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Physiochemical properties of kenaf seed oil microcapsules before and after freeze drying and its storage stability

Hue, W.L. and *Nyam, K.L.

Department of Food Science with Nutrition, Faculty of Applied Sciences, UCSI University, 56000 Kuala Lumpur, Malaysia

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Abstract

Kenaf seed oil contains high amount of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), and bioactive compounds, such as tocopherol and phytosterol. In order to prevent bioactive compounds from oxidation, kenaf seed oil (KSO) was encapsulated by co-extrusion technology. KSO and microencapsulated kenaf seed oil (MKSO) were then subjected to accelerated storage to investigate the effect of microencapsulation on the storage stability of kenaf seed oil. The changes of fatty acids profiles and bioactive compounds in oils were evaluated. Result showed that there was significant decreased ($p<0.05$) of MUFA, PUFA, phytosterol ($p<0.05$) and tocopherol in KSO during accelerated storage. However, MUFA, PUFA, phytosterol and tocopherol contents in MKSO remained constant. Microencapsulation was proven to be able to protect MUFA, PUFA and bioactive compounds in kenaf seed oil from oxidation during prolonged storage.

Introduction

Kenaf (*Hibiscus cannabinus* L.) belongs to Furcuria section of Malvaceae family, which is closely related to okra, cotton and hollyhock. Kenaf is a plant that commonly grown for its fiber. The fiber of kenaf can be used to produce rope, twine, paper and coarse cloth. Apart from fiber, kenaf leaves can be used as sources of animal feed. Besides, woody core of kenaf can be burned as fuel. Kenaf consists of numerous active components, such as fatty acid, phospholipid, tannin, saponins, and alkaloid. This made kenaf prescribed as traditional medicine (Monti and Alexopoulou, 2013). In Malaysia, The National Kenaf and Tobacco Board (LKTN) had identified kenaf as new potential industrial plant to replace tobacco plantation. Kenaf plantation can be found in Perlis and Kedah because of annual rain season in the both states. Due to its potential, automotive manufacturers, such as Honda and Toyota have tried to use kenaf as vehicles bodies (Ishar, 2012).

In kenaf industry, kenaf seed is usually a by-product and treated as agricultural waste (Mariod *et al.*, 2010). Therefore, utilization and processing of by-product and waste have been focused (Nyam *et al.*, 2009). The problem of this agricultural waste can be solved by developing its nutritional and industrial potential. In Malaysia, LKTN is cooperated with Malaysian Agricultural Research and Development Institute (MARDI) to investigate economical potential of kenaf seed and carry out verification trial on it (Ishar, 2012). Kenaf seed oil can be used as biofuel, lubricants, and for cosmetics purpose (El Bassam, 2010). Besides, the high oil content in kenaf seed was suggested to be used to produce edible oil. Recently, the interest in health and wellness of people is increasing (Kim and Kwak, 2015). Kenaf seed oil contains a relatively high amount of tocopherol, phytpsterol, monounsaturated fatty acid (MUFA), and polyunsaturated fatty acids (PUFA), which are nutritionally beneficial for human health (Coetzee *et al.*, 2007).

PUFA play important role in reduction of cardiovascular disease, anti-inflammatory and anti-allergic effect (Calligaris *et al.,* 2015). PUFA is also a component of membrane bilayer, which facilitate assembly and stability of protein complexes, and play important roles in cellular metabolism (Hill *et al.*, 2012). However, the high amount of PUFA in kenaf seed oil made it suspectible to oxidation. Kenaf seed oil is readily oxidized by light, moisture, oxygen and high temperature (Razmkhah *et al.*, 2013). Lipid oxidation will cause deterioration of food quality, degradation of nutritional compounds and affect flavours quality of oil (Kanazawa *et al.*, 2002). Autoxidation is the main oxidation process that leads

*Corresponding author.
Email: *nyamkl@ucsiuniversity.edu.my*

to oxidative deterioration in lipid (Sun *et al.*, 2011). The autoxidation process causes the production of free radical, which may contribute to diseases, such as cancer, stroke, and myocardial infarction or inflammation (Karaś *et al*., 2015).

