

# Quality of Chemical Re-fined Kenaf (*Hibiscus cannabinus* L.) Seed oil during Accelerated Storage

Sook-Chin Chew, Chin Ping Tan, Nyam Kar Lin

**Abstract:** In accelerated stockpiling at 65 °C for 24 days, an oxidative stability test was performed on crudes and re-fined kenaf seed oil. The outcomes revealed which refined oil underwent higher oxidation than the crude oil, as indicated by the peroxide value (40.55 meq/kg), *p*-Anisidine value (18.78) and total oxidation value (99.87) in re-fined oils at day 24. A free fatty acid value in the refined oil did not differ significantly and remained less than 1% during accelerated storage. After accelerated storage, the phenolic substance and anti-oxidant movement of re-fined oil was altogether lesser than crude oil. During accelerated storage, refined oil decreased by 67% tocopherol substance and 12.1 % phytosterol substance. After storage, there was no huge contrast in a content of tocopherol and phytosterol for crude and re-fined oils. The rate of tocopherol and phytosterol degradation in re-fined oil during storage was lesser than in unrefined petroleum (crude oil). Unsaturated fatty acids decreased slightly during storage, together with a slight increase in saturated fats in kenaf seed oil. The refining process reduced the oxidative steadiness of kenaf seed oil, but the refined oil could able to maintain good quality in the estimation of Free Fatty Acid (FFA) and a composition of fatty acid, and to protect tocopherols and phytosterols.

## I. INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is an important fiber plant with a relatively high oil content in kenaf seeds, which has a high substance of Mono-Unsaturated Fatty Acid (MUFA) and Poly-Unsaturated Fatty Acid (PUFA), specifically oleic corrosive (acid) (C18:1) and linoleic corrosive (C18:2). Regular consumption of oil with high content of MUFA and PUFA provides cardio protective effects to human [1]. Research concentrating on the utilization of kenaf seed oils has increased. Previous studies have shown that the kenaf seed oil has an abnormal state of polyphenols, tocopherols and phytosterols, which is notable for its medical advantages also the prevention of aging-related diseases, including cancer and coronary disease [2,3]. Phenolic compounds can scavenge radicals and make a significant contribution to the activity of antioxidants [4]. Tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) are powerful natural antioxidants that contribute to the stability of the oil during capacity (storage) and maintain the time span of usability of edible oils [5].

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A number of plant sterols with specific structures can act as potential anti-polymerization agents for frying oils as they are known to prevent oxidative deterioration of oils [6].

Due to its high nutritional composition, our continuous search for new sources of vegetable oil with improved functional properties has focused our attention on kenaf seed oil. Additionally, kenaf seeds can be used for the marketing of kenaf seeds oil has cheaper raw materials, kenaf seeds are mechanical waste during the handling of kenaf [7] All these interesting characteristics should attract our attention has an another sources of edible oils for the use of kenaf seed. So as to deliver kenaf seeds oil suitable for human consumption, chemical refining has been used to remove these unwanted segments in unrefined petroleum, for example, Free Fatty Acids (FFA), Colour pigments, gums, waxes, phosphates and odoriferous materials. This will help to produce odourless, bland and oxidative stable oils [8,9].

It is generally known that oxidation is a major cause of fat and oils deterioration, especially in PUFA, leading to a loss of nutritional value and the formation of rancid odours, unpleasant flavours and in some cases toxic compounds [10,11]. Hydroperoxides produced by lipid oxidation can be decomposed into products of less molecular weight, such as aldehydes, ketones, alcohols and carboxylic acids, that may affects a flavour on the oil product in some of these volatile compounds [12]. The assessment of oxidative stability is therefore a key factor in the development of the new food oil. Oxidative stability can be determined in the presence of excess oxygen by accelerated methods at high temperatures. To survey the oxidative steadiness of oil products, the Schaal oven test and the dynamic (active) oxygen technique were widely used [13]. However, the study on the refining of kenaf seeds oil and the impact of refining on oxidative dependability and changes in anti-oxidant action and bio-active mixes of refined kenaf seeds oil during capacity is scarce. To the best of our insight, this examination assessed the impact of refining on oxidative stability, anti-oxidant actions and changes in the substance of crude and re-fined kenaf seeds oil in phenolic, tocopherol and phytosterol during accelerated stockpiling.

