Formulation and characterization of chitosan-alginate freeze dried matrices loaded with oleoresin extract of red ginger

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Abstract. A simple method of encapsulation of bioactive compounds that does not involve washing, heating, and degradation of active substance is desired. The encapsulation of red ginger oleoresin in the chitosan-alginate matrix was developed using the freeze drying method to achieve higher matrix yield and loading capacity in the matrix. Bioactive substances that are encapsulated in the chitosan-alginate matrix aim to be released in the colon. Oleoresin red ginger extract was obtained from maceration of dried rhizome powder in ethanol. A mixed solution consisting of chitosan, oleoresin extract, Tween 80 as a surfactant and alginate was lyophilized at -50 C at a vacuum pressure of about 20 atm for 48-72 hours. The X-ray diffraction pattern of the formed matrix shows that the crystalline chitosan and alginate peaks disappear in the chitosan-alginate matrix which shows compatibility. In the Infra Red matrix spectrum the interaction between chitosan and alginate in the matrix is shown by the change of peak height at 1520 and 1385 cm⁻¹. Release assay showed that the matrix with a weight ratio of chitosan: alginate of 1: 0.5 slightly released phenolic compounds from oleoresin in the simulated gastric fluid but highly released in the simulated intestinal and colon fluids.

1. Introduction
Zingiber officinale, generally known as ginger, originated from South-East Asian and introduced to many parts of the globe, has been cultivated for thousands of years as a spice and also for medicinal purposes. The rhizome of this plant has been used as a medicine in Asian, Indian, and Arabic herbal traditions since ancient times also as herbal medicine for the treatment of arthritis, rheumatological conditions and muscular discomfort [1,2]. Some phenolic substances present in ginger, in general, have strong anti-inflammatory and anti-oxidative properties. It also exerts substantial anti-carcinogenic and anti-mutagenic activities. It has been found that ginger supplementation suppresses colon carcinogenesis in the presence of procarcinogen [3,4].

Encapsulation of active compounds in matrices or microparticles with various polymers has been widely known for its use in protecting and acting as carriers of drug or active substance. Chitosan is a common polymer used and known as a biodegradable, biocompatible and non-toxic biopolymer [5] which has a good mucoadhesivity [6]. Alginate is another common biopolymer in oral drug delivery known for its stability in acidic conditions of the stomach [7]. The combination of chitosan and alginate have been previously used in oral controlled drug delivery formulations [8,9]. In this research, the active compounds of ginger oleoresin were loaded into chitosan-alginate
matrices using the freeze drying or also called lyophilization method, which converts a solution into a solid by taking out water molecules through the process of sublimation and desorption in vacuum condition [10]. The concentration of alginate and oleoresin content in the matrices were varied. The matrices had been evaluated for its release characteristics in the in vitro assay using simulated gastrointestinal fluids. The total phenolic content, matrix yield, loading capacity and the morphology of matrices were also evaluated and reported.

2. Materials and methods

2.1. Materials
Fresh red ginger (Zingiberofficinale var. Rubrum) was purchased from a local market in East Jakarta, Indonesia. Pure grade of ethanol 96%, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), acetic acid, Folin-ciocalteu’s (FC) reagent, gallic acid, Tween 80, Sodium carbonate (Na₂CO₃), hydrochloric acid (HCl), potassium chloride (KCl), monopotassium phosphate (KH₂PO₄), and sodium hydroxide (NaOH) were procured from Sigma-Aldrich, Inc. and Merck. The chitosan of MW of 80-120 kDa, particle size of 200-300 mesh, viscosity of 100-200 mPas, was purchased from Chemultiguna, Indramayu, Indonesia.

2.2. Formulation of chitosan-alginate freeze-dried matrices
The red ginger oleoresin extract were resulted from maceration of dry rhizome powder in ethanol 96% for seven days using similar procedure as reported on other publication[11]. The chitosan-alginate matrices were prepared with six variations, i.e. one blank matrix without oleoresin extract and five matrices with variation of alginate and extract composition. All of the matrices were prepared using the freeze drying method reported previously with some modification [12]. A certain volume of chitosan solution (0.1g/50 mL acetic acid 2.5%) was mixed with oleoresin extract. The mixture was stirred using magnetic stirrer (IKA C-Mag HS 7) at 1500 rpm for 10 minutes to form a homogeneous emulsion. For stabilizing the emulsion, 1 mL of 2 % Tween 80 solution was added drop by drop and emulsion was stirred at 1000 rpm for 5 minutes. The alginate aqueous solution of 0.8% (g/mL) was added into the chitosan mixture emulsion and stirred at 1500 rpm for 20 minutes to form a homogeneous emulsion. The emulsion was lyophilized using EYELA FDU-2100 freeze dryer, at -50°C and at vacuum pressure about 20 atm for 48h-72 h, depending on the volume of solution. The formed matrices were grinded into powder (AISHUKA KS-168) and sieved with a 45 mesh metal filter to get particles with size <100 μm.

