In Vitro Release Study of Freeze-Dried and Oven-Dried Microencapsulated Kenaf Seed Oil

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ABSTRACT

Introduction: The high amounts of polyunsaturated fatty acid (PUFA) in kenaf seed oil (KSO) is susceptible to oxidation. However, KSO is rapidly oxidised due to its high PUFA content. Methods: In order to prevent oxidation, kenaf seed oil was encapsulated using a co-extrusion technique to produce microencapsulated kenaf seed oil (MKSO). The shell materials used were formulated from alginate with high methoxy pectin and chitosan. MKSO was freeze-dried and in vitro behaviour of MKSO was investigated and compared with oven-dried MKSO. After in vitro digestion, the antioxidant and bioactive compounds in freeze-dried MKSO were investigated. Results: Freeze-dried MKSO released more oil (95.35%) compared to oven-dried MKSO (83.88%) after in vitro digestion. Total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities showed decreases, compared to before in vitro digestion while 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical-scavenging assay and tocopherol content showed increases in released oil from MKSO after in vitro digestion compared to before in vitro digestion. For phytosterol composition, the decreases in released oil were not significant. Conclusion: The studies showed that microencapsulation allowed for site-controlled oil delivery and protected the bioactive compounds.

Key words: Co-extrusion, polyunsaturated fatty acids, radical scavenging activities, radical-scavenging assay, tocopherol content, total phenolic content

INTRODUCTION

Increasingly, consumers are demanding nutritive and healthy food products (Calvo et al., 2012). Kenaf seed oil (KSO) is rich in nutritional content as it consists of polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and antioxidants including phytosterols. The major PUFA found in KSO is linoleic acid. The high amount of linoleic acid shows that kenaf seed oil could be a good source of essential fatty acids (Nyam et al., 2012). Thus, consumption of kenaf seed oil is potentially beneficial to human health. However, KSO is rapidly oxidised due to its high PUFA content. Microencapsulation is a possible approach to overcome this challenge and protect kenaf seed oil from degradation (Calvo et al., 2012). Microencapsulation is a process whereby sensitive ingredients such as unsaturated fatty acids and phenolic compounds are enclosed and protected against the surrounding environment. Out of numerous microencapsulation methods, co-extrusion is suitable to be used to coat the oil. It can be used to enclose solids, liquids, or gases inside a...
micrometric wall that is made of hard or soft soluble film. This is to reduce the dosing frequency and prevent the degradation of pharmaceuticals. Besides, by encapsulating and protecting health-promoting substances from degradation, the technique is also important in developing an effective system to release the oil to the targeted site during digestion (Zhang, Decker & McClements, 2014).

Alginate is made up of α-l-guluronic and β-d-mannuronic acid residues with varied composition. The gelling ability of alginate is due to the formation of a linkage between guluronic acid of one polymer chain to another. Since alginate is able to form rapid gelation in the presence of calcium cation, it has been widely used as wall material to produce microcapsules. The most common is the ionotropic gelation method, which involves the incorporation of sodium alginate into calcium chloride solution (Li et al., 2002).

Pectin is a polysaccharide that can be found in most plant tissues especially in fruits. Pectin consists of backbone of (1→4)-α-D-galacturonosyl residues which are typically interrupted with a 10% substitution of (1→2)-α-L-rhamnopyranosyl residues. Pectin exists in two forms - high methoxyl pectin (HMP) and low methoxyl pectin (LMP). HMP is more commonly used as it can be easily found in fruits. Chitosan is made up of randomly distributed β-(1,4) D-glucosamine and N-acetyl-D-glucoamine. Chitosan is a soluble in aqueous solution. It is used in different applications such as gel, solution, films or fibres (Rinaudo, 2007).

Based on Zhang et al. (2014), freeze-drying and oven-drying will cause different effects on the structure of microcapsules, thus in vitro behaviours of microcapsules will be different. For in vitro behaviour of oven-drying microcapsules, the study of Chew et al. (2015) was referred to.

In the present study, KSO was encapsulated by alginate, HMP and chitosan using co-extrusion technique to produce microencapsulated kenaf seed oil (MKSO). The in vitro behaviour of freeze-dried microencapsulated kenaf seed oil (MKSO) was investigated and compared with oven-dried MKSO. After subjecting KSO to in vitro digestion, its antioxidant properties and bioactive compounds were determined to examine the protective effects of microencapsulation.