## II. METHODOLOGY

### Material and Chemicals

Kenaf seed's have been gotten from the Malaysian Agricultural Research and Development Institute (MARDI),



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Selangor, Malaysia. Every single used chemical's were logical (analytical) (Merck, Darmstadt, Germany). Products from Sigma-Aldrich (St. Louis, USA) were 5 $\alpha$ -cholestane, standard phytosterols ( $\beta$ -sitosterol, campesterol, and stigmasterol), standard tocopherols ( $\alpha$ - and  $\gamma$ -), and standard fatty acid methyl ester. Corrosive (Acid)-activated bleaching earth was an result of Taiko Clay Marketing Sdn. Bhd. (Perak, Malaysia).

### Solvent extraction of kenaf seed oil

Kenaf seeds are ground within a grinder (Panasonic, Japan) into fine powder. Hexane was used by a Soxhlet extractor at 60 °C for 3 h [sample-to-solvent ratio:1:5, w / v] as a solvent to extract the oils from the kenaf seed powder. The solvent evaporated at 55 °C with a Buchi MultivaporP-6 (BÜCHI Labortechnik AG, Switzerland) pressure of 241 mbar to recover the oils. By flushing with 99.9% purified nitrogen, the lingering (residual) solvent was removed [14].

### Oil Re-finishing Process

The processes of refining were carried out using the previously reported method [7]. The crude kenaf seeds oil are degummed by pre-treating the unrefined petroleum within 0.3% w/w of phosphoric acid for 10 minutes and then treated with 3% w/w of Milli-Q water in a warmed shower at 70 °C for 30 minutes. Centrifugation removed the gums begins with the degummed oil. The degummed oil are then added to an abundance dimension of 0.2 to 0.5% of the stoichiometric amount of sodium hydroxide arrangement at 65 °C and the soapstock was evacuated by centrifugation. To remove an residual soap in the oil, the killed (neutralized) oil was washed with Milli-Q water. The killed (neutralized) oil are agitated at 95 °C at a reduced pressure of 30 min with Taiko Classic corrosive (acid)-enacted/activated dying earth (1.2% w / w). The bleached oil is then deodorized at 200 °C for 1 hour with a glass deodorizer. Steam was generated and transmitted through a vacuum pump into the oil and vacuum was applied.

### Accelerated storage condition

The Schaal oven test, which was used as a rapid method to simulate storage in real conditions, tested the oxidative stability of crude and re-fined kenaf seeds oil in quickened stockpiling conditions. In universal bottles wrapped in aluminum foil with loosely capped, copy tests of crude and re-fined kenaf seeds oil are put away. The oils are then put away in a drying oven (Memmert, USA) for 24 days at 65 °C, whereby one day of capacity at room temperature speaks to multi month of capacity [15]. On day 0, 6, 12, 18 and 24 of the storage, a lot of copied tests was expelled from the oven and analyzed.

### Evaluation of oxidative stability, antioxidant activity and bioactive compound

In accordance with the official AOCS method (AOCS 1998a), the per-oxide values (PV) are determined. The p-Anisidine value (p-AV) was determined using an UV-Vis spectrophotometer (UVmini-1240, Shimadzu Corporation, Japan) at 350 nm in accordance with the AOCS Official Technique Cd 18-90 (AOCS 1998b). The total oxidation values (Totox) were calculated from the Totox values equation= 2PV+ p-AV adapted from O'Connor and others

(2007). Free fatty acids (FFA) were determined by an AOCS (2000) titration. According to the method described by Chew and others (2015), Total Phenolic Content (TPC) was done. Chew et al. (2015) analyzed the substance of tocopherols and phytosterols according as indicated by the recently settled strategy. To analyze the tocopherol content, HPLC (Agilent Technologies 1200 Series, USA) was used within the UV-Vis detector and a Purospher STAR RP-18e column (5  $\mu$ m x 250 mm x 4.6 mm) (Merck, Germany).