The problem of lipid oxidation can be solved by microencapsulation. Microencapsulation is a process in which tiny particles or droplets are completely surrounded in a homogeneous or heterogeneous matrix. Through microencapsulation, physical barrier can be created between core compound and wall material (Silva *et al.* 2014). Among the methods of microencapsulation, co-extrusion method is suitable to be used for microencapsulation of liquids, emulsion and slurries (Garti and McClements, 2012). During the process, a dual fluid stream that made up of liquid core and shell materials is pumped through concentric tube and form capsule (Ghosh, 2006). There are three basic steps involved in co-extrusion. The first step is compound droplet formation. This is follows by shell formation and capsule collection. The capsule formed must be hardened completely by hardening agent once formed in order to maintain shell-core morphology. The process is influenced by flow rate, diameter of nozzle, length of nozzle, and properties of materials.

In this study, sodium alginate, high methoxy pectin (HMP) and chitosan will be used as wall material to encapsulate kenaf seed oil. Alginate has been used in many biomedical applications because of its biocompatibility, low cost, low toxicity and gelation properties (Lee and Mooney, 2012). Alginate hydrogel can be prepared by many cross-linking method. Since its structure is similar to extracellular matrices of living tissue, it is widely used in wound healing and transport of bioactive compound. Pectins are commonly used as wall material in microencapsulation to increase viscosity and get strength. The use of different biopolymer as wall material has shown to produce microcapsule with maximal protection and useful in control release of core substances. Besides, adding pectin to alginate-based microcapsules increase protection on core materials and enhance nutritional value. The uses of sodium alginate and chitosan as wall material were found out to retain drug for longer time in body and hence control release of drug to target site. In the study of microencapsulation of drug by using chitosan and sodium alginate, it was found out that the interaction between sodium alginate and chitosan could further extending drug release for longer time (Li *et al*., 2014).

After MKSO produced by co-extrusion technology, MKSO will be dried by freeze-drying to prevent kenaf seed oil from exposure to light and high temperature. After that, MKSO are subjected to accelerated storage at 65°C for 24 days, which one day of storage at 65°C represent 1 month at ambient temperature.

The objective of this study was to investigate the physical properties of MKSO before and after freeze drying. In addition, the effect of microencapsulation on the storage stability of kenaf seed oil was evaluated.

Materials and Methods

Materials

About 1kg of dried kenaf seeds were obtained from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. Sodium alginate was purchased from Friendemann Schmidt (Australia). High methoxyl pectin (HMP), which was extracted from citrus peel, was purchased from a local food ingredient supplier (VIS Food Tech Ingredient Supplies, Malaysia), while chitosan was purchased from Sigma-Aldrich,Germany. All chemicals used were analytical grade (Merck, Darmstadt, Germany).

Methods

The kenaf seed oils were extracted according to the method by Chew *et al.* (2015). The 200 g of dried kenaf seed powder was added with 1L of n-Hexane. The kenaf seed oil was extracted by Soxhlet apparatus (Favorit, Thailand) at 60°C for 3 hours. The solvent was evaporated by Multivapor P-6 (Buchi, Switzerland) at 55°C and pressure of 251mbar. Kenaf seed oil was transferred to Schott bottle and wrapped with aluminum foil. The residual solvent was removed by flushing with 99% nitrogen gas. The kenaf seed oil was then kept at -20°C for further study.

Microencapsulation of KSO

Sodium alginate solution (1.5% w/w) and 1.5% w/w of HMP solution was mixed at a volume ratio 2:1 and stored overnight at 4ºC (Chew and Nyam, 2016). In preparing hardening solution, 3% w/w $CaCl_2$ was mixed with 0.1% w/w of chitosan and 1g of Tween 80 as surfactant. The KSO was encapsulated using Buchi Encapsulator B-390 (Buchi, Switzerland). Firstly, the inner and outer nozzles of encapsulator with diameter 200 µm and 300 µm respectively were installed. The core (kenaf seed oil) and shell (alginate pectin solution) was connected to encapsulator. The pressure of encapsulator was set at 600mbar to aid in transferring of the core and shell. The frequency

was set at 500 Hz and additional electrostatic field of 2500 V was applied between the nozzle and the hardening solution. The purpose of applying electrostatic field was to prevent coalescence among microcapsules. The microcapsules were incubated in hardening solution mixed with chitosan with constant stirring on magnetic stirrer to prevent clumping of microcapsules. The stirring process was about 10 minutes for complete gel hardening. The microcapsules were then sieved and rinsed with deionised water. Then, the moisture on microcapsules was reduced by placed the sieve on paper. The microcapsules formed were dried by freeze-drier for 21 hours (Christ, Germany).

Water activity

The water activity of microcapsule was determined by using Aqua-Lab Water Activity Meter (Decagon Devices Inc, United State) at 25°C.

