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Original article

Vitamin C encapsulation by a gelation method using deacetylated glucomannan as a matrix

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1. Introduction

Vitamin C is an essential nutrient for the human body. It supports the immune system and plays a role as an antioxidant compound that neutralizes toxins and free radicals in the blood. Vitamin C is available in fruit and vegetables. According to Sherwood (2015), as much as 20–120 mg/day of vitamin C is actively absorbed in the small intestine. Vitamin C, like other vitamins, is sensitive and can be degraded in extreme environmental conditions such as high temperatures and high pH processes and with oxidants (Katouzian & Jafari, 2016). Meanwhile, many food preparations involve these conditions, which decrease the antioxidant activity of vitamin C (Khalid et al., 2014). To deliver into an absorption site, this vitamin also needs protection and controlled release to optimize the absorption level in the human body (Vranić & Uzunović, 2009). One of the proposed methods to minimize the degradation of vitamin C as well as to deliver it to the right site is encapsulation (Katouzian & Jafari, 2016).

Encapsulation is a process that entraps an active compound into another substance to increase the compound's resistance to denaturation. Encapsulation protects the active compound from degradation by reducing its exposure to its environment (Ray et al., 2016). Moreover, encapsulation can deliver and control the release of the active compound at a specific site (Ankit et al., 2011). Several materials have been successfully applied as an encapsulant matrix, including glucomannan. Glucomannan is a water-soluble polysaccharide composed of D-mannose and D-glucose units linked by β-1–4 glycoside bonds. It has acetyl groups at C-6, which improve glucomannan's solubility in water (Chokboribal et al., 2015). This solubility hinders glucomannan's use as an encapsulant using a gelation method, which requires high gelation compounds. Hydrogen bonds in are considered the main interactions responsible for gel formation (Solo-de-Zaldivar et al., 2014). Deacetylation using alkaline solutions such as NaOH, KOH, and $Ca(OH)_2$ has been performed to improve gel formation (Pan et al., 2011). Du et al. (2012) successfully conducted the deacetylation process using a safer chemical agent, i.e., Na_2CO_3 solution, with different concentrations and obtained deacetylated glucomannan (DGM) with various degrees of deacetylation (DD). Ulya et al. (2019) reported that deacetylation increased the encapsulation efficiency of iron. The study of DGM as an encapsulant of vitamin C and its encapsulation performance has not yet been investigated. Therefore, this study was conducted to examine the effect of DD on encapsulating and releasing vitamin C.

2. Materials and methods

2.1. Materials

Glucomannan of *Amorphophallus oncophillus* flour was obtained from a local seller in Madiun (Indonesia), which was purified following Wardhani et al. (2016) and resulted in 91% purity. Ethanol (96%) was purchased from PT Brataco Chemical (Indonesia), while Na_2CO_3, ascorbic acid to represent vitamin C, KOH, HCl, phosphate buffer, $CaCl_2$, and other chemicals were bought from Merck (Germany).

2.2. Glucomannan deacetylation

DGM was obtained heterogeneously following the previous study (Wardhani et al., 2018). Ten milliliters of ethanol (75%) were used to dissolve Na_2CO_3 to give 0.4 M under continuous 100 rpm magnetic stirring. After complete dissolution, 10 g of glucomannan was dispersed gently. The deacetylation was conducted to obtain various DD by varying deacetylation periods (2, 4, 8, 16, and 28 h) at room temperature and 60 °C. The DGM was collected using

Abbreviations: ANOVA, analysis of variance; DD, degree of deacetylation; DGM, deacetylated glucomannan; FTIR, Fourier-transform infrared spectroscopy; IR, infrared; LC, loading capacity; M, molarity; N, normality; SEM, scanning electron microscope; UV–Vis, ultraviolet visible.

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Whatman no. 42 filter paper and washed using 50 mL of 50, 70, and 96% ethanol. The resulting DGM was put in a desiccator at room temperature for drying.