### Fatty acid composition

The composition of Fatty Acids were analyzed according to an previous technique[ 1]. The sample was prepared by 50 mg of oil dissolved in 950  $\mu$ L of n-hexane, trailed by 50  $\mu$ L of methanol sodium methoxide (30% v/v). The best layer (1  $\mu$ L) was infused into a GC with a FID and a BPX70 section(0.32 mm interior distance across, 30 m length and a film thickness of 0.25  $\mu$ m; SGE International Pty. Ltd., Victoria, Australia), with a 50:1 split proportion. The initial temperature of the column oven was modified from 115 °C to 180 °C at 8 °C / min and held for 17 min. Comparing retention times with a Supelco FAME mix, the peaks of fatty acid methyl esters were identified.

### Statistical analysis

All experiments were duplicated and estimations duplicated twice (N= 4). All outcomes were broke down utilizing MINITAB 16 software (Minitab Inc., Pennsylvania, USA) using a one-way analysis of variance (ANOVA) and free T-test. A post-hoc tests of Tukey are utilized to decide the huge difference between the average ANOVA values. The distinctions were viewed as noteworthy at the  $p < 0.05$  level.

## III. RESULT AND DISCUSSION

### Evaluation of Oxidative Stability

As shown in Figure 1, the PV of re-fined kenaf seeds oil at day 0 were significantly lower ( $p < 0.05$ ) to crude kenaf seed oil. However, during 24 days of accelerated capacity, the PV of refined oil expanded fundamentally ( $p < 0.05$ ) from 0.85 to 40.55 meq / kg oil. This is due to the extent of oxidation during lipid oxidation caused by the formation of hydroperoxides. Then again, the PV of unrefined petroleum was expanded from 2.39 to 22.48 meq/kg oil from day 0 to 18 but then decreased to 17.19 during day 24. This might due to the PV in crude oil had reached the maximum when day 18 and start to decompose into secondary oxidation products (aldehydes, ketones, alkenals, etc) [16]. In addition, Iqbal[ 15] explained that the volatilization of some lipid hydroperoxide breakdown products may result in a decrease in PV after reaching its maximum. The PV of crude and refined oils experienced a higher rate of increment from day 0 to 12. After that, the PV were not significant difference from day 12 to 24. This may have helped to maintain the oil quality due to the bio-active mixes in kenaf seeds oil, such as tocopherol and phytosterol. Because of the



removal of phenolic mixes during the re-refining procedure, the refined kenaf seed oil was more oxidized than crude oil could. Zacchi and Eggers [17] clarified that the decrease in an oxidative stability index of rapeseed oil following the refining process is because of the almost complete removal of phenolic compounds during the refining procedure, which have a significant negative effect on the oxidative soundness of the oils.

On accelerated storage, Figure 2 shows the p-AV of crude and refined kenaf seed oil. Refined oil p-AV grew from day 0 to 24. The p-AV of crude oil, however, increased from day 0 to 18, but then slightly decreased to day 24. An elevated p-AV indicated an increase in oil optional oxidation items, including aldehydes, ketones and different substances. The decrease in p-AV of crude oil from day 18 to 24 can be clarified by further oxidation of aldehydes into carboxylic acids, that resulted in a decrease in a number of aldehydes in crude oils (Grill et al. 2006). The p-AV of raw and refined oils at day 0 was not significantly different, but the p-AV of refined oil was essentially higher ( $p < 0.05$ ) than the p-AV of raw oil from days 6 to 24.

