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Evaluation of *In-Vitro* Release of Kenaf Seed Oil from High Methoxyl Pectin-Alginate Microcapsules

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Abstract

The aim of this study was to evaluate the oil release behavior of Microencapsulated Kenaf Seed Oil (MKSO) after simulated *in vitro* digestion, to determine the antioxidant activities, antioxidant compounds and bioactive compounds in the MKSO after simulated *in vitro* digestion. Kenaf Seed Oil (KSO) was encapsulated using co-extrusion technology with the wall materials of High Methoxyl Pectin (HMP)-enhanced alginate solution. It was then oven-dried in conventional oven at 65°C. The MKSO after treated with simulated *in vitro* digestion was compared with bulk (unencapsulated) KSO in terms of their changes in radical scavenging activities (ABTS and DPPH), antioxidant contents (total phenolics content and total flavonoids content) and bioactive compounds such as phytosterols compositions. Results showed that small amount of KSO released in gastric phase digestion while high release of KSO in the intestine phase digestion was observed. The ABTS radical scavenging ability in MKSO after simulated *in vitro* digestion experienced a significant increase. While the DPPH radical scavenging ability in MKSO after simulated *in vitro* digestion experienced a significant decrease compared to bulk KSO. The bioactive compounds such as phytosterols, total phenolics and total flavonoids also experienced a significant decrease in MKSO after simulated *in vitro* digestion compared to bulk KSO. The results of this study showed that the process of microencapsulation could protect the KSO from the external environment that causes lipid oxidation.

Keywords: *Hibiscus cannabinus L.*; Microencapsulation; Co-extrusion; Oil release behavior; Gas chromatography

Abbreviations

ABTS: 2,2'-Azino-Bis-(3-ethylbenzothiazoline-6-sulfonate); AOA: Antioxidant Activity; BSTFA: N,O-Bis(trimethylsilyl) trifluoroacetamide; DE: Degree of Esterification; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FCR: Folin-Ciocalteu Reagent; GAE: Gallic Acid Equivalent, GC-FID: Gas-Chromatographic-Flame Ionization Detection; KSO: Kenaf Seed Oil; MKSO: Microencapsulated Kenaf Seed Oil; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid; TFC: Total Flavonoid Content; TPC: Total Phenolic Content

Introduction

Kenaf (*Hibiscus cannabinus L.*) is a valuable fibre and medicinal plants belong to the family *Malvaceae*, which is native to India and Africa [1]. In the past, kenaf plant has been used as a cordage crop to produce twine, rope, and sackcloth for over six millennia [2]. However, kenaf seeds have always been treated as an agricultural or industrial waste or rendered into animal feed. Kenaf seeds are suggested for use as a good source for edible oil due to presence of high oil content and nutraceutical value [3,4]. KSO contains high amount of mono-(MUFA) and Poly-Unsaturated Fatty Acids (PUFA), which are nutritionally beneficial for human health [5], and has a good source of lipid-soluble bioactive compounds [6]. Besides, KSO contains a high percentage of linoleic acid (Omega-6) PUFA, which is important in reducing risk of cholesterol and heart diseases [3]. KSO also has high concentration of phospholipids, which interact as a natural antioxidant and consequently increase oil stability and shelf life [3]. In addition, KSO has high percentage of phytosterols [3,6], which possess anticancer, antioxidant and lipid lowering cholesterol properties [7]. KSO has proven to have anti-inflammatory and antithrombotic activities [8] due to the presence of α-linoleic acid and it also acts as chemo preventive agent [9]. However, its high concentration of PUFA is undesirable in terms of oil stability, as they are readily oxidized and lead to rancidity [10] and defective nutrition due to degradation products such as reactive oxygen species [11]. Rapid oxidation of oil leads to unfavorable tastes and odors, degrades the nutritional quality and safety of the lipid, reduces the shelf life of the product [12] and may cause harmful effects on human health due to formation of primary and secondary oxidation products [11].

