.4 |
| Kitchen (27-33°C) | 12 | 17.8 | 31.0 | 38.2 | 13.0 |

Data are presented as mean ± standard deviation. n= number of replicates. The values in each column with the same superscript are not significantly different (p > 0.01, t-test).

### Table 8 Carotene content of red palm olein (RPOo) at initial values and values obtained on the 12th month under different conditions.

| Storage condition | Storage Period (month) | β-carotene (mg kg⁻¹) | expressed as β-carotene |
|-------------------|-------------------------|----------------------|------------------------|
| Supermarket (21-23°C) | 0 | 546 ± 12a | 528 ± 5a |
| Kitchen (27-33°C) | 12 | 546 ± 12a | 529 ± 2a |

They found that the carotenoids decreased with storage at 26-32°C with exposure to natural light and 20-25°C in the dark. This study indicates that carotenoids in refined oil are more stable compared to that of crude oil. Another possible reason for carotenoids stability in RPOo is the probable reduction of sensitizers for e.g. chlorophyll due to the new refining process. A storage study for 7 days under various fluorescent lights with varying intensity by Ayu et al. (2017), showed that carotenoids content in refined red palm olein was not degraded. The reason for this is that the refining process was able to reduce sensitizers from 10.0 mg/kg in crude palm oil 8.74 mg/kg in the final product which was red palm olein. Chlorophyll is a known sensitizer which promotes photo-oxidation of carotenoids.

### Conclusion

The low initial quality parameters of RPOo used in this study e.g. PV which was 0.12%, AnV of 1.8 and FFA below 0.1% in this study showed that the oil was a freshly refined oil when it was first acquired at the supermarket. As the storage period progressed, the results of the oxidative parameters showed that there was an onset occurrence of progressive autoxidation. The PV and AnV after a year’s storage were still within the Codex Alimentarius limit indicating the stability of the oil. At the end of twelve-month storage, the storage conditions effect was only observed for AnV and free fatty acid.

It was found that storage under fluorescent light caused more lipid oxidation as indicated by higher p-AnV for supermarket condition. Storing at higher temperatures caused more hydrolytic reactions with time as is observed by the significantly higher FFA for kitchen conditions. The Vitamin E content was found to be degraded at 30% from initial values. During this study, the carotene content remained similar to initial values for both storage conditions. Both oxidative and quality parameters showed good oil stability at the end of storage. It should be noted however that...
additional study will need to be carried out in terms of sensory tests for RPOo at the end of twelve-month storage.

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Conflict of Interest

The authors declare no conflict of interest in the publication of this article.

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