Based on Figure 3, totox values of crude and refined oils undergo a higher rate of increment from day 0 to 12. After day 12, the oxidation in crude and refined oils occurred much slower. The totox value of refined oil from days 6 to 24 were essentially higher ( $p < 0.05$ ). This showed which refined kenaf seed oil had less oxidative stability than the crude oil. It is probably because of the crude oil contains a greater levels of natural anti-oxidants and phosphatides that have synergistic effects on antioxidants [18,19]. Gutierrez and others [20] reported that removal of the phenolic compound from olive oil without altering other antioxidant components resulted in the decrease of 50% of the oil stability. However, Nyam [21] reported that the PV and totox values of refined sunflower oil had reached 173 meq/kg and 360.21 respectively, during the 24 days of accelerated storage at 65 °C, which were much higher than the PV and totox values of kenaf seeds oil in this review. The refined kenaf seed oil in this review therefore showed higher oxidative stability in the current market compared to refined sunflower oil.

Figure 4 shows that during 24 days of accelerated storage, the FFA values of all re-refined kenaf seeds oils was essentially lesser ( $p < 0.05$ ) than crude kenaf seeds oil. As observed from the FFA values at day 0, the refining process removed 49.6 percent of FFA from crude oil. As the carboxylic group accelerates the rate of hydroperoxide decomposition, FFA may go about as pro-oxidants in vegetable oils. In vegetable oil, a higher FFA value can cause an unwanted taste and flavor [ 22]. One of the fundamental worries in the re-refining procedure is therefore to expel the raw oils begin with the FFA content. During accelerated storage, there were no huge distinction in FFA values in crude oil. These results also showed that accelerated storage in kenaf seed oil did not have a significant impact on the FFA content. This could be due to the lower humidity content of the kenaf seed oil. FFA is produced by hydrolytic rancidity caused by the interaction with water molecules of the hydrophilic groups of FFA. During accelerated storage, the FFA content of refined oil remained below 1 percent. The FFA content for edible oil

should not exceed 5%, according to Esuoso and Odetokun [23]. This result shows that the refined oil was acceptable and safe to consume within 2 years.

