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PREPARATION AND EVALUATION OF ALGINATE-CHITOSAN MATRICES LOADED WITH RED GINGER OLEORESIN USING THE IONOTROPIC GELATION METHOD

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ABSTRACT

The non-volatile phenolic compounds of red ginger oleoresin are known to have high antioxidant properties to counteract a number of free radicals. Ginger oleoresin is easily degraded when exposed to air, light, water, high temperature, and low-pH conditions in the gastric area. The objective of this research was to evaluate chitosan-alginate matrices as biodegradable media for the targeted release of red ginger oleoresin in the gastrointestinal tract. The chitosan-alginate matrices were prepared using the ionotropic gelation method with varying weight ratios of chitosan to alginate. The encapsulation efficiencies, loading capacities, and cumulative release profiles were determined based on the total phenolic content of the samples. The *in-vitro* release assays of red ginger oleoresin in simulated gastrointestinal fluids showed that the chitosan-alginate matrices with a weight ratio of chitosan to alginate of 2:1 had a low initial cumulative release (4.3%) in simulated gastric fluid and a moderate final release in simulated colonic fluid (40.7%). The results indicated that chitosan-alginate matrices could be formulated for targeted release of red ginger oleoresin in the gastrointestinal tract and could be used as carriers to deliver bioactive compounds to the colon via oral administration.

Keywords: Alginate; Chitosan; Ionotropic gelation; Oleoresin; Red ginger

1. INTRODUCTION

The root or rhizome of ginger (*Zingiber officinale*) has been used since ancient times as a spice for food and traditional herbal medicine for treating inflammation, arthritis, neurological disease, gingivitis, asthma, stroke, diabetes, and tumors in China, India, and Middle East (Mashhadi et al., 2013; Zhu et al., 2013). A large number of phytochemicals present in the rhizome of ginger, such as phenolic compounds, sesquiterpenes, vitamins, and others, show antioxidant properties against free radicals, protecting cell membrane lipids from oxidation (Al-Nahain et al., 2014). Non-volatile oleoresin extracted from ginger rhizome containing 6-, 8-, 10-gingerol and 6-, 8-, 10-shogaol as well as their derivatives (Sonale & Kadimi, 2014; Varakumar et al., 2017) have been confirmed by using ion tandem mass spectrometry (Tao et al., 2009). Red ginger (*Zingiber officinale* var. Rubrum) is one ginger variant with a red root on the outside and a yellow-to-pinkish cross-section. The rhizome of red ginger contains phenolics and flavonoids in higher amounts than that of white ginger (*Zingiber officinale* var. Roscoe) (Oboh et al., 2012). Ginger oleoresin has been used as a food preservative (Krisanti et al., 2017) and also to cure prostate cancer (Karna et al., 2012), colon cancer (Deol & Kaur, 2013), liver cancer (Habib et al., 2008),

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and lung and cervix cancers (Choudhury et al., 2010).

Ginger oleoresin is susceptible to degradation when exposed to air, light, water, high temperatures, or gastric acid in the stomach (Harimurti et al., 2011). Therefore, it is of interest to find the right carrier to protect oleoresin from low-pH gastric conditions and to adjust the cumulative release of its bioactive compounds in the gastrointestinal tract. Chitosan has been used to prepare micro and nanoparticles or emulsions for the delivery of bioactive compounds through oral and topical applications (Agnihotri et al., 2004; Muharam et al., 2015). Protonated chitosan could react with negative ions in the mucous layer in the mucosa or peptidoglycan tissue (Wen & Park, 2011). This mucoadhesive interaction slows the release of the drug, thereby increasing its bioavailability. Alginate plays a role in protecting chitosan from degradation in acidic digestive conditions by forming an interpolymeric complex with chitosan. This complex swells and slowly releases the drug at a neutral pH (Tonnesen & Karlsen, 2002). In this study, red ginger oleoresin-loaded chitosan-alginate matrices were prepared using the ionotropic gelation method. The effect of varying alginate content on the cumulative release of the oleoresin in synthetic gastrointestinal fluids, corresponding to various conditions of the digestive system, were determined along with the total phenolic content, encapsulation efficiency, drug loading, and scanning electron microscopy (SEM) pictures. It is expected that red ginger oleoresin–loaded matrices could be formulated as a medicinal or health supplement for gastrointestinal diseases.