2.3. Degree of deacetylation

DD was determined following Zhang et al. (2015) with slight modification. Five grams of DGM were placed in a 250 mL Erlenmeyer flask together with 50 mL of ethanol (75%) under continuous stirring at 50 °C for 30 min. Five milliliters of 0.5 M KOH was added for 48 h reaction. The mixture was titrated with 0.1 M HCl using phenolphthalein indicator. DD was determined as in Equation [1].

$$DD = \frac{\omega_o - \omega}{\omega_o} \times 100\% \quad (1)$$

$$\omega_o = \frac{(V_2 - V_o) \times N_{HCl} \times M_{acetyl}}{m} \times 100\% \quad (2)$$

$$\omega = \frac{(V_2 - V_1) \times N_{HCl} \times M_{acetyl}}{m} \times 100\% \quad (3)$$

where ω_o = acetyl content of the native glucomannan, ω = acetyl content of DGM, V_o = volume HCl for native glucomannan, V_1 = volume HCl for DCM, V_2 = volume HCl for the blank, N_{HCl} is the normality of the HCl, M_{acetyl} = 43 g/mol, and m (g) is the sample mass to be titrated.

2.4. Encapsulation of vitamin C

Encapsulation of vitamin C was conducted based on the method of Wang and He (2002). Vitamin C (200 mg) and 5% (w/v) DGM were placed in a 50 mL volumetric flask and topped up with distilled water. This solution was dropped into 200 mL $CaCl_2$ solution (0.2 M, pH 5) using a 1 mL needled syringe from 10 cm above the liquid surface. After an hour, the formed beads were collected and vacuum-dried to obtain encapsulated vitamin C.

2.5. Functional groups, morphology, and particle distribution

Identification of infrared (IR) spectra of the samples was performed using a Perkin Elmer Spotlight 200 (PerkinElmer Inc., US). Absorbance data of each sample were taken in the wavenumber range of 4000–400 cm^{-1}. Samples of native glucomannan, DGM, and encapsulated vitamin C were photographed using a Phenom ProX Desktop Scanning Electron Microscope (SEM) (Thermo Fisher Scientific™, US) to reveal the morphological changes caused by the deacetylation and encapsulation processes. The size of 30 free-touching particles of each sample from the same SEM images magnification was determined using ImageJ image analysis software (Version 1.50i) (Barreto et al., 2019). The particle size distribution was presented as histograms which created using Sigmaplot 14.0 software.

2.6. Loading capacity

Loading capacity (LC) was determined based on the method of Corrêa-Filho et al. (2019). LC of encapsulation was expressed as the mass of entrapped vitamin C per mass of particles [Equation (4)].

$$LC(\%) = \frac{m_{vitaminC}(mg)}{m_{particle}(mg)} \times 100\% \quad (4)$$

Meanwhile, the mass of entrapped vitamin C was determined following the method of Desai et al. (2006) using a UV–Vis spectrophotometer. Encapsulated vitamin C (25 mg) was dissolved in 100 mL 0.1 N HCl. After 30 min, this solution was centrifuged at 2,300 g for 20 min (Hettich EBA 200, Germany) prior to absorbance reading at 244 nm (Shimadzu UV mini-1240, Japan). The mass of vitamin C was determined from the standard curve.

2.7. Vitamin C release and the models

The release of vitamin C was conducted in two solutions, i.e., HCl solution (pH 1.2) and phosphate buffer solution (pH 6.8). Encapsulated vitamin C (6 mg) was dissolved in 20 mL of each solution under stirring at 37 °C. This solution was centrifuged for vitamin C determination as explained in Section 2.6.

Two models were proposed to describe the release of vitamin C from DGM beads, i.e., Higuchi and Korsmeyer–Peppas. The Higuchi and Korsmeyer–Peppas equations are expressed as Equations [5] and [6], respectively:

$$\frac{C_t}{C_{total}} = K_H t^{0.5} \quad (5)$$

$$\frac{C_t}{C_{total}} = a t^n \quad (6)$$

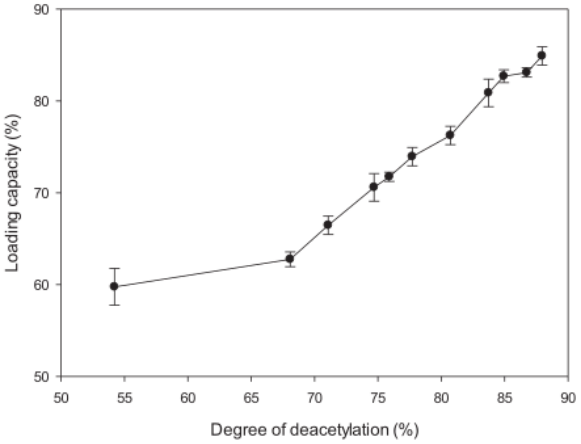

Fig. 1. Loading capacity of vitamin C at various degrees of deacetylation.