Thus, microencapsulation technique is employed to protect the oil from oxidation, which has been widely used for manufacturing of powdered edible oil products comprising oil globules embedded in a homogenous or heterogeneous matrix [13], where the core materials (oils) are protected from deteriorative reactions or adverse environmental condition such as heat, light or moisture, thus help to extend the products shelf life [14]. Microencapsulation also can help to control the release of the core material in specific target part of the body and to mask the core taste or flavor [14]. Microencapsulation by co-extrusion technology can be utilized to prepare spherical

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microcapsules with a hydrophobic core of active ingredient and a hydrophilic or hydrophobic shell produced by interfacial gelling (with calcium-alginate or potassium-carrageenan) or cooling (gelatin or fat) [15]. Co-extrusion technology (also referred as vibration nozzle technology) is based on the principle that a laminar flowing liquid jet breaks up into equal sized droplets by a superimposed vibration [16]. Thus, optimum processing parameters during encapsulation of KSO was employed to produce microcapsules with high Microencapsulation Efficiency (MEE). In addition, not many literature works have been found on the study of the oil release behavior of MKSO. Therefore, the aims of this study were to produce MKSO by co-extrusion technology and to evaluate the oil release behaviour of the MKSO after subjected to sequential simulated *in vitro* digestion. In addition, the released oil from the MKSO was evaluated for the antioxidant activities, amount of antioxidant compounds and bioactive compounds.

Materials and Methods

Materials and chemicals

Kenaf (*Hibiscus cannabinus L.*) seed was obtained from the Malaysian Agricultural Research and Development Institute (Selangor, Malaysia). High Methoxyl Pectin (HMP) was purchased from a local food ingredient supplier and sodium alginate was purchased from Friendemann Schmidt, Australia. All chemicals used were of analytical grade (Merck, Germany).

Methods

Production of microcapsules

Oil extraction: Kenaf seeds were ground into a fine powder using a food grinder (Sharp, USA). The oil was extracted from the seeds with soxhlet extractor using hexane at 60°C for 3 hours [17]. The oil was then recovered by evaporating off the solvent using a rotary evaporator (Rotavapor R-205, Buchi, Switzerland) at 48°C and the residual solvent was removed by flushing with 99.9 % nitrogen.

Preparation of the encapsulant formulations: High Methoxyl Pectin (HMP) solution was prepared by slowly adding 1.5 g of HMP powder to 98.5 g of deionised water and then was gently homogenized at 7000 rpm for 1 minute using a digital Ultra-Turrax® homogenizer (T25, IKA, and Germany). Sodium alginate solution was prepared by slowly adding 3 g of sodium alginate powder to 197 g of deionised water and then was gently homogenized at 12000 rpm for 1 minute using a T25 digital Ultra-Turrax® homogenizer. According to the modified method of [18], the Alginate-Pectin (A-P) solution was prepared by mixing carefully the 1.5 % sodium alginate solution with the 1.5 % HMP solution at a volume ratio 2:1, followed by gentle stirring with a hotplate stirrer (LMS, Japan) at 1200 rpm without heat for 2 minutes to obtain a homogenous shell solution.

Preparation of the hardening solution: 3 % of calcium chloride solution was prepared by dissolving 30 g of calcium chloride dihydrate weighed using an analytical balance (Mettler Toledo, Switzerland) into a 1 L volumetric flask and topped up with deionized water to make up a final volume of 1 L. After thorough mixing, the calcium chloride solution was heated to 50°C, followed by addition of Tween® 80 (calcium chloride/Tween® 80 ratio of 0.1:100) and stirred gently using a hotplate stirrer at 1200 rpm at 50°C for 1 min.

Microencapsulation by co-extrusion technology: The microencapsulation of KSO using co-extrusion technology was carried out according to the modified method of [18,19] using a Buchi Encapsulator B-390 (Buchi, Switzerland). The encapsulator was equipped with a concentric nozzle set for one step core-shell microcapsules production. During microencapsulation, the core fluid (KSO) and the shell fluid (HMP-enhanced alginate solution) were simultaneously pumped into the concentric nozzle by the air pressure to give a core-shell fluid stream which was sprayed out through the nozzle. The air pressure was set at 600 mbar and the frequency was set at 500 Hz. An additional electrostatic field of 1.5 kV was applied between the nozzle and the hardening solution to prevent the potential coalescence of produced microcapsules during the flight or in approaching the surface of the hardening solution. The distance between the nozzle and the hardening solution was adjusted to 13 cm.