**METHODS**

Dried kenaf seeds were obtained from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia.

**Kenaf (KSO) extraction**
The kenaf seed oils were extracted according to the methods by Nyam et al. (2009).

**Microencapsulation of KSO**
Sodium alginate solution (1.5% w/w) was prepared by weighing 1.5g of sodium alginate and dissolving it in distilled water. High methoxyl pectin solution (1.5% w/w) was prepared by dissolving 1.5g of HM pectin powder in distilled water. The alginate-pectin solution was prepared by mixing the alginate solution and high methoxyl pectin (HMP) solution at a volume ratio of 2:1 and stored overnight at 4°C. The hardening solution was prepared by mixing 3% w/w CaCl, with chitosan. The KSO was encapsulated by co-extrusion technology using BuchiEncapsulator B-390 (Buchi, Germany). The microcapsules formed were then freeze dried.

**Swelling and erosion behaviour**
Swelling and erosion studies were carried out according to the method reported by Zvonar, Bolko & Gasperlin (2012) with slight modifications. One gram (mo) of MKSO was soaked in 10mL of solution of pH 3 and pH 6.8 by adding 0.1 M
hydrochloric aid or sodium hydroxide, respectively. The schott bottle was then transferred to a water bath at 37±0.5 ºC. At predetermined time intervals (1, 2 and 3h), MKSO was taken out and lightly wiped with tissue paper to remove the excess test liquid. The swollen microcapsules were then weighed (mt). After that, the microcapsules were dried in the oven at 60ºC for 24 h. The hydrated microcapsules were weighed again to determine the remaining dry weight (mr). The swelling degree and percentage of erosion were calculated at each time point using the following equations:

Swelling degree (%) = \( \frac{mt - mr}{mr} \times 100 \)

% erosion = \( \frac{mo - mr}{mo} \times 100 \)

**In vitro release study**

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to the method reported by Karaca, Nickerson & Low (2013). For SGF, 2 g of NaCl was dissolved in 7 mL of 36 % HCl in 900 mL of distilled water. Then, 3.2 g of purified porcine derived pepsin was added into the solution. The pH of the mixture was adjusted to pH 1.5 with either adding 1M NaOH or 1M HCl. The final volume of solution was made up to 1000 mL with distilled water. To prepare SIF, 6.8 g of K$_2$HPO$_4$ was dissolved in 250 mL of distilled water and mixed well. Then, 10.0 g of pancreatin and 77 mL of 0.2 M NaOH were added into the solution. The pH of the solution was adjusted to pH 6.8 and final volume was adjusted to 1000 mL with distilled water.

For release behaviour of MKSO, MKSO was dispersed in SGF only and in SGF followed by SIF. Two grams of MKSO was incubated in 20 mL of SGF and then transferred to a water bath at 37 ± 0.5ºC with constant agitation at 100 rpm in for 30 min, 60 min and 120 min. Then, the digested MKSO was immediately adjusted to pH 6.8 with 1 M NaOH to inactivate pepsin. After that, 20 mL of SIF was added into the digested MKSO and incubated for 2, 3 and 4 h in a water bath at 37 ± 0.5ºC with constant agitation at 100 rpm. After digestion, the kenaf seed oil was extracted from the digested media according to the previously reported method (Calvo et al., 2012). The % of oil released was calculated based on the following equation.

\[
\text{% released oil} = \frac{\text{Amount of oil released from the microcapsules (g)}}{\text{Total oil in microcapsules (g)}} \times 100
\]

**Determination of antioxidant activities of KSO after in vitro digestion**

**Total phenolic content (TPC)**

Total phenolic content was estimated using Folin-Ciocalteu assay, based on the method described by Wong et al. (2014). The TPC of the samples was expressed in mg GAE (gallic acid equivalents)/100 g oil.

**2,2-Diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging activity assay**

The DPPH radical scavenging activity was determined according to the method decribed by Wong et al. (2014). The antioxidant activity of the oil samples was expressed as mg Trolox equivalents (mg Teq/100 g oil) and percentage of inhibition (%), which was calculated based on the expression \( [1 - (A_s/A_c) \times 100\%] \), where \( A_s \) and \( A_c \) represent the absorbance of the sample and control, respectively.