2. METHODS

2.1. Materials

Fresh red ginger (*Zingiber officinale* var. Rubrum) was purchased from a local market in East Jakarta, Indonesia. Chemicals and reagents used, such as ethanol 96%, HPLC-grade methanol, 2.2-diphenyl-1-picrylhydrazyl (DPPH), sodium tripolyphosphate (Na-TPP), gallic acid, Folin-ciocalteu's (FC) reagent, calcium chloride ($CaCl_2$), sodium carbonate (Na_2CO_3), hydrochloric acid (HCl), potassium chloride (KCl), monopotassium phosphate (KH_2PO_4), and sodium hydroxide (NaOH) were procured from Sigma-Aldrich and Merck. Medium–molecular weight chitosan was purchased from Chemultiguna, Indramayu, Indonesia.

2.2. Preparation of Red Ginger Oleoresin

The fresh red ginger was washed, peeled, cut, and dried at room temperature (25°C). The dried ginger was then mashed to obtain ginger powder. The ginger powder (500 g) was macerated in 96% ethanol using a powder to solvent ratio of 1:3 (g/mL) and incubated at room temperature for seven days. After seven days, the mixture was filtered and evaporated under reduced pressure at 40-0°C for 4 h to produce the ginger oleoresin, which was immediately stored at 4°C.

2.3. Preparation of Oleoresin-Loaded Matrices

The chitosan-alginate matrices loaded with oleoresin were prepared by crosslinking and the ionotropic gelation method (Yu et al., 2009), resulting in the four formulations listed in Table 1. Slurries containing ginger oleoresin (0.1 g/mL ginger oleoresin) were mixed with 2.5% (v/v) acetic acid (50 mL) and 1.0 g of chitosan using a four-blade impeller (IKA Labortechnik) at 1000 rpm for 15 min. The 100-mL solution of 1% (w/v) Na-TPP was dropped into the homogenized chitosan-oleoresin slurry using a 10-mL syringe with 600-rpm stirring for approximately 10 min. The mixed solution was kept at room temperature for 30 min. The matrices of chitosan-oleoresin-TPP in the solution were filtered, rinsed with distilled water, and dried using a vacuum filter. The chitosan-oleoresin-TPP powders from the previous step were added into the alginate solution and stirred with magnetic stirrer at 1000 rpm for 15 min, resulting in a chitosan-alginate suspension. The suspension was then dropped slowly into the 100 mL solution of 6% (w/v) $CaCl_2$ using a syringe. The beads solution was kept for 30 min.

The beads were separated from the solution and rinsed with distilled water using a vacuum filter. The separated matrices were dried using a freeze dryer (EYELA FDV-1200; -47.6°C; 11.1 Pa). The dry particles were ground using mortar and sieved to gain particles with size <100μm. The resulting powders were stored at 4°C.

Table 1. Composition of chitosan-oleoresin-alginate matrices

Sample code	Weight ratio		
	Chitosan	Oleoresin	Alginate
COA0	1	0.1	0
COA10	1	0.1	0.10
COA25	1	0.1	0.25
COA50	1	0.1	0.50

2.4. Chemical Composition of Red Ginger Oleoresin

The determination of the chemical compound of oleoresin was done using liquid-chromatography mass-spectrophotometry (LC-MS). The LC instrument was LC-MS/MS using Acquity UPLC HSS C18 SB Column 1.8μm and MS Detector Xevo G2-XS QTof. The operating conditions used included a methanol:water mobile phase with a flow rate of 0.2 mL/min for a total of 23 min. Ionization detection was performed using an electrospray ionization (ES) system positive ion mode in the range 50–1200 m/z. Input temperature was 100°C, column temperature 40°C, desolvation gas flow rate of 800 L/h, collision energy 25–70eV, and ramp collision energy 25–70eV. Identification was performed using the MassLynx 4.1 analysis tool.

