Properties of Spray-Dried Iron Microcapsule Using Hydrolysed Glucomannan as Encapsulant: Effect of Feed Viscosity
(Sifat Mikrokapsul Besi Sembur-Kering Menggunakan Glukomanan Terhidrolisis sebagai Enkapsulan: Kesan Kelikatan Suapan)

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ABSTRACT
As one of the polysaccharides with high viscosity, even in low concentration, glucomannan could block the nozzle and hinder its application as spray-dried encapsulant. The present research aimed to investigate the effect of viscosity of hydrolysed glucomannan as a spray-dryer feed on properties of encapsulated iron particles. Glucomannan was hydrolysed using cellulase to obtain various viscosities (83-222 cP) and used for encapsulating iron. Enzymatic hydrolysis reduced the glucomannan’s glass transition temperature and transmittance values of O-H, C-O, and C-H groups. Increasing the viscosity lightened the particle colour, and improved encapsulation efficiency and mean particle diameter, however, reduced moisture content and bulk density. The highest encapsulation efficiency (99.95%) was obtained using the most viscous encapsulant (222 cP). Thicker encapsulants produced larger particles with more wrinkles on the surface but performed better in protecting iron. Solubility and swelling of the particles were higher in neutral solution (pH=6.8) than in an acidic one. The degree of iron degradation was around 70% after 10 months of storage. These results suggested the use of an appropriate viscosity of hydrolysed glucomannan not only allow it to be sprayed but also showed a potency to protect the iron from solubility in acid ambient and degradation during the storage.

Keywords: Biodegradable; glucomannan; iron; microencapsulation; viscosity

ABSTRAK

Kata kunci: Besi; glucomanan; kelikatan; mikroenkapsulasi; terbiodegradasi
INTRODUCTION

Over two billion people worldwide suffer from iron deficiency, the most common nutritional disorder (Singh, Siddiqui & Diosady 2018). Food fortification has been proposed as one of the most effective ways to solve nutritional deficiencies at low cost. Adding iron directly to meals implies several limitations, especially for unpleasant odors and tastes. Moreover, exposing ferrous iron to the atmosphere was also susceptible to converting it into ferric iron which has lower bioavailability (Dueik & Diosady 2017).

In the food industry, encapsulation has been common to protect active substances from degradation. Some of the advantages of spray drying encapsulation are a cost-effective and adjustable process that produces good-quality particles (Frascarel et al. 2012). Characteristics of the sprayed product can be controlled by the feed conditions, including its viscosity. Glucomannan, a natural polysaccharide extracted from Amorphophallus sp. tubers, has desirable properties as a protective material for several active compounds but lacks its applications due to its high viscosity even in low concentrations (Wardhani et al. 2022). The viscosity of 1% solution of 99% glucomannan purity is up to 12,345 cP which could interrupt the spray drying process by blocking the atomizer (Wardhani et al. 2022). However, using a less viscous matrix solution reduces encapsulation protective ability of the active substances (Adamiec et al. 2012). Simply using low concentration of glucomannan which fulfills the spray dryer is not an option due to inefficiency. Hence, to maintain spray-dryer efficiency but also support to protect the iron, glucomannan needs to be depolymerized to lower its viscosity to less than 300 cP (Sosnik & Seremeta 2015).

Enzymatic hydrolysis has advantages in glucomannan degradation due to its specificity, controllable degree of polymerization of the oligosaccharides, and environmentally friendly process (Akpinar et al. 2010). β-mannanase successfully hydrolyzed β-(1,4)-D-mannopyranosyl bonds of glucomannan at 50 °C and pH 5.5 for 24 h (He, Zhang & Huang 2001). However, this enzyme is not widely available. Cellulase, a mixture of endoglucanase, exoglucanase, and glucosidase enzyme, is the most common enzyme used for glucomannan hydrolysis. Albrecht et al. (2011) and Wardhani et al. (2022) had been reported to apply this cellulase from A. niger in hydrolysing glucomannan.

