Performance of Deacetyled Glucomannan as Iron Encapsulation Excipient

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Abstract. Encapsulation protects iron from degradation or oxidation possibilities due to its encapsulation material. Glucomannan (GM) is a neutral polysaccharide consist of D-mannose and D-glucose connected with β-1,4 linkage. Deactylation transforms solubility of glucomannan as well as its gel structure. These properties support for excipient application. The aim of this work was to determine performance of deacetylated glucomannan as iron matrix. Deacetylation was conducted heterogeneously. Deacetylation did not change the backbone of GM. Higher alkali concentration has better ability to encapsulate iron. Extended deacetylation time and alkali concentration affect insignificantly on the performance of encapsulation to protect iron from oxidation. The release of iron from the matrix influences by deacetylation degree.

1 introduction

Iron is an element which is very abundant its availability in nature and one of essentials components of every living creature. In the human body, Iron (Fe) is essential for producing red blood cells, i.e. haemoglobin which contributes in oxygen bonding and then it is transported throughout the body. Iron can be obtained from two types of iron, i.e. heme and non-heme. Under certain conditions, Iron is vulnerable to damage due to inhibitors. Besides fitic acid and tannin, some research revealed the inhibitors of Iron is calcium and peptide from digested protein [1].

The requirement of Fe per day varies for each individual. However, the normal Iron diet can not satisfy the Iron requirement which is caused by low its bioavailability (the ability of the body to absorb Iron), i.e. 14% - 18% [2]. Hence, it is needed an additional Iron intake in the form of supplements in order to satisfy the body requirements of Fe. Iron intake which is required by the body can be obtained by consuming the Iron supplements.

In the recent days, there are many variants of supplements form which are used to supply the Iron, for examples capsules, syrup, and even intravenous Iron therapy. One of the forms that is currently developed is encapsulated Iron. Encapsulation aims for giving the maximum protection from the factors of Iron damage, and also minimalise the less common smells and tastes of Iron [3][4].

The study of Iron encapsulation has been conducted by Bezbaruah et al. with modified alginate as an encapsulation material [5]. They used $CaCl_2$ which contributes as strengthen agent and alginate particles stabiliser to make alginate insoluble in water. Iron encapsulation which employs a gelling agent such as modified alginate reduce organoleptic problems and increase the Iron bioavailability [2]. Bioavailability is the Iron ability to be absorbed by the body. Iron encapsulation can increase the bioavailability due to the coating material protects the Iron from degradation by inhibitors and oxidation.

Glucomannan has high viscosity and the molecular weights ranging from 200 to 2000 kDa. This mannan has ability to form a very good coating, stable in cold and hot water, and the ability to absorb water with high absorption relativity index, i.e. 100 gram water per gram glucomannan [6][7], however, it becomes new problems. The ability of glucomannan to absorb water needs to be controlled or reduced through chemical modification, for example through deacetylation. Glucomannan modification using deacetylation is expected as a proper method to be chosen because it reduces the solubility of glucomannan in water, and also minimalise the Fe^{2+} oxidation into Fe^{3+} as the result of Iron contact with O_2 in the air [8]. This modification method is expected to prevent the degradation by inhibitors. The deacetylation

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alters glucomannan form into stronger, elastic, and stable in extreme pH and high temperature [9]. Deacetylated glucomannan could be more difficult to dissolve in water and form gel structures [10]. By forming a gel structure which is a stable solid phase, encapsulation using KGM is more likely to be performed because it can wrap the active agent, insoluble in water, and has a higher boiling point [11]. The increase of deacetylation degree (DD) will cause the glucomannan insoluble in water [12]. These properties affect on Fe^{2+} oxidation into Fe^{3+} as the result of interaction with water which contains dissolved oxygen [13].

The ability of KOH as deacetylation agent is weaker than NaOH because the ionisation degree of KOH is lower than NaOH in the alcohol medium [14. Deacetylated glucomannan using KOH has higher viscosity compared to that of NaOH [15]. The utilisation of glucomannan of A. oncophyllus itself as an excipient of iron has not much explored. Hence, this work aimed to study the effect of deacetylation conditions of glucomannan of A. oncophyllus on iron encapsulation performance. Effects of concentration of alkali (0.1 and 0.25 M) and deacetylation reaction time (2, 8, 16 and 24 h) on the efficiency of encapsulation of Iron, oxidation protection performance and its release were studied.