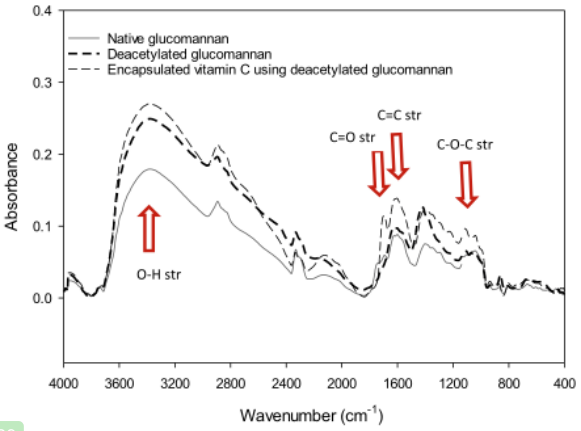

Fig. 2. IR spectra of native glucomannan, deacetylated glucomannan at the highest degree of deacetylation, and encapsulated vitamin C by the highest deacetylated glucomannan.

where $\frac{C_t}{C_{total}}$ is the release fraction of vitamin C at time t in the solution. Here, K_H, a, and n are the constants of each equation. The experimental data were fitted to the models using linear regression.

2.8. Statistical analysis

Data were obtained as the mean of triplicate analyses. Analysis of variance (ANOVA) and mean comparisons were carried out using MS Excel 2019. The significance of comparisons between samples was determined by one-way ANOVA with a significance level of $p < 0.05$.

3. Results and discussion

This work was conducted in two stages, i.e., deacetylation and encapsulation. The deacetylation was performed using Na_2CO_3 heterogeneously in ethanol. This condition allowed higher glucomannan concentrations in the reaction because native glucomannan shows over 50% water absorbance capacity (Wu et al., 2013), which hinders the reaction. The performance of this DGM as an excipient of vitamin C was examined using LC, functional groups, morphology, release kinetics, and the two models.

3.1. Loading capacity of vitamin C

The LC is the ratio between the mass of vitamin C in the encapsulation product and the mass of the product (Equation [4]). The performance of DGM in encapsulating vitamin C was supported by DD. Extending DD led to a positive impact on vitamin C entrapment (Fig. 1).

Deacetylation reduced the solubility of glucomannan due to the acetyl group replacements (Wardhani et al., 2017). More replacement produced higher DD of DGM. This condition promoted the gelation form of glucomannan by creating more hydrogen bonds, hence allowing it to entrap more active compound (Wardhani et al., 2019).