On contact with a 3 % of calcium chloride solution and Tween 80, regular-sized microcapsules formed through the cross-linking mechanism. Tween 80 was added to improve sphericity of microcapsules. The microcapsules were incubated in calcium chloride solution and stirred gently with a magnetic stirrer at speed 2 to prevent clumping of microcapsules for 45 min for complete gel hardening. Next, the microcapsules were collected with nylon sieve and were rinsed with distilled water followed by ethanol at 25°C. Afterwards, the microcapsules were dried in conventional oven (Memmert, Germany) at 65°C for 75 mins. A total of 8 g of KSO was used to run the microencapsulation of every production batch.

In vitro release evaluation of MKSO

***In vitro* gastric and intestinal digestion:** The simulated *in vitro* digestion has been carried out in two phases. First, Microencapsulated Kenaf Seed Oil (MKSO) was exposed to Simulated Gastric Fluid (SGF) containing pepsin and sodium chloride at low pH value [20]. After that, an intestinal digestion was simulated by exposing gastric digestion samples to a Simulated Intestinal Fluid (SIF) containing pancreatin and bile salts [21].

To determine the *in vitro* release of antioxidant compounds and bioactive compounds, the MKSO was subjected to a total of 4 h, 5 h and 6 h sequential simulated *in vitro* digestion. SGF was prepared according to the USP method [20] with slight modifications, and it contained 0.4 g pepsin and 0.25 g NaCl dissolved in deionized water to give a final volume of 125 mL at pH 1.5. Twenty gram of MKSO was first added to 72 mL of SGF and the pH was adjusted to 2.0. The mixture was stirred continuously in a controlled water bath temperature with orbital shaking at 100 rpm for 2 h at 37°C. After that, the pH of the resulting product for gastric digestion was adjusted to 7.0 with 1 M NaOH to inactivate pepsin [20] with slight modifications. The Simulated Intestinal Fluid (SIF) was prepared in 100 mL of 0.1 M Phosphate Buffered Saline (PBS) buffer containing 20 mg pancreatin. The reconstituted bile extract was prepared by dissolving 5 g of bile salts in 100 mL deionized water. SIF (72 mL) was added to the resulting product for gastric digestion followed by sequential addition of 25.2 mL reconstituted bile extract whose pH had been previously adjusted to 7.0. The mixture was stirred continuously in a controlled water bath temperature with orbital shaking at 100 rpm for 2 h, 3 h and 4 h at 37°C to complete the intestinal digestion.

Oil release behavior of MKSO: The released oil after subjecting MKSO to the simulated *in vitro* protocol was estimated using solvent extraction and adapted from the methods reported earlier [20-23]. The oil fraction was extracted from the digestion media by 300 mL hexane (3 independent separations with 100 mL of hexane) using separating funnel. The mixture was shaken vigorously to facilitate the transfer of oil into the n-hexane layer. The n-hexane layer was collected, combined and evaporated using a Multivapor P-6 (Buchi, Switzerland) at 56°C, 125 mbar for 30 min with speed 6. After that, residual solvent was removed by flushing with 99.9 % nitrogen. The weight of the released oil was estimated gravimetrically by weighing the rotary bottles. The result was calculated based on the formula in equation (1) and was expressed in percentage (%).