2 Materials and Methods

2.1. Materials

A. oncophyllus flour was bought from a local farmer at Nganjuk, East Java-Indonesia. Glucomannan was extracted from A. oncophyllus flour following Rahayu method's using 2-propyl alcohol solution (IPA, 70%) [16]. The glucomannan content of the purified flour was 76.3%. KOH and ethanol (95%) were used for deacetylation reaction and washing the deacetylated glucomannan, respectively.

2.2. Deacetylation of Glucomannan

The deacetylation was conducted based on the method of Chen et al. [17]. Ethanol (50%, 100 ml) and glucomannan (5 g) were placed in beaker glass and stirred 250 rpm for 30 min at 50°C. A particular concentration of KOH was added to the mixture while maintained the stirring (225 rpm) for a time corresponding to the variable. The solid which is separated using filter paper was oven-dried at 40°C to obtain deacetylated glucomannan (DGM).

2.3. Functional Groups and Morphology

The morphology and functional groups of the sample after deacetylation was analysed using FEI Inspect S50 device and IR Prestige Shimadzu. The peaks of sample were assigned by comparison with the literature data.

2.4. Formation of Fe bead

The dried deacetylated glucomannan (2 g) for each variable was placed into a beaker glass containing 100 ml of distilled water. $FeSO_4$ (1 g) was added to the mixture. The mixture was dropped into the ethanol (50%) using a syringe. The bead was collected using filter paper, and oven-dried at 40°C to obtain encapsulated Fe.

2.5. Determination of iron

The sample (0.1 g) is placed in a beaker glass together with HCl 6M (10 ml), then heated for 1 h. The solution is filtered and the filtrate is fed into a 10 ml flask. Added distilled water to the boundary marks and homogenized. To each flask (including the distilled water "blank" and the unknowns), pipet in order 1 ml of the hydroxylamine solution,10 ml of the 1,10-phenanthroline solution, and 8 ml of the sodium acetate solution. Swirled each flask to mix the contents, then dilute each solution to the 100 ml mark and mix thoroughly. The solution was shaken and silenced for 120 min, then measured its absorbance using a UV-Vis spectrophotometer at 520 nm.

2.6. Efficiency of Encapsulation

The determination of the efficiency of encapsulation was calculated as follow:

$$Efficiency\ of\ encapsulation = \frac{Bound\ iron}{Total\ iron} x\ 100\% \quad (1)$$

2.7. Encapsulation Protection

The effect of encapsulation again the oxidation effect was determined at ambient temperature. After 5 days incubation, the samples was analyzed for the iron content. The iron calculations was compared before and after exposure for oxidation determination.

2.8. Release of Iron

The in vitro release of iron from its excipient was conducted at free-enzymatic liquid prepared using 0.1 M HCl to simulate the stomach liquid at pH 1.2.[18] This simulated liquid was incubated at 37°C, 150 rpm. After a certain time, some liquid are took and measured for Fe determination. [19]

3 Results and Discussion

3.1. Morphology and Functional Groups of the Deacetylated Glucomannan

Figure 1 shows the FT-IR spectra comparison of the native glucomannan and the deacetylated sample. In general, the peaks of both samples are very similar but difference in the amount of absorbance. This indicates that glucomannan backbone was not changed during deacetylation. The samples had the broad absorption

band at around 3400 cm^{-1} and was attributed to the stretching vibration of O-H groups [20]. This absorption band shifted to a lower wave number with the present of alkali, indicating a gradual increase of intermolecular hydrogen bonding between glucomannan chains. It can be clearly seen that deacetylation decrease the peak at approximate 1730 cm^{-1} which have been assigned to the stretching of C=O of the carbonyl of the acetyl groups, as found in the previous work [21][22].

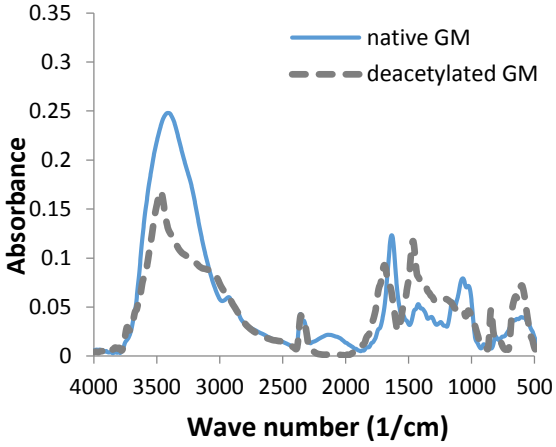

Fig. 1. Comparison of FTIR spectra of native and deacetylated glucomannan.

As a consequence of deacetylation, there was also a significant increase in the intensity of the band in the region of ~1450 and ~880 cm^{-1}, as well as the sift of ~1800 cm^{-1}. This observation suggested that the alkali has been integrated into the sample. A similar phenomenon was reported by Huang et al. in deacetylation using Na_2CO_3 [23].