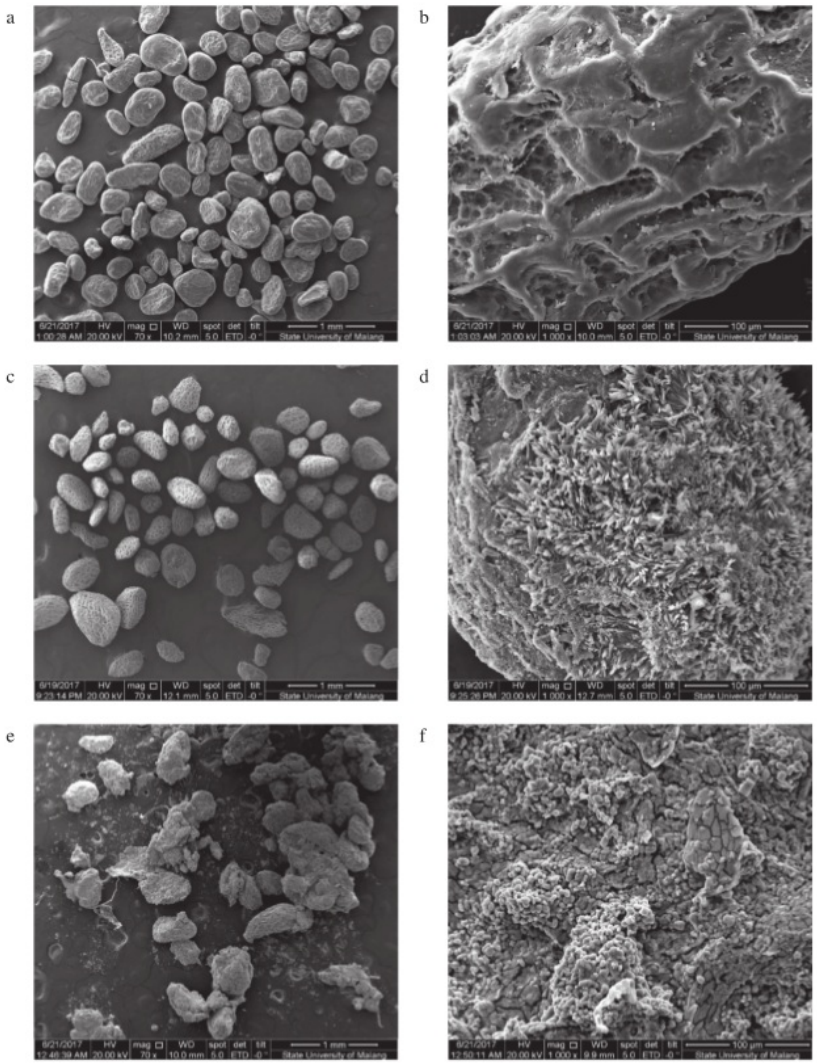

Fig. 3. Morphology of native glucomannan (a, b), deacetylated glucomannan at the highest degree of deacetylation (c, d), and encapsulated vitamin C by the highest deacetylated glucomannan (e, f) at 70 × magnification (left) and 1,000 × magnification (right).

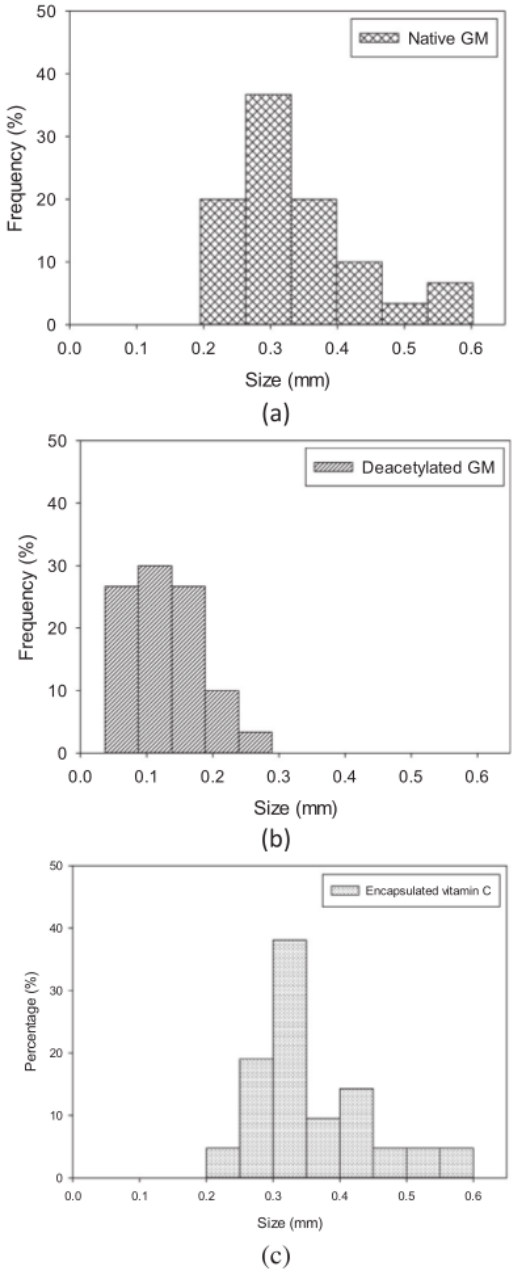

Fig. 4. Particle distribution of native glucomannan (a), deacetylated glucomannan at the highest degree of deacetylation (b) , and encapsulated vitamin C by the highest deacetylated glucomannan (c).