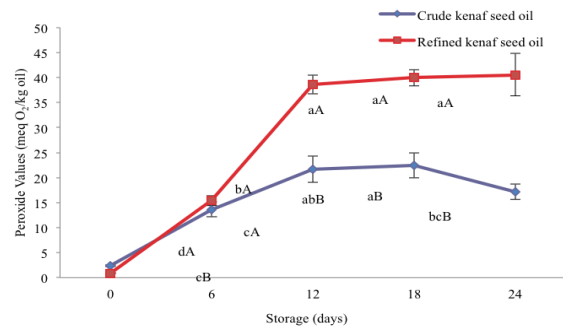


Fig.1 Peroxide values

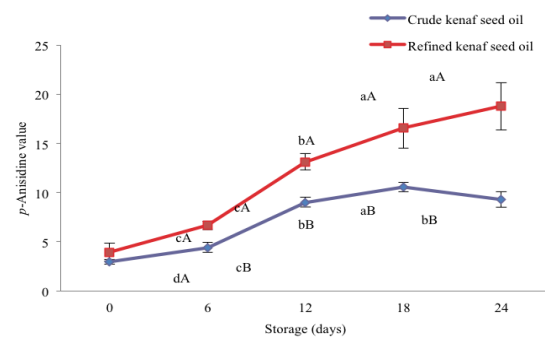


Fig. 2 p-Anisidine values

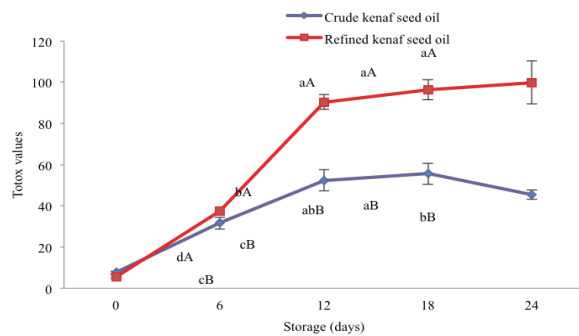


Fig. 3 Total oxidation values

### Evaluation of antioxidant activity and bioactive compounds

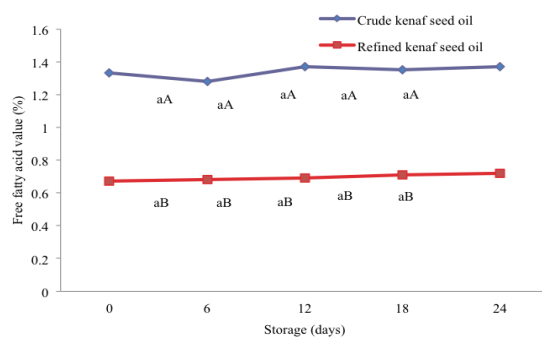
Figure 5 shows that after the refining process, 71.4% of the phenolic content were lost. The phenolic content of raw and refined oils was subsequently significantly reduced from day 0 to 12 ( $p < 0.05$ ). However, an increase in phenolic content in both unrefined (crude) and refined oils was seen during day 18. Crude and refined oils showed that the phenolic content from day 18 to 24 was no essentially unique ( $p > 0.05$ ). An increase in phenolic content can be clarified by the arrival of phenolic mixes begin with bound structures or chemical changes in phenolics at high temperatures [24]. Surjadinata[ 25] explained that in response to environmental stress conditions, the biosynthesis



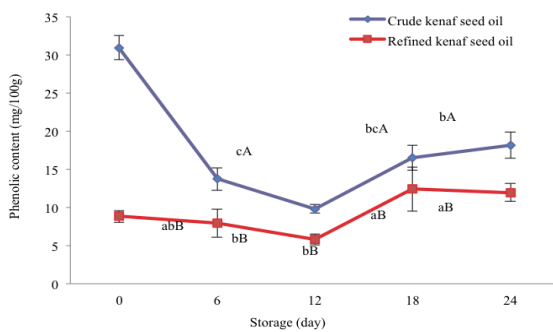


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of phenolic mixes in crude oils may be improved during quickened stockpiling.



**Fig. 4 Free fatty acids in crude and refined kenaf seed oils during accelerated storage.**



**Fig. 5 Total phenolic content**

**Table. 1 Tocopherol Contents in Crude and Refined Kenaf Seed Oils During Accelerated Storage.**

Storage (day)	$\gamma$ -tocopherol (mg/100g)		$\alpha$ -tocopherol (mg/100g)		Total tocopherol (mg/100g)	
	Crude	Refined	Crude	Refined	Crude	Refined
0	50.4 $\pm$ 3.8 <sup>aA</sup>	49.9 $\pm$ 4.6 <sup>aA</sup>	14.4 $\pm$ 2.3 <sup>aA</sup>	15.8 $\pm$ 0.8 <sup>aA</sup>	64.8 $\pm$ 2.5 <sup>aA</sup>	65.7 $\pm$ 5.3 <sup>aA</sup>
6	42.5 $\pm$ 5.0 <sup>bA</sup>	47.6 $\pm$ 5.1 <sup>aA</sup>	10.2 $\pm$ 0.6 <sup>bB</sup>	12.6 $\pm$ 1.4 <sup>bA</sup>	52.7 $\pm$ 5.5 <sup>bA</sup>	60.2 $\pm$ 4.4 <sup>aA</sup>
12	21.5 $\pm$ 3.6 <sup>cA</sup>	25.6 $\pm$ 5.9 <sup>bA</sup>	3.3 $\pm$ 0.7 <sup>cA</sup>	2.6 $\pm$ 0.1 <sup>cA</sup>	24.8 $\pm$ 4.2 <sup>cA</sup>	28.2 $\pm$ 5.9 <sup>bA</sup>
18	15.5 $\pm$ 1.6 <sup>cA</sup>	23.0 $\pm$ 3.8 <sup>bA</sup>	2.7 $\pm$ 0.3 <sup>cB</sup>	3.7 $\pm$ 0.1 <sup>cA</sup>	18.2 $\pm$ 1.5 <sup>cB</sup>	26.7 $\pm$ 3.8 <sup>bA</sup>
24	15.1 $\pm$ 2.8 <sup>cA</sup>	19.0 $\pm$ 1.9 <sup>bA</sup>	2.7 $\pm$ 0.4 <sup>cA</sup>	2.7 $\pm$ 0.1 <sup>cA</sup>	17.8 $\pm$ 3.1 <sup>cA</sup>	21.7 $\pm$ 2.0 <sup>bA</sup>