% released oil = (amount of oil released from the microcapsules (g)/ total oil in the microcapsules (g)) x 100 (1)

Antioxidant activities of kenaf seed oil

2,2'-Azino-Bis (ABTS) cation radical-scavenging assay: The ABTS radical scavenging activity assay was performed according to an established method with some modifications [24]. Ten mL of ABTS (7 mM) and 10 mL of potassium persulphate ($K_2O_8S_2$) (2.45 mM) were mixed manually in a 50 mL amber bottle and the mixture was kept in the dark at room temperature for 12-16 hours for activation. The activated solution was then diluted with ethanol until the absorbance at 734 nm was adjusted to 0.700 ± 0.02 nm using a UV-Vis spectrometer (UviLine 9400, Secomam, France). Then, 50 µL of the KSO samples were added to 1950 µL of ABTS solution in a 15 mL aluminum foil-wrapped test tube and allowed to react for 3 minutes. The steps were repeated for control (ethanol) and standards (Trolox). The absorbance was measured at 734 nm using UV-Vis spectrophotometer against a blank reagent (ethanol). ABTS radical scavenging activity of the KSO was calculated by using the equation (2) and was expressed as percentage inhibition.

Inhibition Percentage (IP) = (blank - KSO)/blank) × 100 (2)

Where blank and KSO are the absorbance values of the blank and the KSO.

Besides, the antioxidant activity of the samples was expressed as mg Trolox equivalents (mg T equiv/100 g oil) through the calibration curve of Trolox with calibration equation (3).

$y = - 3.1091x + 0.6442$ ($R^2 = 0.997$) (3)

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: The free radical scavenging activities of bulk and microencapsulated Kenaf seed oils were measured according to the method [25] with some modifications. Different test tubes were pre-wrapped with aluminum foil to prevent light exposure. KSO samples were dissolved in ethanol with the concentration of 10 mg/ ml. One hundred mg of the KSO sample was weighted and dissolved in 10 mL of ethanol. KSO sample (0.2 ml) was mixed with 2.8 mL of ethanol in the test tubes. One mL of the pre-prepared 0.004% DPPH solution was added and the mixture was shaken vigorously by using a vortex mixer (LMS, Japan). The test tubes were then allowed to stand at room temperature in a dark environment for 30 minutes. After 30 minutes, the absorbance was recorded against ethanol as the reagent blank at 517 nm using a UV-VIS spectrophotometer (UviLine 9400, Secomam, France). The steps were repeated for control (ethanol) and standards (Trolox). DPPH radical scavenging activity of the KSO was calculated by using the equation (4) and was expressed as percentage inhibition

Inhibition Percentage (IP) = (blank – KSO)/blank) × 100 (4)

Where blank and KSO were the absorbance values of the blank and the KSO.

The antioxidant activity of the KSO samples were expressed as mg Trolox equivalents (mg T equiv/100 g oil) through the calibration curve of Trolox with calibration equation (5).

$y = - 1.9888x + 1.6087$ ($R^2 = 0.998$) (5)

Antioxidant compounds of Kenaf seed oil

Folin-Ciocalteu reagent assay (total phenolic content): Total phenolic content of the methanolic extracts was determined with Folin-Ciocalteu colorimetric method [26] with slight modifications. Two gram of KSO sample was weighed into 15 mL Falcon tube, to which was added 5 mL of hexane and the phenolic compounds were extracted with 5 mL of methanol:water (60:40, v/v). Then, the mixture was vortexed using a vortex mixer (LMS, Japan) for 2 min followed by centrifugation at 3500 rpm for 10 min. The lower layer, methanolic phase (200 µL) was pipetted out and diluted with 4.8 mL of deionized water in different aluminium-foil wrapped 15 mL test tubes. For reference (blank) solution, methanol:water solution was used instead of methanol phase. Next, 0.5 mL of Folin-Ciocalteu reagent was added and followed by vortex for 1 min. The mixture was left to stand for 3 min and 1 mL of sodium carbonate solution (35 % v/v) was added after that. Deionized water (3.5 ml) was added to top up the solution to 10 mL and the test tubes were kept in the dark for 30 min. The absorbance of the mixtures was measured at 765 nm using UV-Vis spectrophotometer (UviLine 9400, Secomam, France). Measurements were carried out in duplicate and mean values were calculated. The results were expressed as milligram gallic acid equivalent per 100 grams of oil sample (mg GAE / 100 g of oil) and calculated based on equation (6) formed.