Moisture content

The moisture contents of the microcapsules (0.5 g) were determined gravimetrically by oven drying at 103°C for 24 hr until constant weight was achieved. The weight difference before and after drying was recorded. Results were expressed in (g of water/ 100g of dry solids).

Particle size of microcapsules

The size of MKSO was observed under a Nikon YS100 optical microscope. The MKSO were examined under a magnification of 40x. The diameter of MKSO was measured with a microscope stage micrometer which can measure size within 0.01- 2 mm. 10 microcapsules were examined and pictures of MKSO was taken.

Microencapsulation efficiency (MEE)

The MEE of dried MKSO was determined directly by calculating amount of microencapsulated kenaf seed oil relative to total amount of kenaf seed oil used for each production batch. MEE was calculated by using following equation:

$$MEE(\%) = \frac{Extractable\ oil\ (g)}{Total\ oil\ (g)} \times 100\%$$

Accelerated storage

The oxidative stability of MKSO was tested under accelerated storage conditions by the Schaal oven test, condition at 65°C for 24 days, where 1 day of storage represents 1 month of storage at room temperature. In order to remove the availability of oxygen in the Schott bottle, all the samples in the Schott bottles were flushed with 99.9% nitrogen. At day 0, day 6, day 12, day 18 and day 24 after storage in conventional oven, oxidative stability tests were carried out for each of the day. Unencapsulated kenaf seed oil was used as a control to make a comparison with the MKSO in terms of their oxidative stability.

Extraction of kenaf seed oil from dried microcapsules

The extraction method was carried out based on Sun-Waterhouse *et al.* (2011) with modifications. 5 g of dried microcapsules were first grounded using a mortar and pestle and added with methanol. The resultant mixture was homogenized using a T25 digital Ultra-Turrax homogenizer at the speed of 15 000 rpm for 1 minutes. 150 mL of hexane was added and the mixture was shaken vigorously to facilitate the transfer of oil into the hexane layer. The shaken Schott bottle was left to allow the hexane and methanol to separate into 2 layers. The hexane layer was collected and extraction steps were repeated three times. The obtained hexane layers were mixed together and the oil was recovered by evaporation of hexane using a Multivapor at 55ºC, 241mbar for 30 minutes. The resultant oil was collected and flushed with 99.9% nitrogen

Chromatographic determination of phytosterol and tocopherol composition

Phytosterol content in kenaf seed oil was determined according to the previously established method (Ng *et al.*, 2013). Total lipid (250 mg) and 100 µL of 5α-cholestane (1 mg/mL) were refluxed with 5 mL of ethanolic potassium hydroxide (6 g/100 mL) for 60 mins. The unsaponifiable was first extracted three times with 10mL of petroleum ether. The extracts were combined and washed three times with 10mL of neutral ethanol-water (1:1, v/v) and then dried over anhydrous sodium sulphate. The extract was dried under reduced pressure. N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) (125 µL) with1mL/100 mL trimethylchlorosilane (TMCS) was added to the dry residue. The mixture was vortexed for about 10s and heated at 70ºC in a water bath for 30-60 mins. After cooling, 1µL aliquot was directly injected into the gas chromatography (Agilent Technologies 7890A, USA), equipped with a flame ionization detector and a HP-5 column (30 m x 0.25 mm i.d., 0.32 µm film thickness) with a split ratio 95:1. The initial column temperature was 250ºC and programmed to increase at a rate of 2ºC/min to 300ºC and then held for 12 mins. The phytosterols concentration was calculated from the following equation and expressed in mg/100g oil.

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

Tocopherol content in kenaf seed oil was determined according to the previously described method (Ng *et al*., 2013). HPLC (Agilent Technologies 1200 Series, USA), with a UV-Vis detector and a Purospher STAR RP-18e column (5 µm x 250 mm x 4.6 mm) (Merck, Germany) was used to analyzed the tocopherol content. Separation of all tocopherols was based on 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/100g oil through the calibration curve of a series of standard solutions of α- and γ-tocopherols at a concentration of 0.2 to 1 g/L. Prior to HPLC analysis, the oils were diluted with methanol, filtered with 0.45 µm nylon syringe filter and 20 µL samples were injected.

