Comparative study of crude and refined kenaf (*Hibiscus cannabinus* L.) seed oil during accelerated storage

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Abstract This study assessed the changes of antioxidant activity and bioactive compounds of crude and refined kenaf seed oil during accelerated storage at 65°C for 24 days. 2,2-Diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assays were used to determine their antioxidant activity. The changes of phenolic, tocopherol, and phytosterol contents during the storage were also studied. The phenolic content and antioxidant activity of refined oil were significantly lower than those of crude oil after the accelerated storage. There was a decrease of 72.5% tocopherol content and 31.1% phytosterol content in the crude oil and a decrease of 67% tocopherol content and 12.1% phytosterol content in the refined oil during the accelerated storage. There was no significant difference in tocopherol and phytosterol contents for crude and refined oils after the storage. The rate of degradation of tocopherol and phytosterol contents in refined oil was slower than that in crude oil during the storage.

Keywords: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay, phenolic contents, tocopherol, phytosterol

Introduction

Kenaf (*Hibiscus cannabinus* L.) is a native fiber plant from India and Africa. The uses of kenaf plant are diverse; it is a source of raw material for fiber-based industries and paper making (1). Kenaf seeds represent a potentially unused resource, which is usually discarded as industrial waste. Previous research suggests that kenaf seed oil has a relatively high content of monounsaturated and polyunsaturated fatty acids (MUFA and PUFA), which are nutritionally beneficial for human health and seem to be a good source of lipid-soluble bioactive compounds (2). The total amount of unsaturated fatty acids in kenaf seed oil has been reported to be 74.8% consisting mainly of oleic acid (C_{18:1}), linoleic acid (C_{18:2}), and linolenic acid (C_{18:3}) (3). It has been reported to possess a high level of polyphenols, tocopherols, and phytosterols (2,4). These bioactive compounds possess antioxidants, which are well recognized for their potential in providing health benefits and preventing ageing-related diseases, including cancer and heart disease (5). Vanillic acid, caffeic acid, gallic acid, *p*-hydroxybenzoic acid, ferulic acid, *p*-coumaric acid, and protocatechuic acid were identified as the main phenolic acids in the kenaf seed oil, which contributed to the antioxidant activity for protecting the oil against oxidation (3). Besides that, kenaf seed oil has been reported to exhibit comparable anti-hypercholesterolemic

effect as that exhibited by the commercial hypocholesterolemic drug (6). Thus, research focusing on the application of kenaf seed oil has been increasing.

Solvent extraction is the most commonly used technique to extract crude kenaf seed oil from kenaf seeds. There was 20.8% of oil content found in the kenaf seeds by using solvent extraction method (3). However, the crude oil cannot be consumed directly without proper processing because of the unacceptable color and odor present in the crude oil. The refining process is designed to remove the undesirable components in the crude oil such as free fatty acids, color pigments, gums, waxes, phosphates, and odoriferous materials or reduce them to an acceptable level suitable for human consumption (7,8). However, antioxidant compounds such as phenolics, carotenoids, tocopherols, and phytosterols may be lost during the refining process, subsequently reducing the nutritional value and quality of oil. Thus, a chemical refining process has been applied to minimize the losses of bioactive compounds and protect the oil stability, although chemical refining results in lower yield, higher investment cost, and higher waste compared with the physical refining process (7).

There is an increasing demand for nutritive and functional edible oil in the market. Thus, research that focuses on exploring the new sources of edible oil has increased. Quality and stability represent the

main factors in the production, acceptance, and marketing of edible oil products. Thus, protection from lipid oxidation is a critical factor in oil quality. Lipid oxidation is one of the main processes causing deterioration of food products during processing and storage (9) and results in the change of color, odor, and flavor of food products. It also affects the nutritional value, functionality, and toxicity of the oil (10). The natural antioxidants presented in the oil such as phenolics, tocopherols, and phytosterols can help to preserve the oil quality. To the best of our knowledge, there is a scarcity of research on the antioxidant activity and changes in the bioactive compounds of refined kenaf seed oil during accelerated storage. In this study, the antioxidant activity and changes in phenolic, tocopherol, and phytosterol contents of crude and refined kenaf seed oil were compared during the accelerated storage.