**2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS·+) radical scavenging assay**

The ABTS radical scavenging activity assay was performed according to Wong et al. (2014). The antioxidant activity of the samples was expressed as mg Trolox equivalents (mg Teq/100 g oil). The percentage of inhibition (%) was calculated based on the expression \( [1 - (A_s/A_c) \times \)
100%], where $A_s$ and $A_c$ represent the absorbances of the sample and control, respectively.

**Chromatographic determination of tocopherols and phytosterols**
The tocopherol and phytosterol contents in kenaf seed oil were determined according to the method established by Nyam et al. (2009).

**Statistical analysis**
All experiments were performed in duplicate and measurements were replicated twice ($n = 4$). Results were expressed as mean ± standard deviation. All results were analysed 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 significant at the level of $p < 0.05$.

**RESULTS AND DISCUSSION**

**Swelling and erosion study**
The swelling degree of freeze-dried MKSO gradually increased from the start of incubation (241.34%) and continued until 3 h of incubation (298.69%) (Figure 1). At low pH, hydration of hydrophilic –NH$_3^+$ group occurred in chitosan. Besides, carboxylate groups of alginate were neutralised by the free ammonium group of chitosan in acidic medium. Due to the reaction, the size of membrane pore increased and favoured the penetration of water, thus increasing the swelling degree. Besides, the presence of high methoxyl pectin (HMP) enhanced the swelling degree of microcapsules by conversion in pectinic acid, which has the ability to swell at acidic medium (Sriamornsak, Thirawong & Korkerd, 2007). From the results, the swelling degrees of microcapsules at pH 6.8 were found to be lower than the swelling degrees of microcapsules at pH 3 for the first 2 h. At the last hour of incubation, the swelling degree of microcapsules at pH 6.8 exceeded the swelling degree of microcapsules at pH 3.

The swelling degree of microcapsules at pH 6.8 was due to the complete deprotonation of the carboxyl group (-COOH) of alginate into anionic form (-COO−). Strong electrostatic repulsive forces between -COO− caused the water uptake to increase and resulted in high swelling ratios (Gong et al., 2011). Similar results were obtained by Jao, Ho & Chen (2010) who proposed that the swelling of calcium-alginate beads in SIF was due to the exchange of calcium with cation in the medium. However, the chitosan solubility decreased at higher pH, resulting in rigid microcapsules which become insoluble in water (Dima et al., 2013). At higher pH, COO− of the alginate chain and NH$_3^+$ of chitosan increased. This resulted in more reaction sites taking part in membrane formation and anti-swelling force consequently increased. Thus, the swelling degree of microcapsules at pH 6.8 was lower compared to pH 3 for 1 h and 2 h of incubation. At 3 h of incubation, the microcapsules at pH 3 might approach to its swelling equilibrium, thus the rate of swelling decreased. According to Xu et al. (2007), the swelling equilibrium of alginate-chitosan bead was reached faster in SGF (3 h) compared to SIF (4 h).

The erosion study reflects the amount of polymer dissolved in different media during dissolution process (Sriamornsak et al., 2007). In our erosion study, the percentage of erosion was found to be significantly higher ($p<0.05$) at pH 6.8 compared to pH 3. The percentage of erosion is strongly dependent on pH (Kim, 2015). At low pH, sodium alginate provided a stiff hydrated layer, which resisted erosion (Timmins, Pygall & Melia, 2014).

Based on the results obtained by Chew et al. (2015), the swelling degree of oven-dried MKSO was significantly lower
Figure 1a. Swelling degree of MKSO versus time of incubation at pH 3

Figure 1b. Swelling degree of MKSO versus time of incubation at pH 6.8

Figure 1c. Percentage of erosion of MKSO versus time of incubation at pH 3
Figure 1d. Percentage of erosion of MKSO versus time of incubation at pH 6.8