2.5. Total Phenolic Content in Red Ginger Oleoresin

The total phenolic content (TPC) of the oleoresin was determined using the FC reagent method adapted from a previous report (Singleton, 1999). The quantitative analysis of TPC is expressed by the milligram equivalent of gallic acid equivalent (GAE) per g of dry sample (mg GAE/g sample). A certain concentration of the red ginger oleoresin in methanol (0.1 g/mL) was mixed with 5 mL of 10% FC reagent (v/v). The mixture was allowed to react for 5 min. Then, 4 mL of 1 mol/L Na_2CO_3 was added and mixed well using a vortex. The mixture was incubated at room temperature for 15 min. The absorbance was measured at 765 nm using the UV/VIS-spectrophotometer Spectroquant® Pharo 300. The phenolic content of the samples was analyzed in triplicate.

2.6. Encapsulation Efficiency and Loading Capacity

The encapsulation efficiencies and loading capacities of red ginger oleoresin using chitosan-alginate polymer were obtained based on the TPC from the initial oleoresin compound added to the matrices and the wasted oleoresin in the filtered and rinsed water. The calculation used Equation 1 and Equation 2:

$$\text{Encapsulation efficiency } (\%) = \frac{m_{\text{initial}}^{oleoresin} - m_{wash\ solution}^{oleoresin}}{m_{\text{initial}}^{\text{oleoresin}}} x\ 100\% \quad (1)$$

$$\text{Loading capacity } (\%) = \frac{m_{\text{initial}}^{oleoresin} - m_{wash\ solution}^{oleoresin}}{m_{\text{final}}^{\text{matrix}}} x\ 100\% \quad (2)$$

2.7. Physical and Morphology Characterization

The surface morphologies of the matrices were evaluated utilizing an SEM SEI JEOL-JSM 6510LA using the secondary electron image mode with a 20kV acceleration voltage and 500–10,000× magnification. The interactions between functional groups in chitosan-alginate and red ginger oleoresin were observed using Fourier transform infrared (FTIR) spectroscopy of PerkinElmer UATR two. The IR spectra of red ginger oleoresin and the spectra of chitosan-

alginate matrices loaded with ginger oleoresin were compared to analyze the effect of encapsulation.

2.8. *In-Vitro* Drug Release Study

The release profiles of oleoresin from chitosan-alginate matrices were obtained using synthetic gastrointestinal fluids with a sequential release method (Mulia et al., 2017). The simulated gastric fluid (SGF) was created from 0.2 M KCl and 0.2 M HCl buffer solutions with a ratio of 1:1.7 to obtain pH 1.2. The simulated intestinal fluid (SIF) was created from 0.1 M KH_2PO_4 and 0.1 M NaOH buffer solutions with a ratio of 1:0.782 to obtain pH 7.4. The SCF was created from 0.1 M KH_2PO_4 and 0.1 M NaOH buffer solutions with a ratio of 1:0.448 to obtain pH 6.8. The *in-vitro* release was conducted by immersing 20-mg matrices in 60 mL of synthetic fluid at a 37°C incubation temperature. The buffer change was done after 3 h from SGF to SIF and after 7 h from SIF to SCF. The samples were taken in every 2 h until 24-h immersion in synthetic fluids and determined by UV-spectrophotometry analysis. The profile release curve was obtained by plotting the cumulative percentage of oleoresin (mg/mg) as a function of time immersed in synthetic gastrointestinal fluids.

3. RESULTS AND DISCUSSION

3.1. Composition of Red Ginger Oleoresin

The chemical compounds in the red ginger oleoresin were determined using LC-MS analysis and the LC chromatogram, as shown in Figure 1. The chromatogram showed the peak of the standard 6-gingerol at 11.3 min. The chromatogram of ginger oleoresin extracted from the rhizome also showed a peak of 6-gingerol as well as other peaks. The mass spectroscopy analysis of the ginger oleoresin showed eight main compounds identified as phenolic compounds, as shown in Table 2.