Materials and Methods

Materials and chemicals Kenaf seeds were obtained from Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. Ethanol, Folin–Ciocalteu's phenol reagent, methanol, *n*-hexane, phosphoric acid, potassium hydroxide, sodium carbonate, and sodium hydroxide were obtained from Merck (Darmstadt, Germany). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH[•], 95%), 5 α -cholestane, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [Trolox (TE), 97%], *N,O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1 mL/100 mL trimethylchlorosilane (TMCS), phytosterols standard (β -sitosterol, campesterol, and stigmasterol), tocopherols standard (α - and γ -) were the products obtained from Sigma-Aldrich (St. Louis, MO, USA). Acid-activated bleaching earth was the product of Taiko Clay Marketing Sdn. Bhd. (Perak, Malaysia).

Solvent extraction of crude kenaf seed oil Kenaf seeds were ground into fine powder (<1 mm diam.) using a food grinder (Panasonic, Tokyo, Japan). Crude kenaf seed oil was extracted from the seeds with a Soxhlet extractor using hexane at 60°C for 3 h [sample-to-solvent ratio 1:5, w/v]. Hexane was evaporated using a Buchi Multivapor P-6 (BÜCHI Labortechnik AG, Switzerland) at 55°C under a reduced pressure condition of 241 mbar to recover the oil. Nitrogen gas (99.9%) was flushed to remove the residual solvents in the oil.

Oil refining process The refining process was conducted according to a previously described chemical refining process (4). Crude kenaf seed oil was acid-degummed by pretreating the oil with 0.3% w/w of phosphoric acid (85% concentration) at 70°C for 10 min. Then, Milli-Q water (3% w/w) was added into the oil and agitated in a heated water bath at 70°C for 30 min. After cooling the mixture, the gums were removed from the degummed oil by centrifugation. A stoichio-

metric quantity of sodium hydroxide solution (12 °Baume) with an excess level of 0.2–0.5% was added into the degummed oil to neutralize the free fatty acid. The soapstock was removed by centrifugation at 10,000 $\times g$ for 10 min. The neutralized oil was washed three times with Milli-Q water to remove the residual soap in the oil. The neutralized oil was bleached with 1.2% w/w of Taiko Classic acid-activated bleaching earth using a Buchi Multivapor P-6 at 95°C for 30 min. The bleached oil was followed by the final deodorization step performed using a lab-scale glass deodorizer at 200°C for 1 h.

Accelerated storage condition The crude and refined kenaf seed oils were tested under accelerated storage conditions using the Schaal oven test and conditioned at 65°C for 24 days, where one day of storage represents one month of storage at room temperature (11). Duplicate samples of crude and refined kenaf seed oil were stored in 28 mL loosely capped McCartney bottles wrapped in aluminium foil and stored in a drying oven (Mettler, Schwabach, Germany). A set of duplicated samples was removed from the oven on day 0, 6, 12, 18, and 24 of the storage and subjected to the analyses to assess the changes in the antioxidant activity and bioactive compounds during storage.

Assessment of antioxidant activity and bioactive compounds

Total phenolic content (TPC): TPC analysis was conducted according to a previously described method (12). The TPC of the samples was expressed in mg GAE (gallic acid equivalents)/100 g oil through the calibration curve of gallic acid at concentrations of 0.02–0.1 mg/mL.