Figure 2a. Percentage of oil released from MKSO versus time in SGF

Figure 2b. Increase in paw diameter in different treatment groups in carrageenan induced edema model and % inhibition
(p<0.05) compared to freeze-dried MKSO. The percentages of erosion for oven-dried MKSO at pH 3 and pH 6.8 was significantly lower (p<0.05) compared to freeze-dried MKSO. Different drying methods will have an effect on the size, texture and hardness of the microcapsules. Generally, oven-dried microcapsules are smaller, harder and less fragile compared to freeze-dried microcapsules (Hamoudi et al., 2013). Besides, oven-dried microcapsules are disc shaped and have collapsed centres while freeze-dried microcapsules have porous structures. The porous structures favour the penetration of water into MKSO, thus increasing the swelling degree. As for the percentage of erosion, the amount of polymers dissolved in media increases when porosity increases. The contact area of polymers with media increases, thus more polymers are dissolved in media, leading to a higher percentage of erosion.

**In vitro release behaviour**
The major site of lipid digestion occurs in the small intestines. Therefore, it is important to investigate whether the bioactive compounds in MKSO are able to be delivered to the target site in the gastrointestinal tract (GI). The results showed that the amount of oil released in the gastric phase (15.15%) was significantly lower (p<0.05) lower compared to the intestinal phase (95.35%). The slower release of oil in the gastric phase was due to the higher ability of MKSO to swell in the gastric phase. Besides, there were also positive correlations between erosion rate and release of oil. A higher erosion rate in the intestinal phase resulted in more oil being released from the microcapsules (Sriamornsak et al., 2013). Generally, a high swelling degree with low erosion rate causes a thick swelling gel layer and long diffusion rate (Huanbutta et al., 2013). Besides, sodium alginate is structurally resistant to an acidic environment and is released rapidly in mild alkali condition (Yoo et al., 2006).

In SIF, the release of oil in the small intestines was favoured by digestion activities of hydrolytic enzyme. Pancreatin, that is present in the intestinal phase, was made up of pancreatic amylase and pancreatic lipase. The exposure of MKSO towards pancreatin increased the solubility of wall materials and caused the breakage of MKSO, leading to the release of the oil. The increase in oil release was also favoured by an increase in exposure time of MKSO towards pancreatin (Karaca et al., 2013).

Based on the results of oven-dried MKSO obtained by Chew et al. (2015), the percentage of oil released (83.33%) was significantly lower (p<0.05) compared to freeze-dried MKSO (95.35%). The higher porosity of freeze-dried MKSO also contributed to a higher percentage of oil released. Oven-drying caused the microcapsules to shrink by capillary pressure and led to densification. The densification process of microcapsules led to exudation of oil located near to the surface of the microcapsules and caused the sticky texture. The thin layer of oil made the microcapsules become more hydrophobic. This slowed down the reaction between simulated fluid and microcapsules, thus less oil was released. By comparing the results of oven-dried MKSO and freeze-dried MKSO, freeze-drying was found to be a better method to dry the microcapsules due to its high released rate in the intestine phase. The results were in agreement with Hamoudi et al. (2013).

**Antioxidant activities of KSO after in vitro digestion**
Recently, interest in health benefits of plants has increased because of their antioxidant and free radical scavenging activities observed after in vitro digestion (Nursakinah et al., 2012). Based on the results shown in Table 1, the TPC value of undigested oil (control) was higher (16.28mg GAE/100g oil) compared to released oil from digested MKSO (7.03mg
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Table 1. Antioxidant activities, tocopherols and phytosterols contents of microencapsulated kenaf seed oil (MKSO) after in vitro digestion