Determination of fatty acid composition

Fatty acid compostion was determined based on method reported by Ng *et al.* (2013) with slight modification. Prior to the gas chromatography (GC) analysis, fatty acid composition was determined by the conversion of oils to fatty acid methyl esters (FAME). After preparing sample, 1 µL of the top layer was injected in the form of manual injection into a gas chromatography equipped with a flame ionization detector (FID). A capillary column HP-5 (0 m × 0.25 mm i.d., 0.32 µm film thickness) was used at a column head pressure of 10 psi. Nitrogen (99.99%) was used as the carrier gas at approximately 23 mL min^{-1}. The FID and injector temperatures were both maintained at 220°C. The initial column oven temperature was 115°C, temperature programmed to 180°C at 8°C min^{-1} and held at this temperature until the analysis was completed. Flow rate was set at 1.6943 mL min^{-1}.

Statistical analysis

Analysis of variance (ANOVA) was carried out and the average values were compared with Tukey's post hoc test. Differences were considered statistically significant at $P<0.05$. All statistical analyses were performed using Minitab version 17.

Results and Discussion

Physical properties of MKSO

From Table 1, the water activity (A_w) of microencapsulated kenaf seed oil (MKSO) decreased from 0.995± 0.00 to 0.29± 0.04 after freeze-drying. Aw is defined as the ratio of vapor pressure of water in a system to vapor pressure of pure water at the same temperature (Shafiur, 2009). It is an important indicator to determine growth of microorganism (Maltini *et al.*, 2003). A food with Aw <0.3 can be categorized as microbiologically stable product and lowered lipid oxidation. Before drying, MKSO had Aw of 0.995 indicated the presence of free water around the capsule (Ibarz and Barbosa-Canovas, 2014). The presence of free water is not favourable as it is available for biochemical reaction. Besides, a product with A_w >0.6 is more prone to microbial proliferation (Ng *et al.*, 2013).

Table 1. Physiochemical properties of microcapsules before and after freeze drying

Physiochemical Properties	Before Drying	After Drying
Water Activity	1.00±0.00[a]	0.29±0.04[b]
Moisture content (%)	97.03±1.14[a]	29.99±2.49[b]
Particle size (mm)	700-920	330-500
Microencapsulatioon efficiency (%)	-	65.34 ±2.14

[a] Means ± standard deviations followed by different superscript letters within the same row are significantly different ($p<0.05$) according to Tukey's test

According to the result, the moisture content of MKSO before drying was 97.03 % ±1.14 %. A food product with high moisture content is subjected to rapid deterioration from mold growth, heating, insect damage and sprouting (Pomeranz and Meloan, 2000). Therefore, it is important to reduce moisture content by drying. After freeze drying, the moisture content of MKSO reduced to 29.99%, which showed a significant ($p <0.05$) reduction of 69.09%. Based on the results, the particle size of MKSO was in range of 700-920mm before drying and reduced to 330-500mm after drying. The freeze-drying is a process of drying product through sublimation of ice (Hui, 2006). During the drying process, the ice sublimation creates pores and cause shrinkage of MKSO due to surface forces or gravity (Krokida *et al*., 1998). This shrinkage process reduce the MKSO after drying. Besides, the drying process remove water content in wall material (sodium alginate, high methoxy pectin and chitosan) of microcapsules, thus water activity, moisture content and particle size reduce along with water removal. MEE plays a significant role in this study because MEE refers to the proportion of oil that is surrounded by the shell wall matrix and less exposed to the surrounding environment and thus, having effect on the oxidative stability of the product (Jimenez *et al*., 2004). The microencapsulation

efficiency (MEE) for the MKSO was 65.34 ±2.14%. It was comparable to the previous study done by Chew and Nyam (2016).

Changes of phytosterol composition upon accelerated storage

Phyosterol is beneficial to our health due to its hypocholesterolemic properties. Phytosterol has ability in decreasing serum LDL cholesterol level and protect against cardiovascular disease. Besides, phytosterol has no effect against protective HDL cholesterol (Kaloustian *et al*., 2008). From this study, it was observed that the three main phytosterol compounds found in KSO were campesterol, stigmasterol and β-sitosterol. The main phytosterols in present research using day 0 as base comparison were β-sitosterol (266.30±21.80 mg/100g) followed by campesterol (29.34±4.40 mg/100g) and stigmasterol (14.50±2.64 mg/100g). The results were in agreed with previous study done by Nyam *et al*. (2009) which reported that major phytosterols were β-sitosterol (289.9 mg/100g), followed by of campesterol (58.1 mg/100g), stigmasterol (23.3 mg/100g). However, most of the concentration of phytosterol reported by Nyam *et al*. (2009) was much higher compared to this study. The difference might due to the environmental factors, such as soil conditions, ripeness of seeds and duration of storage (Ng *et al.*, 2013).