Chromatographic determination of tocopherol: Tocopherol content in kenaf seed oil was determined according to a previously described method (4). High-performance liquid chromatography (HPLC) column (1200 Series; Agilent Technologies, Santa Clara, CA, USA) with a UV-Vis detector and a Purospher STAR RP-18e column (5 μ m $\times$ 250 mm $\times$ 4.6 mm) (Merck) was used to analyze the tocopherol content. Separation of all tocopherols was done on the basis of gradient elution when the solvent flow rate was maintained at 1 mL/min. The solvents consisted of (A) methanol/water (95:5, v/v) and (B) methanol. The peaks were quantified by its absorbance at 295 nm. The tocopherols concentration was expressed in mg/100 g oil through the calibration curve of a series of standard solutions of α - and γ -tocopherols at a concentration of 0.2–1 g/L. Prior to HPLC analysis, the oils were diluted with methanol, filtered with 0.45 μ m nylon syringe filter, and injected with 20 μ L samples. Limit of detection was determined using the lowest concentration that could produce peak area with a signal-to-noise ratio of 3. Limit of quantification was determined using the lowest concentration on the calibration curve that could produce peak area with a signal-to-noise ratio of 10 at which quantitative results could be reported with a high level of confidence.

Chromatographic determination of phytosterol: Phytosterol content in kenaf seed oil was determined according to a previously

established method (4). Total lipid content (250 mg) and 100 μ L of 5 α -cholestane (1 mg/mL) were refluxed with 5 mL of ethanolic potassium hydroxide (6 g/100 mL) for 60 min. The unsaponifiable component was first extracted three times with 10 mL of petroleum ether. The extracts were combined and washed three times with 10 mL of neutral ethanol–water mixture (1:1, v/v) and then dried over anhydrous sodium sulfate. The extract was dried under reduced pressure. N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) (125 μ L) with 1 mL/100 mL trimethylchlorosilane (TMCS) was added to the dry residue. The mixture was vortexed for approximately 10 s and heated at 70°C in a water bath for 30–60 min. After cooling, 1 μ L aliquot was directly injected into the gas chromatography column (7890A; Agilent Technologies), equipped with a flame ionization detector and a HP-5 column (30 m $\times$ 0.25 mm i.d., 0.32 μ m film thickness) with a split ratio of 95:1. The initial column temperature was 250°C and programmed to increase at a rate of 2°C/min to 300°C and then be held for 12 min. The phytosterols concentration was calculated from the following equation and expressed in mg/100 g oil.

$$\text{Phytosterol} = \frac{\text{Peak area of oil} \times \text{mg of internal standard}}{\text{Peak area of internal standard} \times \text{mass of oil}} \times 100 \quad (1)$$

Radical scavenging activity assays: DPPH $^{\bullet}$ and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS $^{••}$) radical scavenging activities were determined using previously described methods (12). The DPPH and ABTS radical scavenging activities (%) were calculated using the expression $[1 - (A_s/A_c) \times 100\%]$, where A_s and A_c represent the absorbance of the sample and control, respectively. Measurements were calibrated to a standard curve of Trolox at concentrations of 0.02–0.1 mg/mL and expressed as mg Trolox equivalents (mg Teq/100 g oil).

Statistical analysis All experiments were conducted in duplicate and measurements were replicated two times. Results were expressed as mean $\pm$ standard deviation and analyzed with one-way analysis of variance (ANOVA) and independent T-test using MINITAB 16 (Minitab Inc, State College, PA, USA). The average values were compared with Tukey's post hoc test for one-way ANOVA, considering the significant level at $p < 0.05$.