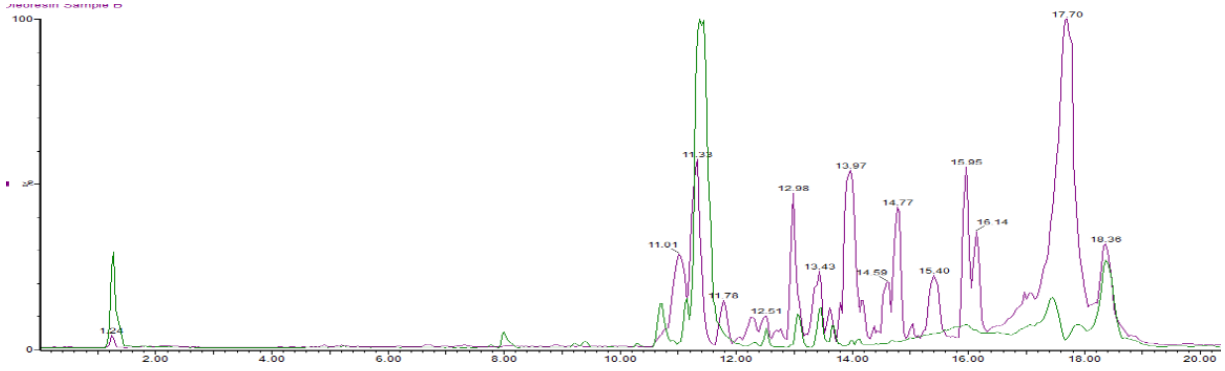


Figure 1 The LC chromatogram of 6-gingerol standard (green) and red ginger oleoresin (purple)

The similar analysis result of oleoresin from red ginger was reported by (Jiang et al., 2005), the gingerols being the major content and the gingerdiols the identity compounds of red ginger. The gingerdiols, the derivatives of gingerols, showed greater cytotoxicity effects on human colon cancer cells HT-29 than 6-gingerols (Tanaka et al., 2015).

The yield of oleoresin obtained was 7.6% (g/g) of dry ginger powder. The TPC of the ginger oleoresin extracted using ethanol was 28 mg GAE/g of dry ginger sample. This result was slightly higher than that reported by Ghasemzadeh et al. (2016) for oleoresin samples from various parts of the ginger plant using vacuum-oven drying. It was reported that the TPC for oleoresin extracted using petroleum ether and chloroform/methanol had values of 52.2 and 60.3 mg GAE/g of dry sample, respectively (Ali et al., 2018). It seems that the TPC of ginger oleoresin was affected by the ginger plant parts used, the type of solvent, and the heating during the process of extraction.

Table 2 Phenolic compounds identified in red ginger oleoresin using mass spectra of LC-MS

Compound Name Formula	Molecular Weight (g/mol)	%-abundance
6-gingerol	293	33.14
3,5-acetoxy-6-gingerdiol	338	6.46
Methyl diacetoxy-[6]-gingerdiol	394	5.66
Methyl 3,5-acetoxy-6-gingerdiol	352	2.39
Acetoxy-6-gingerol	336	2.25
8-gingerol	321	1.75
Diacetoxy 6-gingerdiol	380	1.54
Methyl 6-gingerol	308	0.10
Others	-	46.70

3.2. Encapsulation Efficiency and Loading Capacity

The encapsulation efficiencies and loading capacities values, as calculated using Equation 1 and Equation 2, are given in Table 3. The highest encapsulation efficiency was found in COA1 matrices (79.4%) and the lowest in COA25 matrices (68.9%). The COA1 matrices were prepared from a chitosan:oleoresin:alginate weight ratio of 10:1:1. In general, the encapsulation efficiency obtained ranged from 69% to 79%. The encapsulation efficiency obtained in this study was quite similar with the encapsulation efficiency of the chitosan-oleoresin-alginate matrices reported by Krisanti et al. (2017). The phenolic compounds in oleoresin have low solubilities in aqueous solutions. During the formation of chitosan-alginate matrices following the ionotropic gelation method, the phenolic compounds which are hydrophobic active compounds prefer to be inside the matrices rather than diffuse to the aqueous solution of TPP. This is consistent with the explanation by Sabliov and Astete (2008).

