International Food Research Journal 22(5): 1898-1905 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of microwave pretreatment on stability of kenaf (*Hibiscus cannabinus* L.) seed oil upon accelerated storage

[1*]Nyam, K.L., [1]Tan, C.H. and [2]Long, K.

[1]Department of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, 56000 Kuala Lumpur, Malaysia
[2]Malaysian Agricultural Research & Development Institute (MARDI) PO Box 12301, 50774 Kuala Lumpur

Article history

Received: 9 January 2015
Received in revised form: 29 January 2015
Accepted: 7 February 2015

Keywords

Microwave pretreatment
Oxidative stability
Antioxidant activity
Accelerated storage
Fatty acid composition

Abstract

The aim of this study was to investigate the oxidative stability, antioxidant activity and fatty acid composition of 2 minutes microwave pre-treated kenaf seed oil (MKSO) in comparison with the untreated kenaf seed oil (KSO) under accelerated storage for 24 days. Results obtained on oxidative stability for both KSO and MKSO by the end of storage with PV were 9.83 meq O_2/kg and 8.97 meq O_2/kg, respectively; p-Anv were 17.28 and 13.48, respectively; TOTOX value of 36.94 and 31.42, respectively; IV value were measured 84.50 g of I_2/ 100 g and 84.34 g of I_2/ 100 g oil, respectively; FFA value of 5.67 mg KOH/100g oil and 5.14 mg KOH/100g oil, respectively. Aside from that, the antioxidant activity in MKSO was better than KSO. For the fatty acid composition, the oleic and linoleic acids were affected significantly throughout storage for both KSO and MKSO. MKSO presented a better overall oxidative stability, antioxidant activity and retained higher content of MUFA and PUFA significantly ($p<0.05$) upon accelerated storage.

Introduction

Kenaf (*Hibiscus cannabinus* L., Malvaceae) is an annual herbaceous crop which considered as one of the most valuable fibre crop native to India and Africa (Mohamed *et al*., 1995). Over the last few decades, kenaf seeds have been disposed as agricultural waste or rendered into animal feed during harvesting or processing of kenaf (Ng *et al*., 2013a). However, intensive researches have been done for several years on kenaf plants especially the kenaf seeds. Researchers found that kenaf seeds contain high oil content (20.4%) which made it comparable to those common edible oil such as cotton seed oil and soybean oil (Nyam *et al*., 2009). The abundance of polyunsaturated and monounsaturated fatty acid especially linoleic (45.9%) and oleic acids (29.2%) determined that kenaf seed oil has a quality that will be beneficial to human health due to the presence of cardio-protective properties (Ismail *et al*., 2011). Moreover, the high percentage of oleic acid in oil made it desirable in terms of nutrition and high stability as cooking oil (Nyam *et al*., 2009). Apart from the essential fatty acids, kenaf seed oil also composed of significant levels of tocopherols, phytosterols and phenolic compounds (Ng *et al*., 2013a). These considerable amounts of phytochemicals that possessed antioxidant, cardio-protective and anti-inflammatory activities have been pharmacologically investigated (Sachdewa and Khemani, 2003).

Being highly unsaturated, kenaf seed oil is chemically unstable and susceptible to lipid oxidation especially when exposed to oxygen, moisture, light and high temperature (Ng *et al*., 2013a). With the concern of the adverse effects of lipid oxidation on oil deterioration and human health, a pre-treatment before extraction should be sought to delay the oil deterioration.

In microwave heating, energy is delivered directly to the food materials through molecular interaction with the electromagnetic field (Venkatesh and Raghavan, 2004). Due to the molecular friction and excitation of both water and fat molecules, heat is generated instantaneously within the food materials first and then transfer to the entire volume of food (Yadoji *et al.*, 2003). Thus, this heating mechanism provided many advantages on the pre-treated seed oils such as improvement of extracted oil yield and quality, reduced energy consumption and processing time (Ramanadhan, 2005). Studies have shown that oils that undergo microwave pre-treatment will have better quality in terms of physicochemical properties such as p-Anisidine value, colour and smell (Anjum *et al*. 2006). Microwave roasted rapeseed oils showed

*Corresponding author.
Email: *nyamkl@ucsiuniversity.edu.my*
Tel: +603-91018880; Fax: +603-91023606

a markedly improved oxidative stability due to the increase of phenolic antioxidants (Veldsink *et al.,* 1999). Therefore, this pre-treatment can be applied on kenaf seed oils to delay the lipid deterioration upon storage as to further evaluate the oil quality of 2 minutes microwave pre-treated kenaf seed oil which has been done in preliminary study.