Results and Discussion

Phenolic contents Figure 1 shows the phenolic contents of refined kenaf seed oil to be significantly lower ($p < 0.05$) than crude oil at day 0. This indicated that 71.4% of phenolic content was lost after the refining process. Previous study reported that deodorization step in the refining process caused the marked decrease in the phenolic content of kenaf seed oil because of the high temperature applied (4). After that, the phenolic contents of crude and refined oils decreased significantly ($p < 0.05$) from day 0 to day 12. This indicated

Fig. 1. Total phenolic contents in crude and refined kenaf seed oils during accelerated storage. Means $\pm$ standard deviations with different superscript letters *abc* indicate significant differences ($p < 0.05$) among different days of the same sample and means $\pm$ standard deviations with different superscript letters *AB* indicate significant differences ($p < 0.05$) between two samples on the same day of storage.

the degradation of phenolic contents in kenaf seed oil during storage. Phenolic compounds are very important plant constituents because of their ability to scavenge free radicals through their hydroxyl groups (13). Phenolic compounds may disrupt the initiation and propagation stages of the oxidation mechanism since they react with lipid radicals to form more stable products (14). Thus, phenolic compounds have the ability to delay the lipid oxidation process. Phenolic compounds can exert different antioxidant activities in different oil systems depending on their mode of action (15). It is of interest to investigate the changes in the phenolic contents of vegetable oil during the storage test.

However, an increase in the phenolic contents was observed during day 18 in both crude and refined oils. There was no significant difference in the phenolic contents from day 18 to day 24 in both crude and refined oils. The oil matrix, storage temperature and period, and extraction method of oil may be the factors that influence the phenolic content (16). The increase in the phenolic contents may be explained by the release of phenolic compounds from bound structures or chemical changes of phenolics at elevated temperature (17). Thus, storage at elevated temperature might favor the release of phenolic compounds from oil (16). Surjadinata and Cisneros-Zevallos (18) explained that biosynthesis of the phenolic compounds in bulk oil can be enhanced during accelerated storage as a response to environmental stress conditions.

Tocopherol content Table 1 shows that total tocopherols in crude and refined kenaf seed oil were decreasing during the accelerated storage. The decrease in the total tocopherol content was because of the oxidation and degradation of oil at elevated temperature (2). There was no significant difference in the tocopherol contents of crude and refined oils on day 0, indicating that the chemical refining

process in this study did not have a negative influence on the tocopherol content, which was different from the results of the previous studies, wherein a higher temperature (240°C) applied in the deodorization process caused increased deterioration of bioactive compounds compared with the 200°C temperature used in the deodorization process in this study (7,19). On the last day of storage, the tocopherol contents in refined oil were higher than those in the crude oil although there was no significant difference ($p>0.05$). This showed that both crude and refined oils can preserve the tocopherol contents. Tocopherols are the most abundant natural antioxidants found in vegetable oils, which can exert biological activities and assist in protecting cellular membranes and increase shelf stability of vegetable oils (20). This compound prevents lipid oxidation by acting as captors of lipid peroxyl radicals, inhibiting these radicals to react with the lateral chains of adjacent fatty acids or with membrane proteins. α -Tocopherol interrupts the chain reactions of lipid peroxidation by donating its hydrogen atom from the hydroxyl group to the peroxyl radical ($\text{LOO}\cdot$), producing an α -tocopheryl and a hydroperoxide (21).

The total tocopherols lost were 72.5% in crude oil and 67% in refined oil after accelerated storage. Among the tocopherols in kenaf seed oil, the higher loss was that of the α -tocopherol constituent. The percent of α -tocopherol lost was 81.2% and that of γ -tocopherol was 70% in crude oil after the storage, whereas α -tocopherol loss of 82.9% and γ -tocopherol loss of 62% occurred in refined oil after the storage. The thermal stability of α -tocopherol was found to be lower than γ -tocopherol. This result was in accordance with the results of previous studies. α -Tocopherol presented lower stability than γ -

tocopherol in rapeseed oil during the storage at 40°C for 16 days (22). Player *et al.* (23) reported that α -tocopherol degraded faster than γ - and δ -tocopherols in soybean oil during the storage at 50°C and α -tocopherol was completely destroyed within 16 days. The degree of degradation of tocopherols in rice bran oil at 100 and 180°C showed the following order: α ->(γ + β)> δ -tocopherol, which indicated the α -tocopherol was the least stable (20). However, α -tocopherol possesses the greatest biological activity among tocopherols by its biological activity is double than that of the β - and α - and is 100 times greater than that of δ -tocopherol (24).