Table 3 Encapsulation efficiency and loading of oleoresin

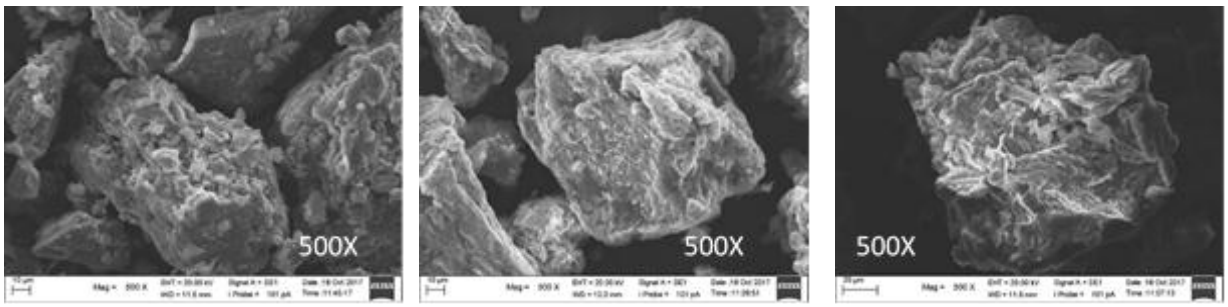
Sample	Encapsulation efficiency (%)	Loading capacity (%)
COA0	71.4	2.0
COA10	79.4	2.1
COA25	68.9	1.8
COA50	69.3	1.9

At a constant amount of oleoresin but with various amounts of alginate, the highest encapsulation efficiency and loading capacity values were with the matrix COA1 in which the weight ratio of chitosan:oleoresin:alginate was 10:1:1, as shown in Table 3. The percentage of LC is generally influenced by the weight of the matrices formed. It is reported that the dense structures due to cohesive interactions between polymer compounds can increase the loading of oleoresin but, on the other hand, minimize the free space between alginate polymer matrices, reducing the chitosan-oleoresin matrix entrapment area (Soliman et al., 2013).

3.3. Surface Morphology of Oleoresin-Chitosan Matrices

The surface morphologies of the matrices were determined based on the SEM pictures using an SEI JEOL-JSM 6510LA instrument. Figure 2 shows the SEM photographs with magnifications of 500× for three matrix variations. The non-alginate chitosan-oleoresin matrices (Figure 2a) appeared more hollow and had coarse surfaces, while the chitosan-alginate matrices (Figures 2b and 2c) showed softer, denser surfaces. The alginate-coated chitosan-oleoresin matrices appeared to have flake-like surfaces and to be homogeneous. This type of surface is thought to be the result of ionic interactions between chitosan and alginate. The morphological surface observations by SEM indicated crosslinking formed between chitosan-oleoresin and TPP. A

similar observation was reported earlier (Krisanti et al., 2017). Matrices of chitosan-alginate-oleoresin-TPP were not spherical, and non-alginate chitosan-oleoresin matrices tended to be irregular.



(a) (b) (c)

Figure 2 Scanning electron micrograph (500× magnification) of chitosan-oleoresin-alginate matrices with various weight ratios: (a) 10:1:0; (b) 10:1:1; and (c) 10:1:5

3.4. Infrared Spectra of Oleoresin-Chitosan Matrices

The structural observation of chitosan-alginate matrices using the FTIR method was based on the presence of absorbance peaks attributed to the vibrations of functional groups in the compounds. Figure 3 shows the FTIR spectra of red ginger oleoresin extracted from the ginger powder and the chitosan-oleoresin matrices with and without alginate.