The morphology of the native and deacetylated is presented in Figure 2. The difference between both samples was insignificantly observed in a 1000 magnification (figure not shown). However, it was detected in 10000 magnificent that the surface of the native sample is rough and covered with the dusk-like which separated each other. While in the same magnificent, the deacetylated sample has a smoother surface in which the dusk-like is connected with some of the melted part.

3.2. Efficiency of iron encapsulation

The efficiency of encapsulation describes ability of the excipient to trap the iron. Effects of KOH concentration and reaction time of deacetylation on the efficiency of encapsulation is presented at Figure 3. Higher concentration of alkali resulted in higher ability to encapsulate the iron. Both concentrations of KOH showed a similar trend in which the highest efficiency obtained after 8 h of deacetylation. However, Figure 3 shows all the efficiencies of encapsulation are less than 50%. This suggests the amount of glucomannan used in this study could be too low to bind the iron added.

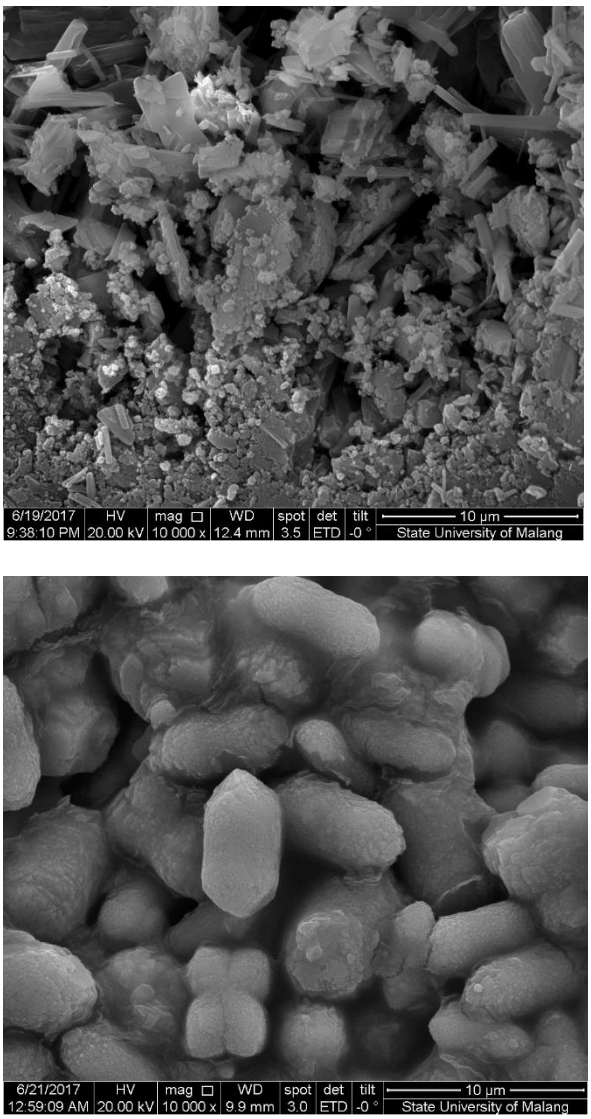

Fig. 2. Comparison of morphology of native glucomannan (top) and deacetylated glucomannan (bottom) in 1000 magnification.

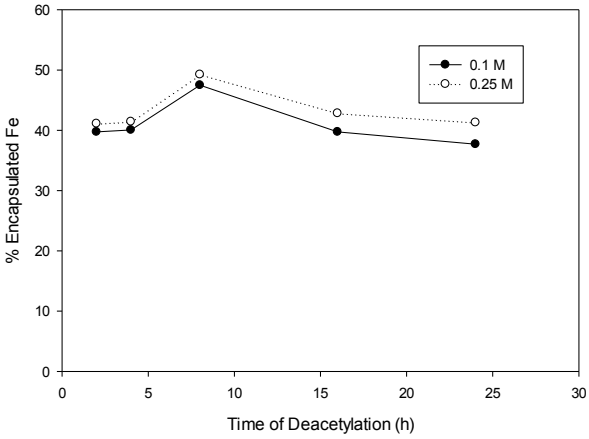

Fig. 3. Effect of time of deacetylation and alkali concentration on Fe encapsulation efficiency.