In this work, vitamin C was mixed with DGM during preparation before encapsulation. It is commonly reported that acid hydrolyzes glucomannan into smaller molecules. However, hydrolysis could occur using strong acid in a long period of reaction (Tanaka et al., 2013). HCl and H_2SO_4 have been reported to successfully hydrolyze glucomannan (Cheng et al., 2010; Huang et al., 2010; Wang et al., 2015). Moreover, Barta et al. (2019) reported that polysaccharides, such as alginate, gum arabic, maltodextrin, and modified starches, are shown as unreacted materials in ascorbic acid encapsulation. Hence, it is suggested that in this study the vitamin C entrapment using DGM could occur physically.

The maximum loading (84.9%) was achieved by glucomannan with 87.95% DD. Better gelling ability of glucomannan was also obtained by Wen et al. (2009) with a higher DD. Less-soluble and better-gelled material creates more stable capsules and prevents loss of vitamin C. Similar correlation of the DD and LC was described by Gupta and Jabrail (2007), who found that increasing drug loading up to 49% was achieved after deacetylation of chitosan by as much as 75%.

3.2. Functional groups, morphology, and particle distribution

Functional groups, morphology, and particle distribution were determined for native glucomannan, modified glucomannan with the highest DD value, and encapsulated vitamin C using DGM with the highest LC. IR spectroscopy was used to investigate the interactions between different species and changes in chemical compositions of glucomannan after deacetylation and encapsulation. IR spectra of the samples are shown in Fig. 2. Briefly, all the samples had similar peaks of functional groups in the range of 4000–400 cm^{-1} but with different absorbance intensities. All the samples had a broad absorption due to the stretching vibration of the O–H group at ~ 3400 cm^{-1}. This band broadened after deacetylation

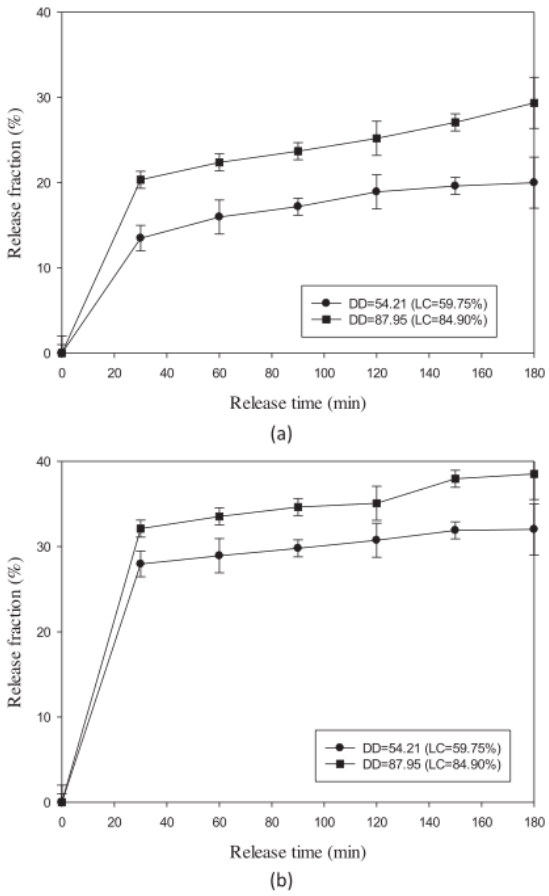

Fig. 5. Release of vitamin C from deacetylated glucomannan matrix of the lowest LC (a,c) and the highest LC (b,d) in two pH solutions, i.e., pH 1.2 (top) and pH 6.8 (bottom).