Thus, this study was initiated to investigate and compare the oxidative stability, antioxidant activity and fatty acid composition of 2 minutes microwave pre-treated kenaf seed oil and untreated kenaf seed oil under accelerated storage condition for 24 days.

Materials and Methods

Sample

Dried kenaf (*Hibiscus cannabinus* L.) seed was obtained from the Malaysian Agricultural Research and Development Institute (MARDI, Serdang, Malaysia) and ground into powder form with a grinder (SHARP, Japan). The particle size of the kenaf seed powder was less than 1 mm.

Microwave roasting of kenaf seeds

Kenaf seeds were placed in Pyrex petri dishes (20.0g in each petri dish) and were roasted in a consumer model microwave oven (Model R-397J(S), SHARP, Malaysia) for 1, 2 and 3 minutes. Kenaf seeds were then allowed to cool to ambient temperature and grinded into powder form by using grinder (Model BL1111AD, Tefal, Indonesia). The particle size of kenaf seed powder was approximately 1 mm.

Kenaf seed oil extraction

The oils were extracted from the seeds with soxhlet extractor using hexane at 60ºC for 3 hours. The oil was then recovered by evaporating off the solvent using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by flushing with 99.9%.

Oxidative stability under accelerated storage

The oxidative stability of bulk oil and MKSO was tested under accelerated storage conditions (65°C for 24 days, for which 1 day of storage represents 1 month of storage at room temperature) by the Schaal oven test. At Day 0, Day 6, Day 12, Day 18 and Day 24, oxidative stability tests were conducted. Bulk kenaf seed oils were kept in a Schott bottle and stored under the same accelerated storage conditions and were used as a positive control for comparison with the MKSO in terms of oxidative stability. To reduce the availability of oxygen in the Schott bottle, all of the samples in the Schott bottles were flushed with 99.9% nitrogen.

Chemical analysis

Peroxide value (PV)

The peroxide values (PV) of the bulk oil and MKSO were determined as described in the method of the Ng *et al.* (2013b). An oil sample (5 g) was weighed into a 250 mL Erlenmeyer flask, and 30 mL of a 3: 2 (v/ v) acetic acid/chloroform mixture was added to the samples. The flask was swirled to dissolve the sample. An aliquot of 1 mL saturated potassium iodide solution was added to the sample and the reaction mixtures were left to stand for 1 min at room temperature with occasional shaking. Thirty millilitres of deionised water was added to the sample and the resulting reaction mixtures were each titrated with 0.01 N standardised sodium thiosulphate until the yellow colour disappeared. The results were expressed as milliequivalents of peroxide per kg of oil sample (meq/ kg) and were calculated based on Eq. (1).

$$\text{PV (milliequivalent peroxide/ kg oil)} = S \times M \times (1000/ \text{g test portion}) \quad (1)$$

Where S= mL $Na_2S_2O_3$ solution and M= molarity of $Na_2S_2O_3$ solution.

p-anisidine value (p-Anv)

The p-anisidine value (p-Anv) of the bulk oil and MKSO were determined as described in the method of the Ng *et al.* (2013b). An oil sample (0.5 g) was dissolved in 12.5 mL of cyclohexane. The solution (Ab) was measured at 350 nm using a UV-vis spectrophotometer (Uviline 9400, Secomam, France). The cyclohexane solvent was used as the reference (blank) solution. An aliquot (5 mL) of the oil mixture or blank was mixed with 1 mL of p-anisidine reagent (0.25 g/ 100 mL glacial acetic acid). The mixtures in the capped tubes were shaken vigorously for 10 min. The absorbance of the mixtures (As) was measured at 350 nm using a UV-vis spectrophotometer. The p-AV was calculated based on Eq. (2):

$$\text{p-AnV} = [12.5 \times (1.2\,\text{As} - \text{Ab})/0.5] \quad (2)$$

Where As = the absorbance of the sample solution after reaction with the p-anisidine reagent.
Ab = the absorbance of the sample.