Phytosterol content Phytosterols have been scientifically proven to reduce low-density lipoprotein cholesterol by including 10–15% of phytosterol as part of a healthy diet (25). Owing to its health benefits, Food and Drug Administration (FDA) and European Union (EU) suggested to include free phytosterols in conventional foods and established labeling guidelines. Thus, the determination of phytosterol profile in vegetable oils is important to avoid adulteration (26). β -Sitosterol, campesterol, and stigmasterol were identified in this study as shown in Table 2. The major type of phytosterol in the kenaf seed oil studied was β -sitosterol followed by campesterol and stigmasterol, which was in agreement with the phytosterol contents reported in previous studies (3,4).

The total phytosterols in crude kenaf seed oil were significantly higher ($p<0.05$) than those in the refined oil on day 0. This showed that the refining process had partly removed the phytosterol contents in refined oil because of the dehydration of phytosterols that occurred in the refining process (4). The phytosterol contents in

Table 1. Tocopherol contents in crude and refined kenaf seed oils during accelerated storage

Storage (day)	γ -Tocopherol (mg/100 g)		α -Tocopherol (mg/100 g)		Total tocopherol (mg/100 g)	
	Crude	Refined	Crude	Refined	Crude	Refined
0	50.4±3.8 ^{aA1)}	49.9±4.6 ^{aA}	14.4±2.3 ^{aA}	15.8±0.8 ^{aA}	64.8±2.5 ^{aA}	65.7±5.3 ^{aA}
6	42.5±5.0 ^{bA}	47.6±5.1 ^{aA}	10.2±0.6 ^{bB}	12.6±1.4 ^{aA}	52.7±5.5 ^{bA}	60.2±4.4 ^{aA}
12	21.5±3.6 ^{cA}	25.6±5.9 ^{aA}	3.3±0.7 ^{cA}	2.6±0.1 ^{cA}	24.8±4.2 ^{cA}	28.2±5.9 ^{aA}
18	15.5±1.6 ^{cA}	23.0±3.8 ^{aA}	2.7±0.3 ^{cB}	3.7±0.1 ^{cA}	18.2±1.5 ^{cB}	26.7±3.8 ^{aA}
24	15.1±2.8 ^{cA}	19.0±1.9 ^{aA}	2.7±0.4 ^{cA}	2.7±0.1 ^{cA}	17.8±3.1 ^{cA}	21.7±2.0 ^{bA}
Limit of detection ($\mu\text{g/mL}$)	0.27		0.31			
Limit of quantification ($\mu\text{g/mL}$)	0.89		1.02			

¹⁾Values in each column followed by different lowercase superscript letters are significantly different ($p<0.05$); ^AValues in each row for γ -, α -, and total tocopherol respectively followed by different uppercase superscript letters are significantly different ($p<0.05$).

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

Storage (day)	Campesterol (mg/100 g)		Stigmasterol (mg/100 g)		β -Sitosterol (mg/100 g)		Total phytosterol (mg/100 g)	
	Crude	Refined	Crude	Refined	Crude	Refined	Crude	Refined
0	72.6±9.1 ^{aA1)}	56.8±5.0 ^{aB}	40.9±5.6 ^{aA}	24.7±2.1 ^{abB}	589.5±19.8 ^{aA}	463.3±47.7 ^{aB}	703.0±31.9 ^{aA}	544.8±54.7 ^{aB}
6	56.9±4.6 ^{bA}	55.3±3.0 ^{abA}	27.3±2.1 ^{bA}	25.0±0.9 ^{abA}	448.8±37.7 ^{bA}	425.8±23.0 ^{abA}	533.0±44.3 ^{bA}	506.1±25.7 ^{aA}
12	55.7±5.1 ^{bA}	54.1±1.9 ^{abA}	26.7±2.3 ^{bA}	23.3±1.6 ^{bA}	440.6±41.2 ^{bA}	420.9±24.2 ^{abA}	523.0±48.3 ^{bA}	498.3±26.4 ^{aA}
18	54.6±4.3 ^{bA}	51.4±2.2 ^{abA}	26.9±1.3 ^{bA}	24.8±3.0 ^{abA}	432.4±25.3 ^{bA}	424.9±16.6 ^{abA}	514.0±30.6 ^{bA}	501.1±21.3 ^{aA}
24	51.3±4.0 ^{bA}	49.4±0.2 ^{bA}	24.4±2.8 ^{bB}	28.7±0.8 ^{aA}	408.5±30.6 ^{bA}	401.0±7.3 ^{bA}	484.2±37.4 ^{bA}	479.0±7.3 ^{aA}