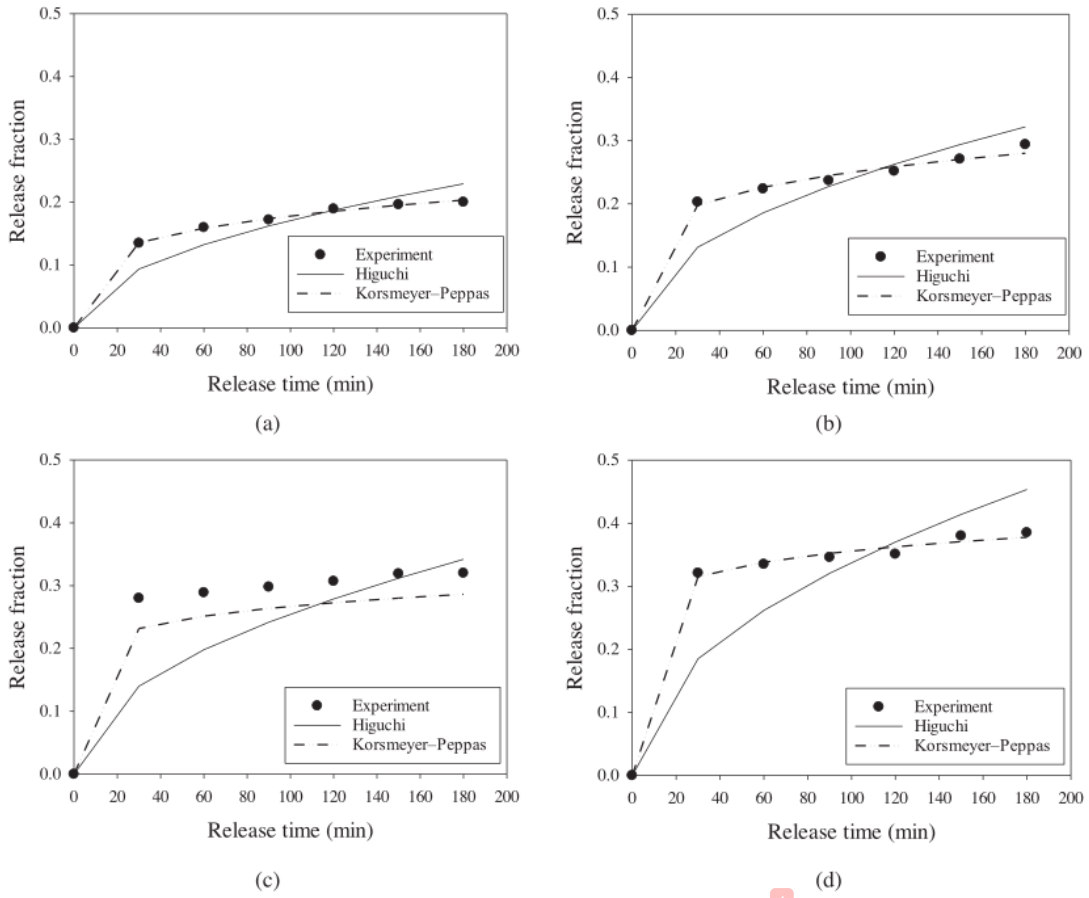

Fig. 6. Plotting models of vitamin C release from the lowest (left) and the highest (right) loading capacity matrix in pH 1.2 solution (a, b) and pH 6.8 solution (c, d).

Table 1
The constants and R^2 values of the release models.

Samples	Solution pH	Higuchi		Korsmeyer–Peppas		
		K_H	R^2	a	n	R^2
DD = 54.21 (LC = 59.75%)	pH 1.2	0.017	0.879	0.063	0.226	**0.992**
	pH 6.8	0.026	0.756	0.155	0.118	**0.772**
DD = 87.95 (LC = 84.90%)	pH 1.2	0.024	0.858	0.102	0.194	**0.944**
	pH 6.8	0.033	0.720	0.224	0.101	**0.892**

suggesting an increase of intermolecular hydrogen bonding between glucomannan molecules (Solo-de-Zaldivar et al., 2014). The addition of vitamin C increased the absorbance of the O–H peak further. The band at ~ 1730 cm^{-1}, which was attributed to stretching of C = O of the carbonyl of acetyl groups, was found in native glucomannan (Solo-de-Zaldivar et al., 2014). This band tended to decrease after deacetylation due to acetyl replacement. However, the addition of vitamin C boosted the absorbance of this carbonyl peak of the five-membered lactone ring. The C = C band at ~ 1660 cm^{-1} and stretch of the C–O–C group at ~ 1100 cm^{-1} increased in vitamin C encapsulation (Sreeja et al., 2015). Alteration of O–H and carbonyl groups suggested the replacements caused by the deacetylation process and the integration of vitamin C.