Total Phenolic Content (TPC) = [(absorbance x 0.0524) x diluted volume x 100]/(16.724 x weight) (6)

Aluminum chloride assay (total flavonoid content): Total flavonoids content of the bulk and microencapsulated kenaf seed oils was determined by aluminum trichloride colorimetric method [27]. Two gram of KSO sample was weighed into 15 mL Falcon tube, to which was added 5 mL of hexane and the phenolic compounds were extracted with 5 mL of methanol:water (60:40, v/v). Then, the mixture was vortexed using a vortex mixer (LMS, Japan) for 2 minutes followed by centrifugation at 3500 rpm for 10 minutes. The lower layer, methanolic phase (100 µL) was pipetted out and mixed with 200 µL of 5% $NaNO_2$ in different 15 mL test tubes which was previously wrapped with aluminum foil and followed by vortexed using a vortex mixer (LMS, Japan) for 1 minute. The mixture was allowed to stand for 5 minutes. After that, 200 µL of 10 % aluminum chloride ($AlCl_3$) and 1 mL of 1 M sodium hydroxide (NaOH) were added to the mixture which was followed by vortexed using a vortex mixer (LMS, Japan) and allowed to stand at room temperature for 15 mins. A blank sample (methanol:water) as the reagent control was

Table 1: Percentage of oil released from MKSO in simulated gastrointestinal fluid model.

Solutions (time)	Released oil (%)
SGF (2 hours)	20.77 ± 0.73^{d}
SGF + SIF (2 hours)	35.77 ± 1.19^{c}
SGF + SIF (3 hours)	56.56 ± 2.07^{b}
SGF + SIF (4 hours)	77.50 ± 2.76^{a}

Means ± standard deviation (n = 4) with different superscript letters ^{abcd} within the same column indicate significant difference ($p < 0.05$).

set up and went through all the steps. The steps were also repeated for standards (Catechin). The absorbance was measured at 510 nm against the reagent blank by a UV-Vis spectrophotometer (Uviline 9400, Secomam, France). The results were expressed as mg of catechin equivalent (mg catechin equiv/100 g oil).

Gas chromatography analysis of bioactive compounds (phytosterols) in Kenaf seed oil

Saponification for sterol analysis: Separation of sterols was performed after saponification of the KSO sample according to the modified method [28]. Total lipid (250 mg) and 100 µL of 5 α-cholestane (1 mg/ml) were refluxed in different conical flasks with 5 mL of ethanolic potassium hydroxide solution (6 g/100 ml) for 60 min by using hotplate stirrer. The unsaponifiables were first extracted three times with 10 mL of petroleum ether using separating funnel; the three extracts were combined and washed three times with 10 mL of neutral ethanol-water (1:1, v/v) and then dried for 30 minutes with anhydrous sodium sulphate. After 30 minutes, the extract was evaporated at 55°C under reduced pressure using Multivapor P-6 (Buchi, Switzerland) until approximately 1 mL of extract left. Then, the 1 mL extract was pipetted into a 15 mL Falcon tube and purged with nitrogen until completely dry.

Preparation of Trimethylsilyl Ether (TMS) derivatives of sterols: N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 mL/ 100 mL Trimethylchlorosilane (TMS) (125 µL) was added to the dry residue, the mixture was vortexed for 10 s and heated at 70°C for 30 mins. After cooling, 1 µL aliquots were directly injected in the form of auto injection into the gas chromatography [6].

Gas Chromatography-Flame Ionization Detection (GCFID): GC-FID analyses were performed using analytical conditions [29]. Briefly, an Agilent Technologies 7890A Gas Chromatography equipped with a split-splitless injector, a FID, and a HP-5 (30 m × 0.25 mm i.d., 0.32 µm film thicknesses) column was used. The initial column temperature was 250°C and programmed to increase at a rate of 2°C/ min to 300°C and then held for 12 min with the flow rate of 2.0 mL min^{-1}. The concentration of phytosterol was calculated from the equation (7) formed [6] and was expressed as mg/100 g.