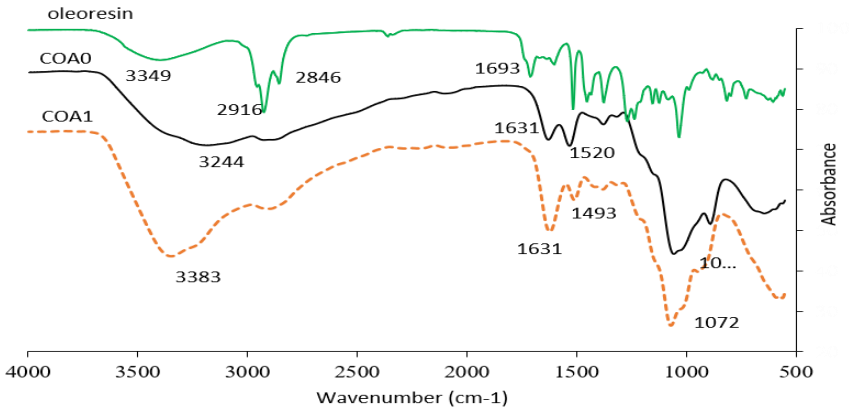


Figure 3 FTIR spectra of red ginger oleoresin and oleoresin-loaded chitosan-alginate matrices

In the red ginger oleoresin spectrum, the presence of broad bands at 3349 cm^{-1} can be attributed to the hydroxyl group (O-H) stretching vibration. Then, the presence of medium- and strong-intensity bands at 2916 cm^{-1} and 2846 cm^{-1} also represented the O-H bond vibrations from the carboxylic acid functional group present in the ginger oleoresin. A medium-intensity band at 1693 cm^{-1} was also observed due to the presence of the carbonyl functional group (C=O) vibration peak of oleoresin (Purnomo et al., 2010). The spectra of the chitosan-oleoresin matrix showed an absorbance peak at 3244 cm^{-1}, identical to the characteristic stretching vibration peaks of OH groups in polysaccharides, such as chitosan and alginate. This peak was more intense when alginate was added, as in the chitosan-oleoresin-alginate matrix. It was shifted to a higher wavenumber of 3383 cm^{-1}, which may be due to the interaction between chitosan and alginate. The stretching vibration peaks of OH groups in the chitosan-alginate matrix without oleoresin was nearly similar (3344 cm^{-1}) as the result reported by Mulia et al. (2017). The presence of interaction between the amino groups of chitosan and the carboxylate anions of alginate was shown by the peak at 1631 cm^{-1} attributed to the asymmetric stretching of -COO- groups. When alginate was added into the chitosan-oleoresin, the intensity at 1631 cm^{-1} was higher than that of the chitosan-oleoresin matrix (Agnihotri et al., 2004; Li et al., 2008; Nnamonu

et al., 2012). The peaks at 1520 and 1493 cm^{-1} in both matrices indicated the characteristics of hydrogen bonds formed between the amine group of chitosan and the phosphate group of the TPP linking agent. The peak at 1065 cm^{-1} was the stretch vibration peak of -C-O-C- in chitosan, which had higher intensity and shifted to 1072 cm^{-1} when alginate was added because alginate also had the -C-O-C group (Mulia et al., 2017).

3.5. *In-Vitro* Drug Release Study

Figure 4 shows the *in-vitro* pH-sensitive release profile of oleoresin out of the chitosan-alginate matrices. The amount of phenolic compounds released into acidic SGF (pH of 1.2) was low. The average phenolic compounds released after 2 h ranged from 1.9–4.3%, except for COA0 (<10%), the non-alginate chitosan-oleoresin matrices. Alginate formed alginic acid at low pH that triggered the formation of a gel or a hydrocolloid layer with high viscosity (Wen & Park, 2011). The formation of gel might prevent the degradation of chitosan matrices so that limited amounts of bioactive compounds were released (Mulia et al., 2017).

