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In-vitro evaluation of kenaf seed oil in chitosan coated-high methoxyl pectin-alginate microcapsules

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ABSTRACT

Kenaf seed oil was encapsulated using co-extrusion technology with the shell formulation of high methoxyl pectin (HMP)-alginate solution and a chitosan coating was applied additionally on the microcapsules. An *in vitro* digestion was used to simulate the human gastrointestinal (GI) digestion to examine the oil release behavior of chitosan-coated microencapsulated kenaf seed oil (CMKSO) and non-coated microencapsulated kenaf seed oil (NMKSO). The changes in antioxidant activities and bioactive compounds of kenaf seed oil in CMKSO before and after digestion were evaluated. The best CMKSO with the highest microencapsulation efficiency was obtained from the concentration of 0.1% w/v chitosan solution, which was hardened through one-step process. The results showed that CMKSO was more resistant to simulated gastric fluid than NMKSO. There was 83.33% of kenaf seed oil released from CMKSO after simulated digestion. Enzymatic breakdown of the GI digests decreased the total phenolic content (47.5% decrease) and phytosterols content (35.4% decrease), but increased their 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging activity (145.1% increase), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^{\bullet+}$) (120.9% increase) radical scavenging activity, and tocopherols content (32.1% increase), compared to the undigested kenaf seed oil. This work showed that the microencapsulation of kenaf seed oil with HMP-alginate and a chitosan coating offers an effective controlled release delivery system.

1. Introduction

Kenaf (*Hibiscus cannabinus* L.), which is from the Malvaceae family is a valuable fiber plant native to India and Africa and currently planted as the fourth industrial crop in Malaysia (Chan and Ismail, 2009; Ayadi et al., 2011). The potential uses of kenaf seeds as a source of edible oil is often overlooked when considering kenaf as a fiber and feed crop (Coetzee et al., 2008). Kenaf seed oil can be considered nutritionally healthy because of the high concentration of polyunsaturated fatty acids and antioxidant activity (Nyam et al., 2009; Ng et al., 2013; Sara et al., 2013).

Currently, there is an increasing demand for nutritive and healthy foods in the market. Thus, research that focused at optimizing the delivery of health-promoting substances like dietary fibers, polyphenols and unsaturated fatty acids in dietary forms to consumers has been increased (Wang et al., 2013). However, the gastrointestinal (GI) environment, combined with the presence of oxygen in high quantities, can favor the oxidative reactions of unsaturated fatty acids. Thus, microencapsulation has been focused recently as another approach in the food industry to provide a physical barrier against adverse environmental conditions in order to suppress the oxidation of unsaturated fatty acids (Rubilar et al., 2012; Ng et al., 2013). Co-extrusion technology that uses a vibrating nozzle device equipped with a concentric nozzle is a promising technology in microencapsulation since it allows high production rate of uniform size microcapsules. This process can be conducted under mild, non-toxic conditions and can easily be scaled up (Homar et al., 2007).

Alginate is the most commonly shell wall material used for microencapsulation due to their ability to form stronger gel in the presence of Ca^{2+}, chemical stability, low toxicity and low immunogenicity (Liu et al., 2002). However, alginate may provide limited protection to core substances as porous structure of alginate microspheres permits diffusion of acid in and out of microspheres easily. Blending alginate with other polymers or coating one polymer layer

Abbreviations: HMP, high methoxyl pectin; GI, gastrointestinal; CMKSO, chitosan-coated microencapsulated kenaf seed oil; NMKSO, non-coated microencapsulated kenaf seed oil; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid).

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on alginate microspheres can be effectively overcomes these disadvantages (Shi et al., 2013). Pectins that are industrially extracted from apple pomace and citrus peels are mostly in the form of high methoxyl pectin (HMP) (>50% degree of esterification, DE) and often used to increase viscosity and gel strength of food products (Thakur et al., 1997). Calcium binding mechanism results from specific and strong interactions between Ca^{2+} and guluronate and galacturonate blocks in alginate and pectin, respectively (Fang et al., 2008). Pectin can be added to alginate-based shell formulations to give multiple advantages like enhance the protection on core substances, increase the consumption of dietary fiber and enhance the nutritional value of microcapsules (Fernandez, 2001). Chitosan is a polysaccharide composed by β (1–4) linked glucosamine units together with some proportion of N-acetylglucosamine units that is obtained through chitin's deacetylation. Chitosan can react with alginate to form a polyelectrolyte complex and the alginate beads become coated by a chitosan layer as a result of this reaction. This can help to improve the mechanical stability and permeability of alginate beads (Peniche et al., 2004).

To the best of our knowledge, no information has been reported on the microencapsulation by using chitosan-coated HMP-alginate as shell formulation. The main scope of this study was to optimize the concentration of chitosan and hardening step that used to produce chitosan-coated microencapsulated kenaf seed oil (CMKSO). CMKSO was compared with the non-coated microencapsulated kenaf seed oil (NMKSO) for swelling and erosion studies, as well as on the *in-vitro* digestibility. The bioavailability of the released kenaf seed oil from CMKSO was evaluated after the simulated digestion process.