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Undigested oil</th>
<th>Released oil from digested MKSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC value (mg GAE /100g oil)</td>
<td>16.28 ± 0.78\textsuperscript{a}</td>
<td>7.03 ± 0.49\textsuperscript{b}</td>
</tr>
<tr>
<td>DPPH radical scavenging assay (mg Trolox equiv/100 g oil)</td>
<td>46.83 ± 2.47\textsuperscript{a}</td>
<td>23.20 ± 2.71\textsuperscript{b}</td>
</tr>
<tr>
<td>DPPH value (% inhibition)</td>
<td>58.46 ± 3.14\textsuperscript{a}</td>
<td>29.53 ± 3.32\textsuperscript{b}</td>
</tr>
<tr>
<td>ABTS radical scavenging assay (mg Trolox equiv/100 g oil)</td>
<td>7.86 ± 1.62\textsuperscript{b}</td>
<td>15.04 ± 0.85\textsuperscript{a}</td>
</tr>
<tr>
<td>α- tocopherol (mg/100g oil)</td>
<td>11.83 ± 2.26\textsuperscript{b}</td>
<td>24.61 ± 4.94\textsuperscript{a}</td>
</tr>
<tr>
<td>γ- tocopherol (mg/100g oil)</td>
<td>43.49 ± 7.21\textsuperscript{b}</td>
<td>75.10 ± 5.30\textsuperscript{a}</td>
</tr>
<tr>
<td>Sum (mg/100g oil)</td>
<td>55.39 ± 9.13\textsuperscript{b}</td>
<td>99.71 ± 8.11\textsuperscript{a}</td>
</tr>
<tr>
<td>Squalene (mg/100g oil)</td>
<td>11.21 ± 3.37\textsuperscript{a}</td>
<td>10.76 ± 5.24\textsuperscript{a}</td>
</tr>
<tr>
<td>Campesterol (mg/100g oil)</td>
<td>29.34 ± 4.40\textsuperscript{a}</td>
<td>25.21 ± 3.99\textsuperscript{a}</td>
</tr>
<tr>
<td>Stigmasterol (mg/100g oil)</td>
<td>14.50 ± 2.64\textsuperscript{a}</td>
<td>12.72 ± 2.92\textsuperscript{a}</td>
</tr>
<tr>
<td>β-Sitosterol (mg/100g oil)</td>
<td>266.30 ± 21.80\textsuperscript{a}</td>
<td>266.10 ± 20.50\textsuperscript{a}</td>
</tr>
<tr>
<td>Sum (mg/100g oil)</td>
<td>321.40 ± 25.60\textsuperscript{a}</td>
<td>314.80 ± 29.50\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Means ± standard deviations followed by different superscript letters within the same row are significantly different according to Tukey’s test.

GAE/100g oil). This indicated that MKSO experienced a significant decrease ($p<0.05$) of about 56.8% after in vitro digestion. According to Pavan, Sancho & Pastore (2014), the decrease in TPC was due to the instability of the phenolic compound at high pH condition. Based on the research done by Settharaksa et al. (2012), the phenolic compound was most stable at a slightly acidic condition (pH 6). However, at a pH of about 7, the TPC value was the lowest. The alkali environment favoured the conversion of the phenolic compound into an unknown or undetected compound. Therefore, since the pH of SIF was adjusted to 6.8, the degradation in phenolic compound caused the reduction in TPC.

The ability of digested oil to scavenge DPPH• free radical is shown in Table 1. The higher % of inhibition indicates the stronger antioxidant activities. From the results, the DPPH radical scavenging assay of undigested oil was 46.83 mg Trolox equiv/100g oil and 58.46 % while released oil was 23.20 mg Trolox equiv/100g oil and 29.53%. The results showed a 50.40 % decrease in the content and 49.50 % in inhibition activity. According to Rodríguez-Roque et al. (2013), the decrease in DPPH radical scavenging assay is due to transformation of substances with antioxidant activities into different structural forms with different chemical properties in alkali condition. Besides, the enzymatic reaction of pepsin and pancreatin (amylase and trypsin) reduced the concentration of phenolic compounds. This led to a reduction in DPPH radical scavenging activity after in vitro digestion (Zhu et al., 2008). It was found that the DPPH radical scavenging assay followed a similar trend to TPC. Both of the assays showed a decrease after in vitro digestion. According to Oktay, Gülçin & Kütrefiöglu (2003), the phenolic compound directly contributed to antioxidant activities.

Based on Table 1, it was found that the ABTS radical scavenging activity in undigested kenaf seed oil (7.86 mg Trolox equiv/100 g oil and 36.28%) was lower compared to the released oil (15.04mg
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Trolox equiv/100 g oil and 64.48%). The ABTS radical scavenging assay increased significantly \((p<0.05)\) to about 47.7% in the content and 43.7% of inhibition. Kamiloglu \textit{et al.} (2014) suggested that the increased in ABTS radical scavenging assay after \textit{in vitro} digestion was due to formation of new compounds with higher antioxidant activities compared to natural compounds present in the sample. From the results obtained, it was found that the trend for ABTS radical scavenging activity was different from the DPPH radical scavenging assay. The enzymatic action of pancreatin caused the gastrointestinal (GI) tract to digest more hydrophilic compounds. Due to increasing polarity, it becomes more difficult for the GI to react with lipid-soluble DPPH radical. However, the increase in hydrophilic property favours the trapping of ABTS radical and thus increases ABTS radical scavenging activities (You \textit{et al.}, 2010).