2.3. Matrix morphology test
Powder X-Ray Diffraction (XRD) of chitosan, alginate, and all samples of freeze dried matrices were obtained using SHIMADZU XRD 7000 MAXIMA-X with Cu tube. All samples were analyzed between 2θ angles of 10° and 80° using the voltage of 40 kV, current of 30 mA, and time per step of 1 second. The crystalline or amorphous character of chitosan, alginate, and obtained matrices were determined through the diffractogram. The Fourier Transform Infra-Red (FTIR) analysis was performed (THERMO SCIENTIFIC NICOLET iS5Spectrophotometer with optic iD5 ATS) using wavelength ranging from 4000 to 400 cm⁻¹. The FTIR analyzed the morphology of matrix, that was based on the absorbance peaks of functional groups vibrations present in oleoresin-chitosan-alginate matrices.

2.4. Total yield and loading capacity of matrices
The yield and loading capacity of the matrices were quantified based on the mass of dried matrices and the oleoresin extract used using the following formulas:

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\text{Yield (\%) = \frac{\text{Mass of dried matrix (mg)}}{\text{Total mass of materials used for matrix (mg)}} \times 100\%}
\] (1)
2.5. Total Phenolic Content determination

The total phenolic content (TPC) was determined using spectrochemistry analysis and Folin-ciocalteu’s (FC) reagent, the procedure was described in previous publication[11]. The absorbance of sample with FC reagent was measured at 765 nm using a UV/VIS-spectrophotometer of Spectroquant® Pharo 300. Phenolic content in samples was expressed as gallic acid equivalent (mg GAE/g sample) and analyzed triplicate.

2.6. In-vitro drug release test in simulated gastrointestinal fluids

The release profile of phenolic compound from the oleoresin-loaded chitosan-alginate matrices was obtained from the sequential series release testing using dialysis membrane tube (Pur-A-LyzerMega dialysis kit Mega 12000, PURG12020, Sigma-Aldrich) in simulated gastrointestinal fluids. Three release media were used: simulated gastric fluid (SGF; pH 1.2), simulated intestinal fluid (SIF; pH 7.4) and simulated colonic fluid (SCF; pH 6.8). The procedure for the preparation of simulated gastrointestinal fluids is as described in a previous publication[5]. 20 mg of matrix particles were immersed in 60 mL of simulated fluids and incubated at 37°C. A sample volume of 5 mL of each fluid media was taken periodically in 2, 4, 6, 8, and 24 hours, to analyze the total phenolic content.

3. Result and discussion

3.1. Formulation of matrices, yield, and loading capacity

Six different matrices have been made. All compositions contain a fixed amount of chitosan, but the ratio of alginate to chitosan and the ratio of oleoresin to chitosan vary from 0.1: 1 to 1: 1. Data obtained from the matrix are shown in table 1. The matrix yields are quite high, with values ranging from 81 % to 96%, which is calculated using equation (1). The matrix yield using the freeze drying method is higher than that formed using other techniques: 29% - 43% are reported for the spray drying method [13] and 34% - 76% are reported for the ionotropic gelation method [14]. This shows that the freeze drying method effectively forms chitosan-alginate matrix.

| Matrix  | Chitosan:Alginate:Oleoresin (w) | Matrix Yield (%) | Loading Capacity (%) |
|---------|--------------------------------|------------------|----------------------|
| A1E0    | 1:0:1:0                         | 91.91            | 0                    |
| A1E1    | 1:0:1:0.1                       | 86.59            | 6.54                 |
| A5E1    | 1:0:5:0.1                       | 81.51            | 5.33                 |
| A10E1   | 1:1:0.1                         | 92.65            | 3.50                 |
| A5E3    | 1:0:5:0.3                       | 95.76            | 11.59                |
| A5E5    | 1:0:5:0.5                       | 94.01            | 17.79                |

As predicted, the loading capacity, which is calculated using equation (2), increases with the decreasing of alginate amount at a constant amount of oleoresin extract, as seen in matrices of A1E1, A5E1, and A10E1. The higher alginate content, the more matrix structure is formed, hence the loading capacity decreases. This result is in agreement with the result reported by Soliman et al. [15]

3.2. Characteristics and morphology of matrices

Figure 1 shows the XRD patterns of pure alginate, chitosan and chitosan-alginate matrices. The XRD pattern of chitosan showed two typical diffraction peaks at 20= 15.45° and 24.49°, whereas the for alginate there was only one peak at 20= 20.47°. These typical diffraction peaks of chitosan and alginate are known as semi-crystalline, which some of it are crystalline and some are amorphous [12]. The XRD pattern of the A5E1 and A5E3 matrices in figure 1 had no
diffraction peaks. This indicates that chitosan and alginate in the matrices had high compatibility, hence the XRD pattern of matrices had a weak broad profile without peaks of individual component. The disappearance of crystalline peaks concluded that the freeze dried matrices formed had amorphous structures. This result is in agreement with the study reported by Wang et al. [16]. Cheng et al. explained the decreased of crystallinity in the matrix chitosan-alginate was attributed to the broken of hydrogen bonding between amino groups and hydroxyl groups in chitosan. When the chitosan-alginate matrix formed, the amino groups of chitosan and hydroxyl groups of alginate form hydrogen bonds, that results in an amorphous structure of matrices [17].