2. Materials and methods

2.1. Solvent extraction of kenaf seed oil

Kenaf (*Hibiscus cannabinus* L.) seeds were obtained from Malaysia Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. The kenaf seeds were ground into fine powder using a food grinder. The oils were extracted from the seeds with a Soxhlet extractor using hexane at 60 °C for 3 h. The oil was then recovered by evaporating off the solvent using Buchi Multivapor P-6 at 55 °C, 241 mbar for 30 min and the residual solvent was removed by flushing with 99.9% nitrogen.

2.2. Microencapsulation of kenaf seed oil using co-extrusion technology

1.5% w/w sodium alginate solution was prepared by dissolving Na-alginate in distilled water, homogenized using a T25 digital rUltra-Turax homogenizer at 12,000 rpm for 2 min. High methoxyl pectin solution (1.5% w/w) was prepared by dissolving HM pectin powder in distilled water and homogenized at 7000 rpm for 1 min using the T25 digital Ultra-Turrax homogenizer. The alginate-pectin solution was prepared by mixing the alginate solution and HM pectin solution at a volume ratio 2:1, followed by gentle stirring with a magnetic stirrer to obtain a homogenous shell solution, and stored overnight at 4 °C.

The microencapsulation of kenaf seed oil using co-extrusion technology was carried out by a Buchi Encapsulator B-390. During microencapsulation, the core fluid (kenaf seed oil) and the shell fluid (HMP-alginate solution) were simultaneously pumped with a flow rate of 0.2 mL/min and 7.0 mL/min, respectively, into the concentric nozzle (200 μm/300 μm) by the air pressure (600 mbar) to give a core-shell fluid stream which was sprayed out through the nozzle under a vibration frequency of 500 Hz with an amplitude of 3 and a voltage of 1.5 kV was applied. The microcapsules obtained were hardened according to the previously described method with some modifications (Zvonar et al., 2012): (a) one-stage procedure in which the microcapsules were simultaneously incubated 10 min in chitosan solution with 3% w/w $CaCl_2$ or (b) two-stage procedure in which the microcapsules were incubated in 3% w/w $CaCl_2$ for 10 min followed by 5 min incubation in chitosan solution. To reduce the surface tension of the hardening solution, the surfactant of 0.1% w/v Tween 80 was added into the hardening solution (Whelehan, 2010). After that, the microcapsules were collected with a 100 μm nylon sieve and rinsed twice with distilled water. Then, the microcapsules were backed by tissue papers until no further moisture was found on the tissue papers. The size, shape and surface morphology of microcapsules were observed under a Nikon YS100 optical microscope (Nikon Corporation, Japan). The microcapsules were then dried in an oven at 50 °C for 2 h until constant weight reached. The microcapsules were kept in Schott bottle and flushed with 99.9% nitrogen. The microcapsules were kept in a freezer (-20 °C) for later user.

Three different concentration of chitosan (0.1, 0.4 and 1.0% w/v) was studied. The optimal hardening process and concentration of chitosan were determined based on the size and microencapsulation efficiency (MEE) of chitosan-coated microencapsulated kenaf seed oil (CMKSO). MEE was calculated according to:

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

Total oil was the total amount of kenaf seed oil used in the microencapsulation per batch. The extractable oil was determined through the extraction of kenaf seed oil from dried microcapsules according to the previously described method (Sun-Waterhouse et al., 2011). Chitosan solution (0.1%) was prepared by dissolving 1 g of chitosan in 900 mL of distilled water containing 10 mL of glacial acetic acid with the aid of a magnetic stirrer. 3% w/w $CaCl_2$ was dissolved in the solution (for one-stage procedure), and its pH was adjusted to 5.0–5.5 with 0.1N NaOH. The chitosan solution was then filtered to remove any insoluble material and the volume adjusted to 1000 mL with distilled water (Ahonkhai et al., 2006).

2.4. Swelling and erosion study

Swelling and erosion studies were carried out according to Zvonar et al. (2012) with slight modifications. Microcapsules (1 g) (m_o) were soaked in two different pH solutions (10 mL of pH 3 and pH 6.8, which prepared by 0.1 M HCl or NaOH), respectively, at 100 rpm and 37 ± 0.5 °C. At predetermined time intervals (1–3 h), microcapsules were taken out and lightly blotted off with tissue paper to remove the excess test liquid. The swollen microcapsules were then weighed (m_t). After the hydrated microcapsules' weight was determined, they were dried in an oven maintained at 60 °C for 24 h before reweighing to determine the remaining dry weight (m_r). Fresh samples were used for each individual time point. The swelling degree and percentage of erosion were calculated at each time point from the following equations:

$$\text{Swellingdegree}(\%) = \frac{m_t - m_r}{m_r} \times 100$$

$$\%\text{erosion} = \frac{m_o - m_r}{m_r} \times 100$$

2.5. In vitro *release studies*

Both simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to the previous reported method (United States Pharmacopeia, 2007; Kamalian et al., 2014; Nieddu et al., 2014). To prepare SGF, 2.0 g of NaCl was dissolved in 7.0 mL of 36% HCl in 900 mL of distilled water, followed by the addition