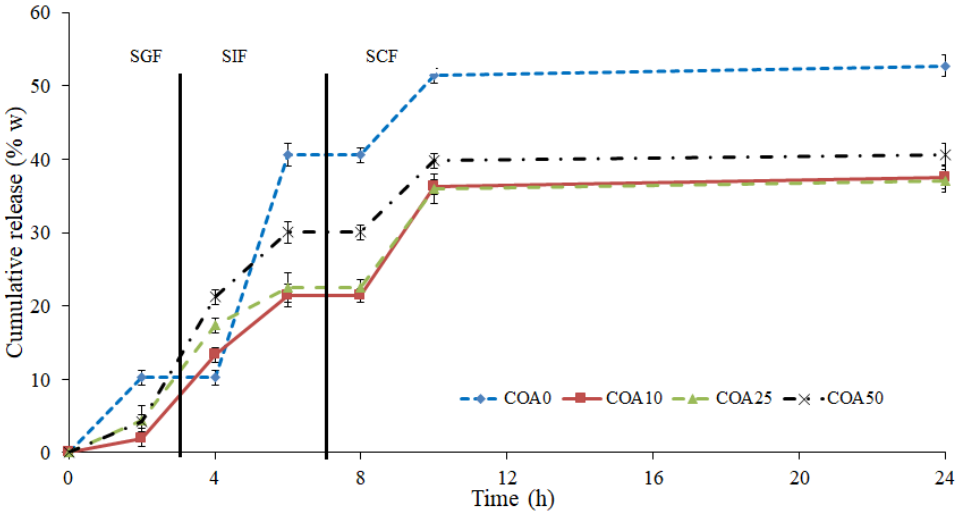


Figure 4 Cumulative release of oleoresin from chitosan-TPP-alginate matrices

Chitosan swelling occurred due to protonation of amine groups at low pH mainly in the first three h. This swelling condition occurred significantly for the COA0 sample, therefore showing high release of phenolic compounds under gastric conditions (10.2%). Similar high release of active compounds from chitosan matrices was has been reported by other researchers (Remunan-Lopez et al., 1998; Zhang et al., 2002). The cumulative release of phenolic compounds in SIF (pH of 7.4, 4 h) reached about 40.6% for the non-alginate matrix. It seems that the degradation of chitosan initiated by swelling that allowed the release of phenolic compounds in SIF, as shown by the steep slope of release in this medium.

The release of phenolic compounds at three h in media with pH 1.2 (SGF) for matrices with alginate was significantly lower than in non-alginate matrices (COA0). In media with pH 7.4 (SIF), the cumulative release of phenolic compounds for all matrices increased. The largest cumulative release from the non-alginate matrix (COA0) was 52.7%. The second was the 40.6% of COA50, which was followed by COA10 and COA25, 37.5 and 37.1%, respectively.

It can be concluded that the release value is influenced by the presence of alginate in the chitosan-oleoresin matrix. Under low pH conditions, the formation of alginate hydrogels protects chitosan from swelling. However, at higher pH conditions (6.8–7.3) and over a longer period, alginate can increase the release of active compounds. This phenomenon may occur because in high pH the carboxyl alginate group is more ionized and the electrostatic repulsion between the ionized group increases the rate of swelling (Zhang et al., 2002). In matrices with relatively high alginate compositions, alginate hydrogels can also swell under neutral pH

conditions, which softens the surface of the polymer matrix. This condition is followed by withdrawal of water by microspheres which causes even more massive diffusion (Siegel & Rathbone, 2012). This might explain observations of higher releases in matrices with higher alginate content (COA50) than in matrices with lower alginate content (COA25 and COA10).

Figure 4 also shows the phenolic compounds were released significantly in 8–10 h, but only small amounts of phenolic compounds were released in the 10–24 h period. It seemed the swelling of the chitosan matrix that caused the release of active compounds from the inside matrix to the outer media was no longer the limiting step. The solubility of active compounds in SCF media seemed to have more control over the release of active compounds from the matrix when the release was conducted over a longer period in the SCF media. The chitosan-oleoresin–matrix formulation without alginate might be quite suitable for gastric release targets. This result indicated the potential of chitosan-alginate matrices as carriers to deliver phenolic compounds of red ginger oleoresin to colon target sites via oral administration.

4. CONCLUSION

The TPC in red ginger rhizome powder was found to be 28 mg GAE/g of dry sample powder, while the encapsulation efficiency was as high as 79% and loading capacity as high as 2% (weight ratio of chitosan:oleoresin:alginate of 10:1:1). The *in-vitro* release assays of ginger oleoresin in SGF showed that the chitosan-alginate matrices with a weight ratio of chitosan to alginate of 2:1 had low release in SGF (4.3%) and moderate release in SCF (40.7%). By selecting the formulation of the chitosan-alginate matrices, the targeted area of release of red ginger oleoresin in the gastrointestinal tract could be designed. The chitosan-alginate matrices had the potential to be carriers to deliver bioactive compounds to the colon via oral administration.

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