TOTOX value

The TOTOX values of the bulk oil and MKSO were calculated from the PVs and p-AnVs of the oil

samples using Eq. (3), which was adapted from Ng *et al.* (2013b).

$$\text{TOTOX value} = 2\text{PV} + \text{p-AnV} \quad (3)$$

Iodine value (IV)

The iodine values of the bulk oil and MKSO were determined as described in the method of IUPAC (1987) with slight modification, 0.3 g ± 0.01 g of oil sample was weighed into a 250 mL conical flask and dissolved with 20 mL of acetic acid (glacial): cyclohexane (1:1, v/v). 25 mL of Wij's solution was then pipetted into the conical flask. It was shook well in the fumehood cupboard (Kenkyuu, Malaysia) and stored in the dark for 30 minutes. After that, 15 mL of 10% potassium iodide solution and 100 mL of deionised water were added into the conical flask. The solution in conical flask was titrated with 0.1 M standard sodium thiosulphate solution until the solution changed to pale yellow colour. During the titration, the conical flask was swirled constantly and vigorously. Then, few drops of starch indicator were added into the solution and the titration was continued until the blue colour of solution disappeared. The volume of titrant was recorded. The steps were repeated for blank at which no oil sample was added into it. The iodine value was expressed as g iodine / 100 g oil and was calculated based on the Eq. (4).

$$\text{Iodine Value (IV)} = \frac{(B-S)}{Wt} \times 12.69 \times N \quad (4)$$

Where B – S = The difference between the volumes of sodium thiosulphate solution required for the blank and for the sample, respectively, in mL; N = Normality of the sodium thiosulphate solution; Wt = Mass of the oil sample (g).

Free fatty acid (FFA)

Free fatty acids (FFAs) were determined using the direct titration method of the Ng et al. (2013b). Neutralised alcohol was prepared with 1.25 mL of absolute ethanol, 2 drops of oil and 50 µL phenolphthalein indicator in a clean, dry flask. The flask was placed in a water bath at 60ºC until warm. Sufficient sodium hydroxide (0.01 N) was added to produce a faint permanent pink colour. An oil sample (1.41 g) was added to the neutralised alcohol and titrated with sodium hydroxide until the same faint permanent pink colour appeared (phenolphthalein indicated the endpoint). The FFA content was calculated using Eq. (5) and was expressed as the percentage (%) of oleic acid.

$$\%\ \text{FFA (as oleic acid)} = [(V_{NaOH} \times N_{NaOH} \times 282.46)/W] \times 100 \quad (5)$$

Where V = the volume of NaOH titrant (mL), N = the normality of NaOH titrant (mol/ 1000 mL), 282.46 = the MW of oleic acid (g/ mol) and W= the sample mass (g).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay

The antioxidant activity of the kenaf seed extract was determined by using the 2,2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging capacity assay as described by Razmkhah *et al*. (2013).

Total phenolic content (TPC)

Phenolic compounds were extracted according to Nyam *et al*. (2013) extraction method with some modifications. Firstly, 1.75g ± 0.01 g of oil sample was weighed into a 50 ml Falcon tube. Next, 5ml of hexane was transferred into it and vortexed using a vortex mixer (VTX-3000L, LMS, Japan). Then, 10 ml of methanol: water (60:40, v/v) was added to extract the phenolic compounds and vortexed for 5 minutes followed by centrifugation at 4500 rpm for 5 minutes. The lower layer which was the methanolic phase was collected while the upper layer, hexane phase was extracted twice with previous step to ensure the phenolic compounds were completely extracted. After that, methanolic phase from 3 extractions was transferred into another Falcon tube and mixed with 10ml of hexane in order to eliminate the residual oil samples in separating funnel and allowed to stand for 10 minutes. The lower fraction, methanolic fractions were subsequently transferred into a round bottom flask were then undergone rotary evaporation with rotary evaporator (R-200, BUCHI Labortechnik AG, Switzerland) at 45ºC until dried. The solution was then reconstituted with10 ml of methanol: water (60:40, v/v) for day 0; 5 ml of methanol: water (60:40, v/v) for day 6 and 12; 4ml of methanol: water (60:40, v/v) for day 18 and 2 ml of methanol: water (60:40, v/v) for day 24 respectively.