Phytosterol = (peak area of standard x mg of IS)/(peak area of IS x weight of oil) x 100 (7)

Where IS = Internal Standard

Statistical analysis

All experiments were performed in duplicate and measurements were replicated two times (n = 4). Results were expressed as mean ± standard deviation. All results were analyzed using MINITAB 16 (Minitab Inc, Pennsylvania, USA). One way Analysis of Variance (ANOVA) and independent T-test were carried out. The average values were compared with Tukey's post hoc test for one way ANOVA. The differences were considered significantly at the level of $p < 0.05$.

Results and Discussion

In vitro release evaluation of Microencapsulated Kenaf Seed Oil (MKSO)

At the gastric digestion phase, the amount of released oil content from the MKSO was minor in SGF (2 hours), which was 20.77 ± 0.73 % (Table 1). After sequential digestion of MKSO in SGF for 2 hours and followed by in SIF for 2 hours, the amount of released oil was the least (35.77 ± 1.19 %) at 4 hours of incubation in SIF. After 3 hours of incubation of MKSO in SIF, the amount of released oil was 56.56 ± 2.07 %. Lastly, after 4 hours of incubation of MKSO in SIF, the result showed that higher percentage of oil was released from the microcapsules (77.50 ± 2.76 %). During 4 hours of incubation of MKSO in SIF, the amount of released oil has increased to 53.78 % than that of 2 hours of incubation of MKSO in SIF. These results indicated that KSO could continuously be released from the MKSO in SGF and the release of KSO from the MKSO in SIF was much higher than that in gastric phase. When the incubation time of MKSO in SIF increased, the results showed an increasing trend of that amount of oil released from the MKSO.

From the results shown, the observed increase in amount of released KSO from the HMP-enhanced alginate microcapsules at acidic (pH 2.0) and basic pH values (pH 7.0) was most likely due to the solubility of HMP-enhanced alginate microcapsules shell, resulting in the loss of capsule integrity/structure with concomitant KSO released. Besides, these results might be due to the effects of the hydrolytic enzymes (pepsin and pancreatin [amylase and trypsin]) and acidic pH (1.2) on protein and carbohydrate oligomer hydrolysis of the capsule wall, which caused the breakage of the microcapsules and oil released. The digestion of lipids only takes place in small intestine due to presence of hydrolytic enzymes (pancreatic lipase and bile salts), and the relatively high efficiency (usually > 90%) of the absorption of digested lipids and lipid-soluble molecules that occur in the small intestine [30].

Antioxidant Activities (AOA) of MKSO after simulated *in vitro* digestion

The ABTS assay is used in hydrophilic and lipophilic systems while the DPPH is used in lipophilic system to measure the free radical scavenging activity. The results showed that the ABTS radical scavenging activity was increased for MKSO for AOA if compared to bulk (unencapsulated) KSO (Table 2). The AOA of MKSO was 61.10 ± 1.57% and 12.04 ± 0.34 mg Trolox equiv/100 g oil, respectively, which were higher than the KSO (17.95 ± 1.00 % and 2.71 ± 0.20 mg Trolox equiv/100 g oil). The ABTS radical scavenging activity of MKSO showed a 70.62 % increase in percentage inhibition and a 77.49 % increase in Trolox equivalent if compared to bulk KSO, indicating that antioxidant polymers with even stronger antioxidant activities were produced during the simulated gastrointestinal digestion. Similar results were also obtained by You et al. [31]. According to You et al. [31], the changing trend of the $ABTS^{\cdot+}$ radical scavenging activity of full term GI digestion was different from that of

Table 2: Antioxidant Activities (AOA) and antioxidant compounds of Microencapsulated Kenaf Seed Oil (MKSO) after simulated *in vitro* digestion.