**Tocopherol composition in MKSO after \textit{in vitro} digestion**

Based on Table 1, \(\alpha\)-tocopherol content in the digested MKSO was 24.61 mg/100g. The amount of \(\alpha\)-tocopherol increased significantly \((p<0.05)\) in the released oil. The increase in \(\alpha\)-tocopherol was due to enhanced bioavailability of \(\alpha\)-tocopherol by unsaturated fatty acids (Failla \textit{et al.}, 2014). Kenaf seed oil is rich in unsaturated fatty acids, such as linoleic acid, oleic acid and palmitic acid which all belong to long chain fatty acids. During digestion, long-chain fatty acids tends to accumulate at droplet surfaces and prevent the lipase to interact with \(\alpha\)-tocopherol within the droplet core (Yang & McClements, 2013). Besides, it was observed that both \(\alpha\)-tocopherol and \(\gamma\)-tocopherol were stable in gastric and intestinal phases. According to Mandalari \textit{et al.} (2013), more than 90% of \(\alpha\)-tocopherol and \(\gamma\)-tocopherol were released in gastric phase and about 100% became available for absorption at the duodenal phase during \textit{in vitro} digestion of a pistachio sample. This suggests that digestive enzymes such as pepsin and pancreatin (amylase, trypsin and lipase) do not cause degradation of tocopherol. Overall, the amount of tocopherol content available after \textit{in vitro} digestion also showed the protective effect of the wall component of MKSO towards tocopherol.

**Phytosterol composition of MKSO after \textit{in vitro} digestion**

From Table 1, the amount of squalene that was present in the undigested oil was 11.21 mg/100g, decreasing to 10.76 mg/100g in released oil. As mentioned above, the enzymatic digestion of pancreatin caused increasing polarity of GI tract leading to the reaction with lipid-soluble squalene decreased (You \textit{et al.}, 2010). The decrease in squalene concentration after \textit{in vitro} digestion was only about 4.01%; however, the decrease was not significant \((p>0.05)\). Squalene is a bioactive compound that is used in the treatment of diabetes, cancer and tuberculosis. Besides, it also exhibits antifungal and antioxidant properties. Campesterol was the second abundant phytosterol that was present in undigested oil and released oil after \textit{in vitro} digestion. The amount of campesterol in undigested oil (29.34 mg/100g) was higher compared to released oil from digested MKSO (25.21/100mg). Although the released oil showed a decrease of about 14.1%, the difference was not significant \((p>0.05)\). A similar trend was found in the amount of stigmasterol and \(\beta\)-sitosterol after \textit{in vitro} digestion where the decrease in stigmatesrol and \(\beta\)-sitosterol was not significant \((p>0.05)\). Campesterol, stigmasterol and \(\beta\)-sitosterol are easily degraded by oxidation. The results showed that the campesterol, stigmasterol and \(\beta\)-sitosterol in kenaf seed oil were protected by microencapsulation during \textit{in vitro} digestion. The phytosterol was protected within the wall material which prevented
its oxidation. From the results, the slight decrease in phytosterol composition in released oil from digested MKSO suggests that the bioavailability of phytosterol is improved by microencapsulation.

**CONCLUSION**

In conclusion, microencapsulation could control the release of kenaf seed oil from microcapsules in human gastrointestinal tract. By comparing different drying methods, freeze-dried MKSO had higher oil released compared to oven-dried MKSO. This can be explained by larger pore size formed in the freeze-dried MKSO. Therefore, the swelling, erosion and oil release behaviour of freeze dried MKSO during *in vitro* digestion were higher than oven-dried MKSO. Besides, microencapsulation can prevent the degradation of bioactive compounds during *in vitro* digestion.

**REFERENCES**