Total phenolic content of oil samples were determined by Folin-Ciocalteu assay described by Wong et al. (2014). A 300 µL aliquot of a 10 mg/ mL sample was added to 1.5 mL of 10-fold diluted Folin-Ciocalteu reagent (FCR) and mixed well. The mixture was allowed to stand at room temperature for 5 min. Next, 1.2 mL of sodium carbonate (7.5%, w/v) solution was added, and the sample was mixed thoroughly with a vortex mixer and allowed to stand for 30 min. After 30 min, the absorbance was

Table 1. Changes in peroxide values (PV), p-anisidine values (p-Anv), total oxidation (TOTOX) values, iodine values (IV) and free fatty acid values (FFA) of untreated kenaf seed oil (KSO) and microwave pre-treated kenaf seed oil (MKSO) under accelerated storage at 65°C for 24 days

Chemical analysis	Days	Samples	
		KSO	MKSO
PV (meq O_2 / kg of oil)	0	1.49 ± 0.04^{eB}	1.94 ± 0.03^{eA}
	6	3.88 ± 0.03^{dB}	4.04 ± 0.01^{dA}
	12	5.15 ± 0.09^{cA}	5.02 ± 0.05^{cB}
	18	5.88 ± 0.01^{bA}	5.54 ± 0.02^{bB}
	24	9.83 ± 0.03^{aA}	8.97 ± 0.07^{Ab}
p-Anv	0	4.89 ± 0.09^{eA}	4.61 ± 0.05^{eB}
	6	9.34 ± 0.05^{dA}	8.53 ± 0.04^{dB}
	12	12.27 ± 0.01^{cA}	9.83 ± 0.01^{cB}
	18	14.19 ± 0.04^{bA}	10.92 ± 0.07^{bB}
	24	17.28 ± 0.12^{aA}	13.48 ± 0.13^{aB}
TOTOX	0	7.87 ± 0.01^{eB}	8.49 ± 0.03^{eA}
	6	17.71 ± 0.05^{dA}	16.61 ± 0.04^{dB}
	12	22.57 ± 0.02^{cA}	19.87 ± 0.05^{cB}
	18	25.95 ± 0.01^{bA}	22.00 ± 0.02^{bB}
	24	36.94 ± 0.04^{aA}	31.42 ± 0.07^{Ab}
IV (g of I_2 / 100 g oil)	0	86.72 ± 0.02^{aA}	85.23 ± 0.06^{aB}
	6	85.92 ± 0.03^{bA}	85.02 ± 0.07^{bB}
	12	85.46 ± 0.05^{cA}	84.87 ± 0.04^{cB}
	18	85.06 ± 0.01^{dA}	84.85 ± 0.03^{cB}
	24	84.50 ± 0.04^{eA}	84.34 ± 0.03^{dB}
FFA (mg KOH/100g oil)	0	3.57 ± 0.03^{eB}	4.17 ± 0.07^{eA}
	6	4.16 ± 0.02^{dB}	4.39 ± 0.01^{dA}
	12	4.54 ± 0.04^{cA}	4.56 ± 0.06^{cA}
	18	4.97 ± 0.09^{bA}	4.67 ± 0.04^{bB}
	24	5.67 ± 0.05^{aA}	5.14 ± 0.03^{aB}

*Values are means ± standard deviation (n=4).

[a] Means in each column followed by different superscripts letters (a-e) are significantly different (*$P < 0.05$).

[A] Means in each row followed by different superscripts letters (A-B) are significantly different (*$P < 0.05$).

measured against a reagent blank (ultra-pure water mixed with FCR and sodium carbonate) at 765 nm with a UV-VIS spectrophotometer (Model XTD 5, Secomam, France). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample.

Instrumental analysis

Fatty acids composition

Analysis of fatty acid composition was done adapted from the method of Nyam *et al.* (2009) by using gas chromatography (GC) with flame ionization detector.

Statistical analysis

All experiments were performed in duplicate and measurements were repeated two times. Analysis of variance (ANOVA) was performed, and the average values were compared with Fisher's Multiple Comparison Test. Differences were considered statistically significant at $p<0.05$. All statistical analyses were performed using Minitab 16 for Windows.

Results and Discussion

The peroxide value (PV) of KSO increased rapidly ($p<0.05$) throughout the storage from day 0 to day 24 due to the extent of oxidation caused by the formation of hydro peroxides during fat oxidation. The PV value of KSO had the highest rate of increment on day 18 of storage. This condition was most probably due to the declined on bioactive compounds as protective agents against oil deterioration at the end of storage. From day 12, the PV of KSO started to exceed all the PV of MKSO. On the last day of storage, KSO showed higher PV than MKSO. This finding is shown that MKSO had a better oxidative stability than KSO. The nutritional contribution of bioactive compounds such as phenolic contents along the storage may have conferred this greater oxidative stability.