Deacetylation using alkali is necessary for the gelation of glucomannan. The physical change in gelation due to deacetylation is quite remarkable. The initial glucomannan solution which has not been treated with alkali does not form a gel [24]. One of the changes could be the ability to trap iron in the gels. Wardhani et al. reported deacetylation of glucomannan using higher alkali concentration results in the more deacetylation substitution which led to the higher value of the degree of deacetylation [25]. This influences stronger interchain attractions among the glucomannan molecules as well as the more number of junctions, which results in a denser and more flexible physical network with higher [20]. The junctions could provide sites to trap the iron. Wandrey et al. suggested deacetylated glucomannan has ability to encapsulate active agent by forming a gel which is a stable and soluble matter [11]. Hence, it is expected that higher degree of deacetylation results in better performance in iron encapsulated. However, Figure 3 demonstrates overextended the deacetylation time reduces the performance of the encapsulation. This suggests the forming junctions are not the only condition that affected the iron binding. After reaching certain deacetylation degree, additional acetyl substitution could become a steric hindrance for iron to be trapped in the junctions.

3.3. Ability of Deacetylated Glucomannan to Protect Iron Oxidation

Fe^{2+} of $FeSO_4$ is easily being oxidised into Fe^{3+} during storage, due to contact $FeSO_4$ with oxygen. However, Fe^{3+} has lower bioavailability in the human body [26]. One of the aims of iron encapsulation is to protect direct contact between the iron source with oxygen.

Figure 4 shows the samples of encapsulated Fe2+ using deacetylated glucomannan after incubated for 5 days. The result showed higher alkali concentrations have better ability to protect the oxidation. However, extended deacetylation time did not demonstrate a significant difference in protecting the oxidation. This research has demonstrated that encapsulated iron using deacetylated glucomannan was able to prevent or minimise the oxidation of the iron. All of the encapsulation samples were able to protect >95% of iron oxidation after 5 days. This means deacetylated glucomannan as excipient of iron works very well in protecting iron interaction with oxygen.

3.4. Release of Iron in pH 1.2

It is expected that the encapsulation is able to deliver the iron into the absorption site which is in the small intestine. Hence the excipient is designed to protect the release of iron in the stomach condition by deacetylation in various alkali concentration and time of deacetylation.

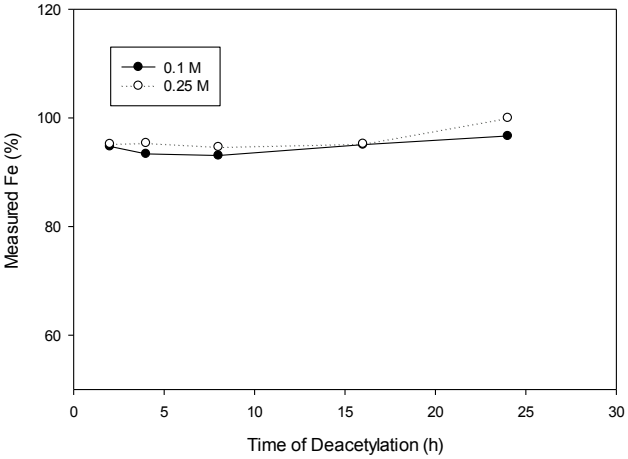

Fig. 4. Effect of time of deacetylation on Fe concentration in encapsulation after 5 days storage.

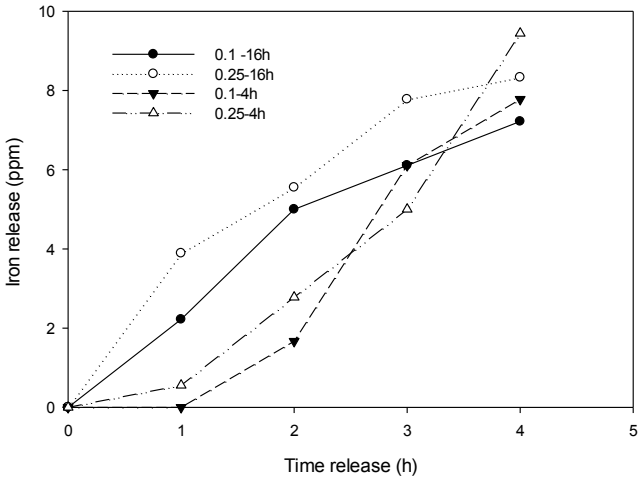

Fig. 5. Profile of iron release at pH 1.2.

Figure 5 shows the release of iron encapsulated in pH 1.2 of HCl solution using a different degree of deacetylated glucomannan as the excipient. Extended deacetylation time resulted in the more iron released. Interestingly, the higher release of iron was observed using excipient with higher degree of deacetylation. This profile supports the result in efficiency encapsulation in the previous discussion.

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