Phytosterols has been deductively demonstrated to decrease lipoprotein cholesterol in low thickness by including 10-15% of phytosterols in a healthy diet [28]. Because of its medical advantages, the Food and Drug Administration (FDA) and the European Union (EU) recommended that free phytosterols should be included in incorporated into traditional nourishments and marking rules established. In order to avoid adulteration, it is therefore important to determine the phytosterol profile in vegetable oils (Inchingolo and others 2014). Table 2 shows that  $\beta$ -sitosterol were the main phytosterol in kenaf seeds oil, trailed by campesterol and stigmasterol in this study, which was consistent within the phytosterol content revealed in past investigations [2,7].

Total phytosterols were significantly higher in crude kenaf seeds oil ( $p < 0.05$ ) than refined oil at days 0. This demonstrated which phytosterol content in refined oil was partially removed by the re-refining procedure. In crude kenaf seeds oil, the phytosterol content was significantly reduced ( $p < 0.05$ ) from day 0 to 6 and the phytosterol content reduced slightly until day 24. The phytosterol content in crude oil from day 6 to 24 was not significantly different.

Table 1 shows that after accelerated storage, the total tocopherol content decreased by 72.5% in crude oils and 67% in re-refined oil. The reduction in total tocopherols was caused by oxidation and oil degradation at high temperatures. The largest loss of tocopherol in kenaf seeds oil was  $\alpha$ -tocopherol. After storage,  $\alpha$ -tocopherol was lost 81.2 percent and  $\gamma$ -tocopherol was lost 70 percent in crude oil, while  $\alpha$ -tocopherol was lost 82.9 percent and after storage, 62 percent was lost in refined oil. This study showed that the warm security of  $\alpha$ -tocopherol was lower than  $\gamma$ -tocopherol. This outcome was consistent with the past investigation, which showed that  $\alpha$ -tocopherol was less stable in rapeseed oil [26] than  $\gamma$ -tocopherol. In addition, the tocopherol content of unrefined and refined oils at day 0 was not significantly different. This showed that the chemical refining process in this examination did not negatively affect the substance of tocopherol, which was unique in previous studies. Previous studies have demonstrated that the refining procedure diminished the content of tocopherol due to the high temperature (240 °C) connected in the deodorization procedure [8,27]. The content of tocopherol in re-refined oil was higher at the last days of storage than crude oil, inspite of the fact that there was no critical contrast. This has shown that re-refined oil can save the content of tocopherol and crude oil.

After accelerated storage, 31.1% of the phytosterol content was lost in crude oil. On the other hand, the content of phytosterol in refined kenaf seeds oil were slightly reduced by a loss of 12.1% during 24 days of quickened capacity.

However, a phytosterol content in refined oil did not differ significantly from day 0 to 24. During quickened capacity, phytosterol degradation were greater stability than tocopherol. The outcomes demonstrated that during storage, the re-refined oil may well prevent the phytosterol content. In spite of the fact that refining reduced the anti-oxidant activity and phenolic content of refined oil during capacity (storage), the bioactive compounds were protected by refined oil compared to crude oil.