In Table 1, the p-anisidine value (p-AnV) of KSO exceeded the p-AnV of MKSO from day 0 onwards until the day 24 of storage. The higher p-AnV of KSO at each day of storage was a consequence of a higher rate of secondary lipid oxidation product formation (Ali *et al.*, 2013). As for day 0 and day 6, the PV of KSO were lower than that of MKSO, however, the p-AnV of KSO was higher than that of MKSO from day 0 to day 24 of storage. This can be explained by the increment in p-AnV was a result of rapid decomposition of the unstable hydroperoxides from primary oxidation at elevated temperature (Yildirim, 2009). The lower p-Anv of MKSO compared to KSO at 24 days of storage proved that microwave pre-treatment can improve the oxidative stability of kenaf

Table 2. Changes in 2-Diphenyl-1picrylhydrazyl (DPPH) radical scavenging capacity assay and total phenolic content (TPC) of untreated kenaf seed oil (KSO) and microwave pre-treated kenaf seed oil (MKSO) under accelerated storage at 65°C for 24 days

Antioxidant activities	Days	Samples	
		KSO	MKSO
DPPH (mg trolox/ 100g oil)	0	9.34 ± 0.04^{aB}	9.87 ± 0.04^{aA}
	6	7.24 ± 0.02^{bB}	7.89 ± 0.08^{bA}
	12	5.10 ± 0.06^{cB}	6.87 ± 0.01^{cA}
	18	4.93 ± 0.02^{dB}	6.86 ± 0.06^{cA}
	24	3.51 ± 0.08^{eB}	6.69 ± 0.02^{dA}
TPC (mg gallic acid/ 100g oil)	0	49.22 ± 0.12^{aB}	75.71 ± 0.07^{aA}
	6	39.03 ± 0.03^{bB}	71.50 ± 0.02^{cA}
	12	36.50 ± 0.14^{cB}	71.18 ± 0.09^{bA}
	18	34.10 ± 0.05^{dB}	69.50 ± 0.13^{dA}
	24	31.38 ± 0.08^{eB}	67.70 ± 0.17^{eA}

* Values are means ± standard deviation (n=4).
[a] Means in each column followed by different superscripts letters (a-e) are significantly different (*P < 0.05).
[A] Means in each row followed by different superscripts letters (A-B) are significantly different (*P < 0.05).

Table 3. Changes in fatty acid composition of untreated kenaf seed oil (KSO) under accelerated storage at 65°C for 24 days

Fatty Acid	Storage (Days) 0	6	12	18	24
Palmitic Acid (C16:0)	22.8 ± 0.2^{d}	26.6 ± 0.3^{cA}	27.6 ± 0.1^{cA}	29.6 ± 0.0^{bA}	32.4 ± 0.2^{aA}
Palmitoleic Acid (C16:1)	0.6 ± 0.3^{a}	0.5 ± 0.6^{b}	0.4 ± 0.2^{cB}	0.4 ± 0.1^{cB}	0.3 ± 0.5^{dB}
Stearic Acid (C18:0)	3.6 ± 0.2^{d}	4.6 ± 0.0^{c}	5.8 ± 0.1^{bA}	5.7 ± 0.2^{bA}	6.6 ± 0.1^{aA}
Oleic Acid (C18:1)	36.6 ± 0.4^{aB}	35.7 ± 0.3^{bB}	35.0 ± 0.2^{cB}	34.6 ± 0.0^{dB}	33.2 ± 0.2^{eB}
Linoleic Acid (C18:2)	36.0 ± 0.2^{aA}	32.3 ± 0.1^{bB}	30.9 ± 0.2^{cB}	29.5 ± 0.3^{dB}	27.3 ± 0.6^{eB}
Linolenic Acid (C18:3)	0.4 ± 0.1^{a}	0.3 ± 0.0^{b}	0.3 ± 0.2^{b}	0.2 ± 0.3^{c}	0.2 ± 0.1^{c}
SAT	26.4	31.2	33.4	35.3	38.9
MUFA	37.2	36.2	35.4	35.2	34.2
PUFA	36.4	32.6	31.2	29.4	26.9

*Values are means ± standard deviation for duplicate analyses of 2 replicates (n=4).
[a] Means in each row followed by different superscripts letters (a-e) are significantly different (*P < 0.05).
[A] Means in each column followed by different superscripts letters (A-B) are significantly different (*P < 0.05) with microwave pre-treated kenaf seed oil (MKSO) in Table 4.

seed oil significantly.