AOA/Antioxidant compounds	KSO*	MKSO
ABTS (mg Trolox equiv/100 g oil)	2.71 ± 0.20[b]	12.04 ± 0.34[a]
ABTS (% inhibition)	17.95 ± 1.00[b]	61.10 ± 1.57[a]
DPPH (mg Trolox equiv/100 g oil)	61.83 ± 1.46[a]	51.70 ± 0.82[b]
DPPH (% inhibition)	76.33 ± 1.79[a]	63.86 ± 1.01[b]
TPC (mg GAE/100 g oil)	8.70 ± 0.13[a]	4.42 ± 0.12[b]
TFC (mg Catechin equiv/100 g oil)	23.83 ± 0.15[a]	9.41 ± 0.16[b]

Means ± standard deviation (n = 4) with different superscript letters [ab] within the same row indicate significant difference ($p < 0.05$).
*without simulated *in vitro* digestion.
ABTS (2,2'-Azino-Bis (ABTS) cation radical-scavenging assay), DPPH (2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay), TPC (Total Phenolic Content), TFC (Total Flavonoid Content).

the DPPH radical scavenging activity. This is because the increase of hydrophobic properties of GI digestion after pepsin treatment makes them less likely to react with water-soluble $ABTS^{\bullet+}$ radical. However, the increase of hydrophilic property of GI digestion after pancreatin treatment favors their trapping of the $ABTS^{\bullet+}$ radical.

The DPPH radical scavenging activity was decreased for MKSO (after simulated *in vitro* digestion) for AOA if compared to bulk (unencapsulated) KSO. The AOA of MKSO showed 63.86 ± 1.01 % and 51.70 ± 0.82 mg Trolox equiv/100 g oil respectively, which lower than the AOA of KSO that showed 76.33 ± 1.79 % and 61.83 ± 1.46 mg Trolox equiv/100 g oil. Antioxidant compounds present in the KSO and MKSO reacted with DPPH, which is a nitrogen-centered radical with a characteristic absorption at 517 nm and is being converted into 1,1-diphenyl-2-picrylhydrazine, due to its hydrogen binding ability at a very fast rate [32].

As the concentration of phenolic compounds decreases after simulated *in vitro* digestion as a result of enzymatic reactions (pepsin and pancreatin [amylase and trypsin]) on the KSO, the DPPH radical scavenging activity would also decrease. The DPPH radical scavenging activity of MKSO showed a 16.38 % decrease in percentage inhibition and a 16.34 % decrease in Trolox equivalent compared to bulk KSO. This is because the increased polarity of the GI digest makes the phenolic contents more difficult to react with the lipid-soluble DPPH radicals [33]. Therefore, the DPPH radical scavenging activity of MKSO was decreased after simulated *in vitro* digestion.

Antioxidant compounds of MKSO after simulated *in vitro* digestion

The results showed that the Total Phenolic Content (TPC) experienced a significant decreased ($p < 0.05$) in MKSO when compared to bulk (unencapsulated) KSO. This indicates that the phenolics stability is strongly affected by pH [34]. Generally, pH > 7.4 is unfavourable for phenolic compounds and the effects of high pH are worsened by lengthy exposures. The number of hydroxyl (-OH) groups in benzene ring of simple phenolics can also be critical clues for phenolic stability. The TPC followed a trend similar to that DPPH radical scavenging assay in (Table 2), which decreased after simulated gastrointestinal digestion process. However, simple carbohydrates or amino acids may be present in the KSO and may interfere with the TPC assay, hence the total percentage loss of DPPH and TPC varied in this study. Similar results were supported by Wong et al. [35], dietary polyphenols are highly sensitive to the alkaline pH conditions in the small intestine. The proportion of these acids was transformed into their corresponding degradation products during digestion in the duodenum. Besides, it is possible that the pancreatin digestion liberates compounds (macromolecules as proteins and fibre) that are able to associate with phenolic compounds.

The results showed that the TFC experienced a significant decreased ($p < 0.05$) in MKSO if compared to bulk KSO. The sharp decrease of TFC in MKSO can be explained by the susceptibility of flavonoids towards the high air temperature during oven drying as flavonoids are susceptible to drying temperatures above 50°C and may decompose and combine with other plant components [36]. Similar results have been obtained by some studies showing that the antioxidant capacity of flavonoid compounds decreased dramatically when they are exposed to acidic or alkaline regions of the Gastrointestinal (GI) tract [37]. Other than the phytosterols, phenols and flavonoids, tocopherols in Kenaf seed oil also believed to be able contribute to its antioxidant activity [6].