**Table. 2 Phytosterol contents in crude and refined kenaf seed oils during accelerated storage**

Storage (day)	Campesterol (mg/100g)		Stigmasterol (mg/100g)		β-Sitosterol (mg/100g)		Total phytosterol (mg/100g)	
	Crude	Refined	Crude	Refined	Crude	Refined	Crude	Refined
0	72.6 ± 9.1 <sup>aA</sup>	56.8 ± 5.0 <sup>aB</sup>	40.9 ± 5.6 <sup>aA</sup>	24.7 ± 2.1 <sup>abB</sup>	589.5 ± 19.8 <sup>aA</sup>	463.3 ± 47.7 <sup>abB</sup>	703.0 ± 31.9 <sup>aA</sup>	544.8 ± 54.7 <sup>abB</sup>
6	56.9 ± 4.6 <sup>bA</sup>	55.3 ± 3.0 <sup>abA</sup>	27.3 ± 2.1 <sup>bA</sup>	25.0 ± 0.9 <sup>abA</sup>	448.8 ± 37.7 <sup>bA</sup>	425.8 ± 23.0 <sup>abA</sup>	533.0 ± 44.3 <sup>bA</sup>	506.1 ± 25.7 <sup>aA</sup>
12	55.7 ± 5.1 <sup>bA</sup>	54.1 ± 1.9 <sup>abA</sup>	26.7 ± 2.3 <sup>bA</sup>	23.3 ± 1.6 <sup>bA</sup>	440.6 ± 41.2 <sup>bA</sup>	420.9 ± 24.2 <sup>abA</sup>	523.0 ± 48.3 <sup>bA</sup>	498.3 ± 26.4 <sup>aA</sup>
18	54.6 ± 4.3 <sup>bA</sup>	51.4 ± 2.2 <sup>abA</sup>	26.9 ± 1.3 <sup>bA</sup>	24.8 ± 3.0 <sup>abA</sup>	432.4 ± 25.3 <sup>bA</sup>	424.9 ± 16.6 <sup>abA</sup>	514.0 ± 30.6 <sup>bA</sup>	501.1 ± 21.3 <sup>aA</sup>
24	51.3 ± 4.0 <sup>bA</sup>	49.4 ± 0.2 <sup>bA</sup>	24.4 ± 2.8 <sup>bbB</sup>	28.7 ± 0.8 <sup>aA</sup>	408.5 ± 30.6 <sup>bA</sup>	401.0 ± 7.3 <sup>bA</sup>	484.2 ± 37.4 <sup>bA</sup>	479.0 ± 7.3 <sup>aA</sup>

#### IV. CONCLUSION

The process of refining is a mandatory process for the production of edible oil. Following 24 days of quickened stockpiling, the value of PV, p-AV and TOTOX of refined kenaf seed oil was significantly higher than crude oil. However, during the accelerated storage, re-fined kenaf seeds oil remained the FFA content suitable for edible. The process of refining greatly affects changes in anti-oxidant activity during capacity. Be that as it may, the content of tocopherol and phytosterol in crude and re-fined kenaf seeds oils after quickened capacity was not significantly different. This demonstrated refined kenaf seeds oil was able to protect bioactive compounds in comparison to crude oil during storage. The composition of fatty acids in refined oil has shown that it is suitable for cooking oil. The MUFA and PUFA decreased 0.1% and 0.96%, respectively coupled with an increase of 1.06% in saturated fatty acids after accelerated storage. This study showed that the refined kenaf seed oil showed less oxidative stability than the crude kenaf seed oil, but the refined oil maintained good quality in FFA, tocopherols, phytosterols and fatty acids. Future work can be considered looking for the optimum parameters of refining process to get the re-fined kenaf seeds oil with improved oxidative stability.

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