Both TOTOX values from KSO and MKSO were similar to the changes trend in PV and p-AnV throughout 24 days of storage as indicated. Overall, the MKSO was observed to have a better oil quality compared to KSO. Iodine value (IV) of KSO was higher as compared to the IV of MKSO throughout the storage period from day 0 to day 24. On top of that, the lower value of IV for MKSO from day 0 can be explained due to the exposure in microwave pre-treatment might attributed to the reductions in the number of unsaturation as result of oxidation, polymerization or breakage of the long-chain fatty acid as the storage time increasing (Anjum *et al*., 2006). Meanwhile, IV of MKSO had smaller rate in decrement throughout storage as compared to IV of KSO. The slower rate of decrement in IV of MKSO may due to the induction period where oil was oxidized slowly showing initiation stage of auto oxidation reaction (Nasirullah *et al*., 1991). This condition might also due to the protective role of the natural antioxidants induced by the presence of treated kenaf seed oil resulted in a smaller decrease in double bonds.

The free fatty acid (FFA) value of KSO was lower than that of MKSO at day 0 and day 6. The increment of FFA in MKSO might attribute to hydrolysis of triacylglycerol (TAG) by microwaves to produce FFA (Uquiche *et al*., 2008). On day 12 of storage, the FFA value of KSO and MKSO were not significant different ($p>0.05$). This can be assumed that MKSO with the potential in delaying the free

Table 4. Changes in fatty acid composition of microwave pre-treated kenaf seed oil (MKSO) under accelerated storage at 65°C for 24 days

Fatty Acid	Storage (Days) 0	6	12	18	24
Palmitic Acid (C16:0)	22.9 ± 0.3^{d}	25.5 ± 0.2^{cB}	26.5 ± 0.2^{bB}	27.1 ± 1.7$^{b\,B}$	29.0 ± 0.3^{aB}
Palmitoleic Acid (C16:1)	0.7 ± 0.4^{a}	0.6 ± 0.2^{b}	0.6 ± 0.1^{bA}	0.6 ± 0.2^{bA}	0.5 ± 0.4^{cA}
Stearic Acid (C18:0)	3.7 ± 0.2^{c}	4.1 ± 0.5^{c}	4.9 ± 0.4^{bB}	5.1 ± 0.1^{bB}	5.9 ± 0.3^{aB}
Oleic Acid (C18:1)	37.6 ± 0.5^{aA}	36.8 ± 0.2^{bA}	36.0 ± 0.6^{cA}	35.9 ± 0.1^{cA}	35.2 ± 0.8^{dA}
Linoleic Acid (C18:2)	34.7± 0.0^{aB}	32.7 ± 0.2^{bA}	31.7 ± 0.2^{cA}	31.0 ± 0.4^{dA}	29.2 ± 0.3^{eA}
Linolenic Acid (C18:3)	0.4 ± 0.1^{a}	0.3 ± 0.6^{b}	0.3 ± 0.1^{b}	0.3 ± 0.2^{b}	0.2 ± 0.4^{c}
SAT	26.6	29.6	31.4	32.2	34.9
MUFA	38.3	37.4	36.6	36.5	35.7
PUFA	35.1	33.0	32.0	31.3	29.4

*Values are means ± standard deviation (n=4).

[a] Means in each row followed by different superscripts letters (a-e) are significantly different (*P < 0.05).

[A] Means in each column followed by different superscripts letters (A-B) are significantly different (*P < 0.05) with untreated kenaf seed oil (KSO) in Table 3.

fatty acid formation during storage. The FFA value of KSO became higher than that of MKSO significantly ($p<0.05$) from day 18 to day 24 of storage. Meanwhile, the lower FFA of MKSO was assumed to be a result of a successful in inactivation of lipid splitting enzymes by microwave pre-treatment which subsequently leads to better oxidative stability. The enzyme lipase is responsible for the splitting of ester bonds in lipids that causes the increment of free fatty acids. Ideally, oil should have a low content of free fatty acids as they had significant effect on the shelf-life (Mgudu *et al.*, 2012).