According to Figure 1, total phytosterol composition showed decreasing trend upon accelerated storage from day 0 to day 24. Campestrol, stigmasterol and β-sitosterol are known as "unsaturated sterol" due to the presence of double bond in their chemical structure. The presence of double bond made phytosterol prone to oxidation during prolonged storage (Rudzińska *et al*., 2014). The co-extrusion process increases the contact of kenaf seed oil with air and lead to degradation of phytosterol. However, the results indicated that co-extrusion process did not have great effect in degradation of phytosterol. In addition, the total phytosterol composition was higher in MKSO compared KSO from day 12 until day 24. The total loss of phytosterol in KSO during storage was 59.76% while total loss of phytosterol in MKSO was 32.86%. This proved that the rate of degradation of phytosterol was faster without microencapsulation. The wall material used in microencapsulation reduces the contact of phytosterol with surrounding environment and thus protect it from degradation.

Changes in tocopherol content upon accelerated storage

Tocopherol is commonly used in food, feed, pharmaceuticals, cosmetics and resins (Nyam *et al*., 2009). Tocopherol is an important fat-soluble

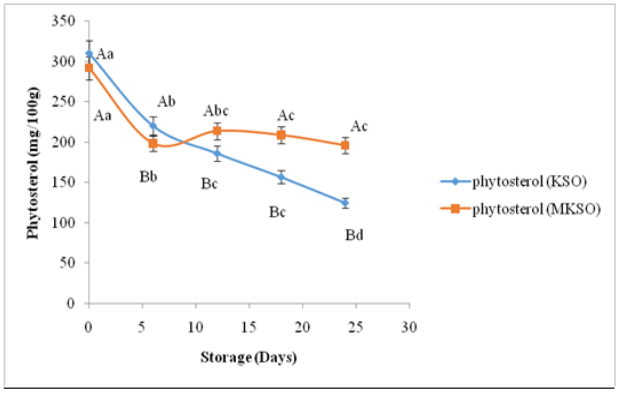

Figure 1. Phytosterol in KSO and MKSO upon accelerated storage. The phytosterol concentration is the sum of β-sitosterol, campesterol and stigmatserol that presented in kenaf seed oil. Means ± standard deviations (n = 4) with different superscript letters abcde indicate significant differences ($p<0.05$) among different days of the same sample and means ± standard deviations (n = 4) with different superscript letters AB indicate significant differences ($p< 0.05$) between two samples at the same day of storage.

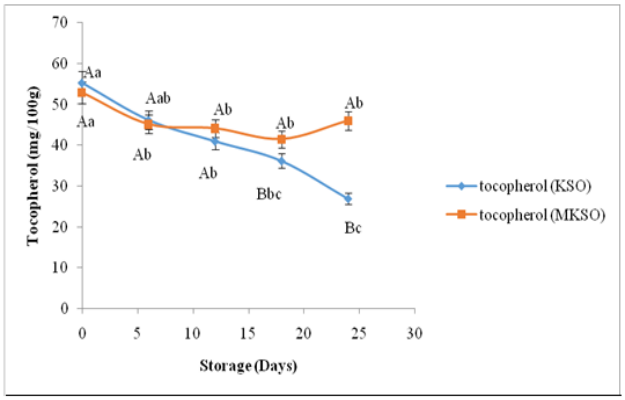

Figure 2. Tocopherol in KSO and MKSO upon accelerated storage. The tocopherol concentration is the sum of α-, γ- and δ- tocopherols that presented in kenaf seed oil. Means ± standard deviations (n = 4) with different superscript letters abcde indicate significant differences ($p<0.05$) among different days of the same sample and means ± standard deviations (n = 4) with different superscript letters AB indicate significant differences ($p< 0.05$) between two samples at the same day of storage.

biological antioxidant that brings various benefits to human health. Tocopherol is proven can be used to delay Alzheimer's disease and aid in prevention of cancer (Nyam *et al*., 2009). Some studies also proposed high intakes Vitamin E are correlated with reduced risk of coronary heart disease (Karmowski *et al*., 2015). From the results, γ-tocopherol was the most predominant tocopherol in bulk oil (43.49± 7.21 mg/100g) following by α-tocopherol (11.83 ± 2.26 mg/100g). Results obtained were agreed with previous study by Nyam *et al.* (2009), which reported that the amount of γ-tocopherol was the most predominant tocopherol than α-tocopherol, which were 63.9