The morphology of glucomannan granules was observed using a scanning electron microscope at two different magnifications. Fig. 3a, 3c, and 3e displays 70 × magnification of samples in which their sizes were relatively similar. These figures suggested that the deacetylation process in a heterogeneous system did not change the particle size. A similar relation between deacetylation and particle size was previously found by Liu et al. (2010). Larger magnification (1,000 ×) of the particles revealed the surface appearance of the samples. A rougher surface was observed in DGM. Encapsulated vitamin C in Fig. 3e tended to be crumbed, possibly due to the drying process of the glucomannan–vitamin C beads. This drying process produced irregular particles of encapsulated vitamin C. Deacetylation could cause surface transformation, as described by Kurt and Kahyaoglu (2017). The deacetylating agent disrupted the particles' surface, resulting in eroded surfaces. The addition of vitamin C produced brittle particles. Therefore, this fragility also impacted on the uneven particle surface as shown in Fig. 3f.

Particle distribution of each samples is presented in Fig. 4. Particle size of native glucomannan was in the range of 0.2–0.6 mm and dominated by the diameter of ~ 0.3 mm. Deacetylation reduced the particle into<0.3 mm. (Fig. 4b). This reduction could be due to erosion of the particle as shown on the Fig. 3d. Entrapment of vitamin C using gelation method increased the size of particle which dominated by the diameter of ~ 0.3 mm (Fig. 4c).

3.3. Release of vitamin C

Release of vitamin C from the lowest and highest LC encapsulants was determined in pH 1.2 and pH 6.8 solutions which simulated the stomach and small intestine fluid without enzyme, respectively (Ghaffarian et al., 2016). The release rate of vitamin C was lower in pH 1.2 solution than in pH 6.8 for both samples (Fig. 5). Both solutions showed two stages of release rate. In the first 30 min, vitamin C was released fast followed by a slower rate. This pattern could be due to the high driving force of dissolution in the initial release condition. Meanwhile, low burst release represented a slow decay of the matrix encapsulant, reducing the rate of release. The burst release was difficult to prevent except other active substances were added (Yeo and Park, 2004).

Encapsulated vitamin C in lower DD showed a lower rate than that of higher DD. Wardhani et al. (2018) reported that higher DD produced a higher solubility of deacetylated glucomannan. Moreover, when more vitamin C was entrapped, this vitamin disrupted the intermolecular binding of DGM. These two conditions facilitated more loss of vitamin C from the matrix, hence increased vitamin C concentration in the solution.

The release profiles of vitamin C were determined using the linearized forms of the two models: Higuchi and Korsmeyer–Peppas. The Higuchi model describes the release rate of solute from the matrix where loading of solute exceeds its solubility in the matrix into the surrounding fluid (Paul, 2011). In contrast, the Korsmeyer–Peppas model describes solute release from the matrix when more than one mechanism is involved (Bruschi, 2015). The model with the highest R^2 value is the most suitable model to represent the release kinetics of vitamin C from DGM.

A plot of the release models of vitamin C from the DGM matrix is shown in Fig. 6, while the constants of the model are presented in Table 1. The Korsmeyer–Peppas model showed the highest R^2 value for all samples ($R^2 > 0.772$), which indicates that this model is fit to describe the release profile of vitamin C from the matrix bead. The n value of this model ($n < 0.45$) suggested that the Fickian diffusion mechanism occurred on vitamin C release (Dash et al., 2010) in which the drug release was governed by diffusion. In this case, the release due to diffusion was more dominant than that of the process of polymeric chain relaxation (Bruschi, 2015).

4. Conclusions

Deacetylation increased loading capacity to entrap vitamin C. This process caused modification in the functional groups, morphology, and particle distribution of glucomannan as displayed by IR spectra and SEM images. The highest DD of glucomannan (87.9%) has 84.9% LC in encapsulating vitamin C. However, this highest DD allowed to release faster vitamin C than the lower one. Release rate of vitamin C from DGM was higher in a pH 6.8 buffer solution than that of pH 1.2 solution. The Korsmeyer–Peppas model was fitted to the release of vitamin C from deacetylated glucomannan at pH 1.2 ($R^2 > 0.892$) and at pH 6.8 ($R^2 > 0.772$). This study suggested the strong potential of deacetylated glucomannan as an encapsulating agent for vitamin C using a gelation method.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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