Attempts to apply hydrolysed glucomannan as spray-dried encapsulant have been reported. Guerreiro et al. (2019) used acid hydrolysed glucomannan as an encapsulant of antitubercular drugs. Wardhani et al. (2020a) reported the temperature effect of spray-drying on iron encapsulation using hydrolysed glucomannan. However, the viscosity of the feed solution also affected the spray drying process as it related to the pressure of atomization stage, as reported by Pistre et al. (2013) which used various viscosities of maltodextrin. The impact of the glucomannan viscosity as spray dryer feed on the iron encapsulation properties including its protection performance has not been reported. Hence, this study aimed to investigate the appropriate viscosity of the feed that not only allows to be sprayed in the dryer but also results in supporting the iron encapsulation product that has protection performance. In this study, various viscosities of hydrolysed glucomannan were produced by controlling the duration of the hydrolysis and fed to the spray dryer. Properties of the encapsulated iron were studied.

MATERIALS AND METHODS

MATERIALS

Glucomannan (97.81%) of Now Foods was used for encapsulating material (Wardhani et al. 2020a). Cellulase of A. niger (C1184, Sigma-Aldrich, St. Louis, MO, USA) has activity ≥0.3 units/mg solid. Iron (II) sulfate heptahydrate (FeSO₄·7H₂O) and other pro-analyst compounds were Merck (Kenilworth, NJ, USA).

IRON ENCAPSULATION

Glucomannan (2%) was hydrolysed using 20 ppm of cellulase at room temperature to obtain various viscosities under constant stirring. The solution was boiled for 10 min to deactivate cellulase at the end of hydrolysis. The viscosity of the hydrolysed glucomannan solution was measured using Cannon Fenske Kapillar Viskometer size 100 (Schott AG, Mainz, Rhineland-Pakatubatem Germany) by comparing its flow rate with distilled water at 25°C. The hydrolysed glucomannan (500 mL) was mixed with 1.5 g of FeSO₄·7H₂O under continuous stirring for 15 min prior to be spray-dried using Mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland). This spray drying process was conducted at 140 °C of inlet drying air temperature.
with 30 °C feed temperature, 667 L/h dry air flowrate, 0.18 L/h liquid feeder pump flow, and constant aspirator (90%).

**MORPHOLOGY OF THE PARTICLE SURFACE, FUNCTIONAL GROUPS, AND PARTICLE SIZE**

Particle surface morphology was scanned using Scanning Electron Microscopy (SEM) (JSM-6510 LV, JEOL, Tokyo, Japan) at 20 k with 3,000 magnifications. The distribution of iron elemental on the surface area of the particle was analysed using Scanning Electron Microscopy- Energy Dispersive X-ray (SEM-EDX) (JSM-6510 LV, JEOL, Tokyo, Japan) at 20 k. SEM-EDX is used to analyse sample compositions qualitatively and quantitatively based on the spectral analysis of characteristic X-ray radiation emitted from sample atoms in irradiation with electron beams. The functional group analysis of the sample was performed using Perkin Elmer Frontier FT-IR (Waltham, MA, USA) version 10.6.1. in the range of 4000-500 cm⁻¹. Meanwhile, the particle size of the encapsulated iron was determined using Laser Scattering Particle Size Analyzer (PSA) HORIBA Partica LA-960 (HORIBA, Ltd., Kyoto, Kyoto, Japan). The static light scattering method was applied.

**BULK DENSITY AND MOISTURE CONTENT**

Bulk density determination was carried out by gently placing 1 g of encapsulated powder into an empty 2 mL-graduated cylinder. If necessary, carefully level up the powder without compacting. The ratio of bulk mass product to bulk volume product was calculated. The determination of moisture content of iron-loaded powder was conducted by oven-dried the samples at 105 °C up to constant weight.

**IRON ENCAPSULATION EFFICIENCY**

Iron encapsulation efficiency (EE) was determined by dissolving 0.2 g of sample into 20 mL of distilled water. Ten mL of this solution, 1,10-phenanthroline (10 mL, 1 g/L), sodium acetate (8 mL, 1.2 M), and hydroxylamine hydrochloride (1 mL, 100 g/L) were mixed before diluting to 100 mL. After 10 min, the absorbance of the solution was read at 508 nm. The EE was calculated following Wardhani et al. (2019) using Equation (1).

\[
EE (%) = \frac{m_{\text{encapsulated iron}}}{m_{\text{initial iron added}}} \times 100\% \quad (1)
\]