The DPPH value of KSO was lower than that of MKSO from day 0 to day 24 of storage. This result reflected the permanent ruptured on the oilseed cell membrane by microwave pre-treatment that produced higher oil yield and enhanced the concentration of bioactive compounds to be released in kenaf seed oil (Azadmard-Damirchi *et al.*, 2010). Therefore, microwave pre-treatment caused an increase in terms of antioxidant activity of kenaf seed oils.

The total phenolic content (TPC) value of KSO was lower as compared to that of MKSO throughout storage from day 0 to day 24 (Table 2). This was because the phenolic compounds were reported to pass into the oil phase better if the oil was obtained from treated seeds (Durmaz *et al.*, 2010). Meanwhile, this phenomenon also caused by the released of phenolic compounds from bound structures or chemical alteration of phenolics at elevated temperatures (Wijesundera *et al.*, 2008). Other than that, Durmaz *et al.* (2010) suggested that Maillard reaction products (MRPs) formed during the roasting process might have lead to the increase in antioxidant activity. As the results from both DPPH value and TPC value of KSO and MKSO that decreased significantly throughout storage. This trend was due to the positive correlation between the depletion of phenolic antioxidants and loss of antioxidant activity of the oil (Jiang and Wang, 2005). In addition to that, Razmkhah *et al.* (2013) also suggested the DPPH radical scavenging activity would decreased as the phenolic compounds declined during storage indicated the result of thermal and oxidative distress.

Changes in fatty acid composition of untreated kenaf seed oil (KSO) and microwave pre-treated kenaf seed oil (MKSO) were shown in Table 3 and Table 4. Oleic acid was the predominant fatty acid in kenaf seed oil followed by linoleic acids and palmitic acids. The total monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in both KSO and MKSO was found to be decreased from day 0 to day 24 upon accelerated storage due to the ease breakage of double bond of unsaturated fatty acids which lead to lipid oxidation (Ng *et al.*, 2013a). In spite of this, the composition was characterized by increment of saturated fatty acids such as palmitic acid and stearic acid due to the declined proportion in unsaturated fatty acids (Anjum *et al.,* 2006). KSO and MKSO had greater impact on oleic and linoleic acids as compared to palmitic and stearic acids in kenaf seed oil during accelerated storage. MKSO has higher palmitoleic acid as compared to KSO on day 12, 18 and 24 (0.6%, 0.6% and 0.5 %) and (0.4%, 0.4% and 0.3%), respectively. This difference indicated that MKSO had better resistance on the reduction of unsaturated bond along storage. However, lower linoleic acid content was found (34.7%) in MKSO as compared to linoleic acid content (36.0%) in KSO at 0 day of storage. This was

because linoleic acid is more susceptible to oxidation (Nyam *et al*., 2009). On top of that, the linoleic acid content of MKSO measured remained higher than that of KSO throughout the storage period. The higher concentration of the natural antioxidants present in MKSO would contribute to a smaller decrease in the double bonds even higher amount of PUFA present. For the palmitic acid content, MKSO had increased in a lower rate significantly ($p<0.05$) started from day 6 to the last day of storage, respectively. The palmitic acid content in MKSO was lower as compared to KSO started from day 6 to the last day of storage, respectively. This condition can be explained as such MKSO able to retained higher amount of PUFA from being reduced into saturated fatty acids like palmitic acids along storage. The lower amount of stearic acid present in MKSO most probably due to the less reduction of MUFA and PUFA along storage. On the whole, the total contents of MUFA and PUFA in MKSO were slightly higher than in KSO at the end of the storage. Therefore, MKSO was considered to have a better oil quality with higher amount of unsaturated fatty acids which indicated microwave pre-treatment able to delay oil deterioration upon accelerated storage.

Conclusion

With the microwave pre-treatment applied, kenaf seed oil was more resistant towards oxidative deterioration upon storage as compared to the untreated kenaf seed oil. This was due to the better retention of bioactive compounds such as the phenolic antioxidants that showed markedly improved in oil stability along the storage. Aside from that, microwave pre-treatment can be served as a convenient and practical method to minimize the undesired enzymatic reactions which consequently retained the quality storage shelf life of kenaf seed oil.

Acknowledgements

Financial support of this work by internal funding from UCSI University is gratefully acknowledged.

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