Table 2a. Fatty acid compositions (%) in kenaf seed oil (KSO) upon accelerated storage

Fatty acid	Storage				
	0	6	12	18	24
C14:0	0.54±0.04[c]	0.75±0.02[b]	0.78±0.04[ab]	0.81±0.03[ab]	0.81±0.02[a]
C16:0	20.27±0.03[e]	21.67±0.13[d]	22.78±0.13[c]	25.13±0.06[b]	26.23±0.30[a]
C18:0	0.71±0.03[a]	0.82±0.04[a]	0.91±0.02[c]	1.39±0.03[b]	2.22±0.05[a]
C18:1	33.20±0.08[a]	32.30±0.11[b]	31.63±0.21[c]	30.74±0.07[d]	30.33±0.37[d]
C18:2	40.11±0.04[a]	39.07±0.05[b]	38.34±0.09[c]	37.03±0.16[d]	35.42±0.16[e]
C18:3	3.56±0.07[a]	3.51±0.10[ab]	3.35±0.08[b]	3.18±0.06[c]	3.01±0.03[d]
C20:0	0.70±0.02[e]	0.83±0.04[c]	1.04±0.06[b]	1.28±0.04[a]	1.31±0.02[a]
C20:1	0.31±0.03[a]	0.36±0.07[a]	0.30±0.02[ab]	0.29±0.01[ab]	0.23±0.02[b]
C24:0	0.59±0.02[b]	0.77±0.03[a]	0.79±0.03[a]	0.79±0.01[a]	0.82±0.03[a]
SAT	22.8	24.8	26.3	29.4	31.4
MONO	33.5	32.7	31.9	31.0	30.6
POLY	43.7	42.6	41.7	40.2	38.4

[a] Means ± standard deviations followed by different superscript letters within the same row are significantly different ($p<0.05$) according to Tukey's test.

Table 2b. Fatty acid compositions (%) in microencapsulated kenaf seed oil (MKSO) upon accelerated storage

Fatty acid	Storage				
	0	6	12	18	24
C14:0	0.57±0.02[a]	0.61±0.03[a]	0.61±0.04[a]	0.62±0.03[a]	0.63±0.04[a]
C16:0	22.51±0.03[a]	22.51±0.05[a]	22.55±0.01[a]	22.67±0.09[a]	22.67±0.16[a]
C18:0	0.89±0.03[a]	0.91±0.02[a]	0.91±0.02[a]	0.92±0.01[a]	0.92±0.03[a]
C18:1	32.00±0.12[a]	32.00±0.11[a]	32.00±0.13[a]	32.11±0.05[a]	32.00±0.08[a]
C18:2	39.15±0.10[a]	38.85±0.11[b]	38.85±0.08[b]	38.72±0.10[b]	38.87±0.09[b]
C18:3	3.51±0.03[a]	3.69±0.02[ab]	3.73±0.05[bc]	3.63±0.07[ab]	3.58±0.03[a]
C20:0	0.65±0.03[b]	0.72±0.02[ab]	0.75±0.02[a]	0.77±0.02[a]	0.77±0.04[a]
C20:1	0.23±0.02[a]	0.24±0.01[a]	0.24±0.03[a]	0.24±0.02[a]	0.22±0.01[a]
C24:0	0.63±0.01[a]	0.65±0.02[a]	0.67±0.02[a]	0.64±0.02[a]	0.66±0.02[a]
SAT	25.3	25.4	25.5	25.6	25.7
MONO	32.2	32.2	32.2	32.4	32.2
POLY	42.7	42.5	42.6	42.3	42.5

[a] Means ± standard deviations followed by different superscript letters within the same row are significantly different ($p<0.05$) according to Tukey's test.

mg/100g and 20.0 mg/100g, respectively. However, tocopherol content reported by Nyam et al. (2009) was much higher than this study. This may also due to different in seed condition and storage condition. Furthermore, the column and mobile phase that used also led to different in tocopherol content reported. In this study, column used was reversed phase C18 column with methanol and water as mobile phase. This was different from Nyam *et al.* (2009) who used Purospher STAR NH_2 with hexane and dioxane as mobile phase.