The results of samples analysis using Fourier Transform Infra-Red spectroscopy or FTIR is shown in figure 2. Matrices of A5E5, A5D3 and A5E1 have the same alginate and different oleoresin, while A1E1, A5E1, and A10E1 have the same oleoresin and the different amount of alginate. The specific broad peaks of chitosan-alginate matrix are on the wavenumber ranged 3244-3433 cm\(^{-1}\) corresponding to the stretching vibration peaks of hydroxyl groups (\(\text{O-H}\)) and amine (\(\text{N-H}\)). The peaks at 1520 and 1385 cm\(^{-1}\) are belong to stretching of carbonyl (\(\text{C}=\text{O}\)) group and bending of secondary amide (\(\text{N}-\text{H}\)), respectively, as the consequence of interaction between chitosan and alginate. The peak at 1107 cm\(^{-1}\) is due to the stretching vibration of \(\text{-C-O-C}\) groups in chitosan and alginate. The vibration of hydroxyl (\(\text{OH}\)) phenolic groups in oleoresin is shown in peak at wavenumber of 595 cm\(^{-1}\). Similar peaks of absorption of chitosan-alginate matrices and ginger oleoresin are reported by other researchers [8,18,19].
However, when the alginate increased the height of both peaks reduced. This might corresponds with the interaction occurred in the matrices between chitosan and alginate, which encapsulate the ginger oleoresin.

3.3. Total phenolic content and invitro release study
The total phenolic content (TPC) of oleoresin extract was reported previously as 40.6 mg GAE/g dry dried ginger powder, while the antioxidant activity determined as the IC50 had a value of 17 ppm was reported previously[11]. The in vitro release profile of oleoresin out of the chitosan-alginate matrices are shown in figure 3. After two hours in buffer solution with pH 1.2 (SGF) the cumulative release of TPC was low. As reported by Wen et al. alginate in the matrix can form alginic acid at low pH condition, which trigger forming gel or hydrocolloid layer with high viscosity[20]. The hydrogel layer could prevent the degradation of chitosan matrices hence the active compounds, such as oleoresin, slightly release out of the matrices [5].

The release rateis faster in SIF of pH 7.4 and slower from 8 hour to 24 hour in SCF of pH 6.8. When the content of alginate is high, as in matrix A10E1 with weight ratio of chitosan to alginate 1:1, the alginate hydrogel in alkaline condition might swell that cause the polymer (matrix) surface becomes softer. This could be followed by water uptake by matrices that cause massive diffusion and high release of active compounds [9,21]. Zhang et al. also found out that in high pH solution the carboxyl groups of alginate are more ionized and the electrostatic repulsion between ionized groups increases swelling rate [22]. The release profile of matrices A10E1 from 3 hours to 7 hours period was inline with the previous explanations. However, in long period from 8 hours until 24 hours the release rate was low, and reach cumulative release of 90% at 24 hours.

![Figure 3. Oleoresin release profile from chitosan/alginate matrices. Effect of alginate and oleoresin in matrices of A1E1; A5E1; A10E1 in SGF, SIF and SCF.](image-url)

The release profile shows that matrices formulations will affect the release characteristics of bioactive compounds in gastrointestinal fluids (digestive system). The chitosan-alginate matrices with weight ratio of chitosan: alginate 1:0.5, that were formed using freeze-drying method, showed slightly release in gastric condition (pH 1.2) as low as 8.5 %. This formulation of matrices released the phenolic compounds of oleoresin extract up to 61% in colonic condition (pH 6.8). The result indicates that chitosan-alginate matrices prepared by freeze-drying method, has the potential to be used as a carrier to deliver phenolic compounds of oleoresin extract to target area in colon via oral administration.

4. Conclusion
The freeze-drying method successfully had been used to form chitosan-alginate matrices that encapsulated oleoresin extract with a relatively high yield (90%) and loading percentage (18%).
The XRD patterns of matrices showed that lyophilization process reduced the crystallinity of chitosan and alginate by forming the matrices with amorphous structures. The FTIR spectrum showed the specific absorbance peaks of various functional group for each matrix that support the presence of strong interaction between chitosan and alginate in the matrix, as it is shown by the absorbance peaks at 1520 cm\(^{-1}\) and 1385 cm\(^{-1}\). The in vitro release profile of oleoresin from chitosan-alginate matrix showed the dependency of pH conditions. The presence of alginate in the matrix suppresses the release of oleoresin in acidic conditions but increase the release in higher pH conditions. Certain composition of matrix, such as weight ratio of chitosan to alginate of 1:0.5, resulted in low release of oleoresin in gastric condition (around 8%) and high release in neutral or high pH condition (around 61%). Therefore, the formulation of chitosan-alginate matrix can be designed to be used as a carrier for extended release of oleoresin extract to the sites in the colon via oral administration.

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