¹⁾Values in each column followed by different lowercase superscript letters are significantly different ($p<0.05$); ^AValues in each row for campesterol, stigmasterol, β -sitosterol, and total phytosterol respectively followed by different uppercase superscript letters are significantly different ($p<0.05$).

crude kenaf seed oil significantly decreased ($p < 0.05$) from day 0 to day 6 and then slightly decreased until day 24. There was no significant difference in the phytosterol contents in crude oil from day 6 to day 24. There were 31.1% of phytosterol contents lost in the crude oil after the accelerated storage. In contrast, the phytosterol contents in refined kenaf seed oil decreased slightly and there was no significant difference in the phytosterol content in refined oil from day 0 to day 24. There were 12.1% of phytosterol contents lost in the refined oil after the storage. The degradation of phytosterol during accelerated storage was more stable compared with that of tocopherol. The results showed that the refined oil can preserve the phytosterol contents well during the storage. The initial composition, present of minor compounds of antioxidant or pro-oxidant characteristics, degree of processing, and their storage conditions will affect the deterioration of oils. Refining process helps to remove the pro-oxidants such as chlorophyll, free fatty acids, and trace metal ions in the crude oil (27). Although refining decreased the antioxidant activity and phenolic content of refined oil during storage, it was able to protect tocopherol and phytosterol in a better manner compared with crude oil.

DPPH[•] radical scavenging activity assay DPPH[•] is a stable free radical containing an odd electron in its structure and usually used for the assessment of radical scavenging activity in chemical analysis. It is known that DPPH[•] free radical scavenging by antioxidants is because of their hydrogen donating ability (28). Figure 2 shows that the DPPH radical scavenging activity of crude kenaf seed oil decreased significantly ($p < 0.05$) from day 0 to day 18 but then it increased slightly until day 24. However, there was no significant difference in the DPPH radical scavenging activity in crude oil for day 18 and day 24. In contrast, DPPH radical scavenging activity of refined kenaf seed oil showed a decreasing trend during the 24 days of accelerated storage. The decreasing DPPH radical scavenging activity was because of the degradation of antioxidant components in kenaf seed oil during the accelerated storage. Previous studies showed that the phenolic content correlated well with DPPH radical scavenging activity in the oil sample (29,30). However, a good correlation between TPC and DPPH radical scavenging activity was not observed in this study. This is because the DPPH radical scavenging activity is not only contributed by phenolic compounds but also by the synergism of phenolic compounds with other antioxidant compounds present in the sample contributing to the overall antioxidant activity in the sample (31). Besides that, DPPH assay is normally applicable to hydrophobic systems (32). Phenolics are considered as hydrophilic compounds (33). Thus, DPPH assay is mainly affected by lipophilic antioxidants such as tocopherols, phytosterols, carotenoids, and chlorophyll. A greater loss of DPPH radical scavenging activity occurred on day 12 for refined oil, which same with the greater loss of tocopherol contents in refined oil at day 12.