The results showed that the total amount of tocopherol was decreased upon accelerated storage. KSO and MKSO were subjected to 65°C upon storage and tocopherol was thermally-degraded when exposed to high temperature. The degradation of tocopherol is favoured by increasing the duration of exposure (Mohd Fauzi and Sarmidi, 2010). Besides, results also showed that total tocopherol content was higher in KSO compared to MKSO from day 0 to day 6 of storage, but the differences was not significant ($p>0.05$). The lower amount of total tocopherol in MKSO in day 0 was due to process of co-extrusion. However, the total tocopherol content in KSO decreased (51.28%) dramatically during prolonged storage. For MKSO, there was only minor decreased (12.9%) in tocopherol upon accelerated storage. Ng *et al.* (2013) reported that the amount of tocopherol in MKSO does not significantly change throughout the storage period. Microencapsulation is effective in protecting tocopherol from oxidztion. Upon accelerated storage, microcapsules coating protect kenaf seed oil from oxidation and thus degradation of tocopherol decreased.

Changes in fatty acid composition upon accelerated storage

According to Table 2a, KSO contained a relatively high percentage of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). From the results of day 0, the predominant fatty acid in KSO were linoleic acid (C18:2) and oleic acid (C18:1), which made up of 40.11% and 33.20%, respectively. However, Nyam *et al.* (2009) reported that oleic acid (37.1%) was the main fatty acid in KSO followed by linoleic acid (36.6%). The differences was due to difference in column used during analysis. The column used in this study was HP-5 column while the column used by Nyam *et al.* (2009) was BPX70 column.

From Table 2a, MUFA showed decreased of 8.66% while PUFA showed decreased of 12.13%

upon accelerated storage. The decreased in MUFA and PUFA were due to breakage of double bond. In addition, PUFA were found out more prone to oxidation compared to MUFA as number of double bond increased. The composition of saturated fatty acid (SAT) in KSO increased from 22.8% to 31.8%. The increased in SAT was caused by decrease in proportion of MUFA and PUFA. For MKSO, it was observed that the composition of all types of fatty acids remained stable throughout the storage period. Although KSO showed higher percentage of unsaturated fatty acid in the day 0 and day 6 of storage, the amount of unsaturated fatty acid in MKSO was higher starting from day 12 until day 24. Therefore, the protective effect of microencapsulation was proven. Since consumption of unsaturated fatty acid is beneficial to our health, it is important to encapsulate kenaf seed oil due to its potential in protecting unsaturated fatty acid.

Conclusion

In conclusion, freeze-dried kenaf seed oil microcapsules had low water activity and moisture content. The particle size of microencapsulated kenaf seed oil (MKSO) was between 330mm- 500mm with microencapsulation efficiency of 65.34%. Although MKSO showed lower amount of bioactive compounds compared unencapsulated kenaf seed oil (KSO) due to co-extrusion process, the bioactive compounds that remained were stable upon accelerated storage. Therefore, microencapsulation was shown to slower degradation of unsaturated fatty acid, phytosterol and tocopherol during prolonged storage.

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References

Calligaris, S., Ignat, A., Biasutti, M., Innocente, N. and Nicoli, M.C. 2015. Cheese fortification using saturated monoglyceride self-assembly structures as carrier of omega-3 fatty acids. International Journal of Food Science and Technology 50(9): 2129-2134.

Chew, S.C., Tan, C.P., Long, K. and Nyam, K.L. 2015. In vitro evaluation of kenaf seed oil in chitosan coated-high methoxyl pectin-alginate microcapsules. Industrial Crops and Products 76: 230-236.

Coetzee, M., Haag, M. and Kruger, M.C. 2007. Effects of arachidonic acid, docosahexaenoic acid, prostaglandin E2 and parathyroid hormone on osteoprotegerin and RANKL secretion by MC3T3-E1 osteoblast-like cells. The Journal of Nutritional Biochemistry 18: 54-63.

El Bassam, N. 2010. Handbook of bioenergy crops, p. 227. London: Earthscan.

Garti, N. and McClements, D. J. 2012. Encapsulation technologies and delivery systems for food ingredients and nutraceuticals, p. 131-133. Philadelphia: Woodhead Pub.

Hill, S., Lamberson, C.R., Xu, L., To, R., Tsui, H.S., Shmanai, V.V., Bekish, A.V., Awad, A.M., Marbois, B.N., Cantor, C.R., Porter, N.A., Clarke, C.F. and Shchepinov, M.S. 2012. Small amounts of isotope-reinforced polyunsaturated fatty acids suppress lipid autoxidation. Free Radical Biology and Medicine 53: 893-906.