Gas chromatography analysis of phytosterols of MKSO after simulated *in vitro* digestion

(Table 3) shows that the squalene in MKSO experienced a significant decreased ($p < 0.05$) compared to the squalene in bulk KSO. This might be due to solubility of squalene towards digestive enzymes particularly pancreatic lipase as squalene is one of the fat soluble antioxidants. Alternatively, these results suggested that the squalene might be susceptible to autoxidation.

Campesterol in MKSO experienced a significant decreased ($p < 0.05$) compared to the campesterol presented in bulk KSO. The amount of stigmasterol in bulk KSO was 2.77 ± 0.68 mg/ 100 g of oil, while it decreased to 2.16 ± 0.14 mg/ 100 g of oil in MKSO after simulated gastrointestinal digestion. β-sitosterol was the most abundant phytosterol found in the bulk KSO without simulated *in vitro* digestion and MKSO after simulated *in vitro* digestion. Results

Table 3: Phytosterol compositions of Microencapsulated Kenaf Seed Oil (MKSO) after simulated *in vitro* digestion.

Phytosterol compositions	KSO* (mg/100 g)	MKSO (mg/100 g)	Total loss of phytosterol compositions (%)
Squalene	2.65 ± 1.42[a]	0.55 ± 0.07[b]	79.25
5 α-cholestane	3.94 ± 0.02[a]	3.93 ± 0.04[a]	0.25
Campesterol	5.84 ± 0.83[a]	4.71 ± 0.05[b]	19.35
Stigmasterol	2.77 ± 0.68[a]	2.16 ± 0.14[b]	22.02
β-sitosterol	51.33 ± 9.25[a]	45.22 ± 1.44[b]	11.9
Sum	66.53	56.57	

Means ± standard deviation (n = 4) with different superscript letters [ab] within the same row indicate significant difference ($p < 0.05$).
*without simulated *in vitro* digestion.

also showed that the amount of β-sitosterol in bulk KSO was 51.33 ± 9.25 mg/ 100 g of oil, while it decreased to 45.22 ± 1.44 mg/ 100 g of oil in MKSO after simulated gastrointestinal digestion. β-sitosterol that presented in MKSO experienced a significant decreased ($p < 0.05$) compared to β-sitosterol that presented in KSO. These results showed that phytosterol is slightly soluble in edible oil and insoluble in water. Besides, these results suggested that phytosterol might be easily degraded by autoxidation or photooxidation.

Conclusion

In this study, KSO was successfully microencapsulated by co-extrusion technology with BUCHI Encapsulator B-390 in the wall materials made up of sodium alginate and High Methoxl Pectin (HMP). The MKSO was found to be stable in the gastric digestion phase while most of the KSO was still being protected and encapsulated when it was subjected to acidic medium. Only small amount of KSO had been released in the SGF indicating the relatively high sustained release property of MKSO in the stomach. High release of KSO in the SIF was observed. Hence, *in vitro* release of the KSO was improved by the microencapsulation technique. After simulated *in vitro* digestion, the contents of bioactive compounds such as phenolic and flavonoid compounds in MKSO were found to be significantly decreased ($p < 0.05$) if compared to bulk (unencapsulated) KSO. Besides, the DPPH radical scavenging ability of the MKSO was found to be significantly decreased ($p < 0.05$) if compared to bulk (unencapsulated) KSO. In contrast, the ABTS radical scavenging ability of the MKSO was found to be significantly increase ($p < 0.05$) if compared to bulk (unencapsulated) KSO. Phytosterol content of MKSO was discovered to be significantly decreased ($p < 0.05$) after simulated *in vitro* digestion, which was due to the enzymatic reaction that took place in gastrointestinal transit. Thus, microencapsulation can help to protect the antioxidants and bioactive compounds in the KSO from degradation or enzymatic reactions. Besides, it also contributes to controlled release of these nutritional compounds in human digestive system.

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