However, the DPPH radical scavenging activity of crude oil was significantly higher ($p < 0.05$) than that of refined oil on the last day of

Fig. 2. DPPH radical scavenging activity in crude and refined kenaf seed oils during accelerated storage. Means $\pm$ standard deviations with different superscript letters *abc* indicate significant differences ($p < 0.05$) among different days of the same sample and means $\pm$ standard deviations with different superscript letters *AB* indicate significant differences ($p < 0.05$) between two samples on the same day of storage.

storage although the DPPH radical scavenging activity of refined oil was higher than that of crude oil on day 0. This may be because the refined oil is less oxidatively stable than crude oil as the refining process removes most of the phenolic compounds in the refined oil. This has been proven by a study that showed that the oil containing phenolic extract was more oxidatively stable than the oil added with an equimolar concentration of α -tocopherol (14). A higher level of natural antioxidants such as phenolics, carotenoids, and phosphatides are present in the crude oil, which has synergistic effects on antioxidants (34). However, refining process reduced these compounds during the process, which resulted in the less protective ability of the refined oil.

ABTS^{•+} radical scavenging activity assay ABTS assay is based on the generation of a blue/green ABTS^{•+} by converting ABTS into its radical cation (ABTS^{•+}) through the addition of sodium persulphate. ABTS^{•+} is soluble in both aqueous and organic solvents, so ABTS assay is applicable to systems containing both hydrophilic and lipophilic components. ABTS assay assessed the antioxidant activity better in a plant food containing hydrophilic, lipophilic, and high-pigmented antioxidant compounds than DPPH assay (32). Thus, ABTS radical scavenging activity in this study might be affected by the contents of compounds that contribute to the antioxidant activity such as phenolics, tocopherols, phytosterols, carotenoids, and chlorophyll.

The trend of ABTS radical scavenging activity was same as the trend of DPPH radical scavenging activity. Figure 3 shows that the ABTS radical scavenging activity of crude kenaf seed oil decreased significantly ($p < 0.05$) from day 6 to day 18, but there was a slight increase in ABTS radical scavenging activity from day 18 to day 24. In contrast, ABTS radical scavenging activity of refined kenaf seed oil showed a decreasing trend from day 0 to day 24. The decreasing

Fig. 3. ABTS radical scavenging activity in crude and refined kenaf seed oils during accelerated storage. Means±standard deviations with different superscript letters *abc* indicate significant differences ($p<0.05$) among different days of the same sample and means±standard deviations with different superscript letters *AB* indicate significant differences ($p<0.05$) between two samples on the same day of storage.

ABTS radical scavenging activity was because of the degradation of antioxidant components in kenaf seed oil during the accelerated storage. The increase of ABTS radical scavenging activity in crude oil from day 18 to day 24 might be because of the increase of phenolic contents in the crude oil. Phenolic metabolism might be stimulated by imposing environmental stress, which might activate the key enzyme in the biosynthesis of phenolic compounds, resulting in the increase of phenolic contents in crude oil during accelerated storage (18). However, the ABTS radical scavenging activity of crude oil was significantly higher ($p<0.05$) than that of refined oil on the last day of storage although the ABTS value of refined oil was higher than that of the crude oil on day 0. This might be because the phenolic contents in crude oil were higher than those in refined oil.

Refining is a process to remove impurities from vegetable oil to make it suitable for human consumption. The phenolic contents and antioxidant activity, evaluated by DPPH and ABTS assays in refined kenaf seed oil, were significantly lower than those in crude kenaf seed oil after accelerated storage. This indicated that the refining process has a greater impact on the changes of antioxidant activity during storage. However, there was no significant difference in the tocopherol and phytosterol contents in crude and refined kenaf seed oils after accelerated storage. The degradation of tocopherol and phytosterol contents in refined oil was slower than that in crude oil during the storage. This showed that refined kenaf seed oil was able to protect the bioactive compounds during the storage compared with crude oil.

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