Hui, Y.H. 2006. Handbook of Food Science, Technology and Engineering, p. 1064. Boca Raton: Taylor and Francis.

Ibarz, A. and Barbosa-Canovas, G.V. 2014. Introduction to food process engineering, p.154. Boca Raton: CRC Press.

Ishar, Z. 2012. Exploring kenaf's economic potential. Sarawak: Borneo Post.

Kaloustian, J., Alhanout, K., Amiot-Carlin, M. J., Lairon, D., Portugal, H. and Nicolay, A. 2008. Effect of water cooking on free phytosterol levels in beans and vegetables. Food Chemistry 107: 1379-1386.

Karaś, M., Baraniak, B., Rybczyńska, K., Gmiński, J., Gaweł-Bęben, K. and Jakubczyk, A. 2015. The influence of heat treatment of chickpea seeds on antioxidant and fibroblast growth-stimulating activity of peptide fractions obtained from proteins digested under simulated gastrointestinal conditions. International Journal of Food Science and Technology 50: 2097-2103.

Karmowski, J., Hintze, V., Kschonsek, J., Killenberg, M. and Böhm, V. 2015. Antioxidant activities of tocopherols/tocotrienols and lipophilic antioxidant capacity of wheat, vegetable oils, milk and milk cream by using photochemiluminescence. Food Chemistry 175: 593-600.

Kim, M.K. and Kwak, H.S. 2014. Influence of functional information on consumer liking and consumer perception related to health claims for blueberry functional beverages. International Journal of Food Science and Technology 50: 70-76.

Krokida, M.K., Karathanos, V.T. and Maroulis, Z.B. 1998. Effect of freeze-drying conditions on shrinkage and porosity of dehydrated agricultural products. Journal of Food Engineering 35: 369-380.

Maltini, E., Torreggiani, D., Venir, E. and Bertolo, G. 2003. Water activity and the preservation of plant foods. Food Chemistry 82: 79-86.

Mariod, A.A., Fathy, S.F. and Ismail, M. 2010. Preparation and characterisation of protein concentrates from defatted kenaf seed. Food Chemistry 123: 747-752.

Mohd Fauzi, N.A., and Sarmidi, M.R. 2010. Storage stability of α- tocopherol extracted from heated and unheated plam oil mesocarp. Journal of Food Science and Technology 2: 111-120.

Monti, A. and Alexopoulou, E. 2013. Kenaf, p. 2-11. London: Springer.

Ng, S.K., Jessie, L.L.Y., Tan, C.P., Long, K. and Nyam, K.L. 2013. Effect of Accelerated Storage on Microencapsulated Kenaf Seed Oil. Journal of the American Oil Chemists' Society 90: 1023-1029.

Ng, S.K., Wong, P.Y., Tan, C.P., Long, K. and Nyam, K.L. 2013. Influence of the inlet air temperature on the microencapsulation of kenaf (*Hibiscus cannabinus* L.) seed oil. European Journal of Lipid Science and Technology 115: 1309-1318.

Nyam K.L., Tan C.P., Lai O.M., Long K. and Che Man Y.B. 2009. Physicochemical properties and bioactive compounds of selected seed oils. LWT- Food Science and Technology 42: 1396-1403.

Pomeranz, Y. and Meloan, C.E. 2000. Food analysis, p. 575. Gaithersburg, MD: Aspen Publishers.

Razmkhah, S., Tan, C.P., Long, K. and Nyam, K.L. 2013. Quality Changes and Antioxidant Properties of Microencapsulated Kenaf (*Hibiscus cannabinus* L.) Seed Oil During Accelerated Storage. Journal of the American Oil Chemists' Society 90: 1859-1867.

Rudzińska, M., Przybylski, R. and Wąsowicz, E. 2014. Degradation of phytosterols during storage of enriched margarines. Food Chemistry 142: 294-298.

Shafiur R. M. 2009. Food properties handbook, p. 10. Boca Raton: CRC Press.

Silva, V., Vieira, G. and Hubinger, M. 2014. Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. Food Research International 61: 132-143.

Sun, Y.E., Wang, W.D., Chen, H.W. and Li, C. 2011. Autoxidation of Unsaturated Lipids in Food Emulsion. Critical Reviews in Food Science and Nutrition 51: 453-466.

Sun-Waterhouse, D., Zhou, J., Miskelly, G.M., Wibisono, R. and Wadhwa, S.S. 2011. Stability of encapsulated olive oil in the presence of caffeic acid. Food Chemistry 126: 1049-1056.