**SOLUBILITY AND SWELLING**

Solubility and swelling of the encapsulated iron were determined according to Wardhani et al. (2019). The sample (0.1 g) was diluted in 10 mL of distilled water and heated for 30 min at 60 °C. The mixture was centrifuged using Hettich EBA 20 at 1538 × g for 10 min. The supernatant and paste were then separated and dried. Their weight in wet and dried conditions were recorded for the solubility and swelling calculation as shown in Equations (2) and (3), respectively.

\[
\text{Solubility (\%) = } \frac{\text{weight of dried supernatant}}{\text{weight of supernatant}} \times 100\% \quad (2)
\]

\[
\text{Swelling = } \frac{\text{weight of wet paste}}{\text{weight of dried sample}} \quad (3)
\]

**COLOUR**

Colour of the encapsulated powder was determined using Chroma meter (CR-300, Minolta Co., Ltd, Osaka, Japan) to obtain the values of L*, a*, and b* of the sample. The colour difference (ΔE) was calculated as Equation 4.

\[
\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (4)
\]

where \(L_0, a_0,\) and \(b_0\) are the values of native glucomannan.

**GLASS TRANSITION TEMPERATURE**

To investigate the effect of glucomannan viscosity on the glass transition temperature, the powder sample (5 mg) was scanned by the Differential Scanning Calorimeter (DSC-60, SHIMADZU, Japan). The cleaning gas and the pan were dry nitrogen (10 mL/min) and sealed aluminum, respectively. The scanning temperature range used was 30 to 350 °C with a heating rate of 10 °C/min.

**IRON DEGRADATION**

The iron content was determined after 10 months of storage in a closed container at ambient temperature (~27 °C). Sample powder (0.1 g) was dissolved in 20 mL of distilled water. After completely dissolved, 10 mL of the solution was added by 1,10-phenanthroline (5 mL, 1 g/L), sodium acetate (4 mL, 1.2 M), and hydroxylamine hydrochloride (0.5 mL, 100 g/L) and diluted to 100 mL. The absorbance of the solution was read at 508
nm and plotted to the iron standard curve. The obtained iron content value was compared to the initial value after the spray-drying process to calculate the degree of degradation.

**STATISTICAL ANALYSIS**

The presented data were mean of triplicates. Values were expressed as means ± standard deviation. Differences between variables were tested for significance using one-way analysis of variance using the Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows. A difference was considered statistically significant at p<0.05.

**RESULTS AND DISCUSSION**

As one of the polysaccharides with high viscosity properties, glucomannan requires to be modified before being used as spray drying feed. Glucomannan was pre-treated by enzymatic hydrolysis to decrease its viscosity. Performance of various glucomannan viscosities as spray-dried encapsulant of iron was determined subsequently.

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A drop in transmission intensity after hydrolysis was observed at ~3400 cm\(^{-1}\) of O-H group, ~1026 to ± 1305 cm\(^{-1}\) of C-O group, and ~675 to ~995 cm\(^{-1}\) of C-H group. The drop suggested that additional of these groups were created during the hydrolysis that produced shorter glucomannan oligosaccharide chains which increased the presence of O-H, C-O, and C-H groups. Although there was a change in the transmittance intensity of the functional groups, however, the peaks found on IR spectra of hydrolysed glucomannan were merely similar to the native one in their wave numbers. The similar peaks indicated that the hydrolysis only performed chain-breaking activity which showed by reducing the peaks intensity, rather than creating new groups. The unchanged chemical structure of glucomannan was also found by Komiya et al. (2010) after conducting a hydrolysis process.

**GLASS TRANSITION TEMPERATURE**

Glass transition temperature (Tg) is the temperature at which a solid material transforms into a liquid, soft, rubbery phase. Tg is essential mechanical property to describe food texture, such as crispiness, stickiness, rigidity, thickening, and viscosity. It also affects the mobility of water in the product, which affects food stability during the storage period (Imtiaz-Ul-Islam & Langrish 2009).

A comparison of DSC results between the native glucomannan and the encapsulated iron using hydrolysed glucomannan is presented in Figure 1-bottom. Hydrolysis, which released some simpler molecular chains of glucomannan oligosaccharides, modified the glucomannan’s structure from amorphous into semicrystalline. The hydrolysis reduced Tg from 120 °C of the native to 94 °C of the 222 cP sample. Higher Tg (±100 °C), has often been reported to stabilize dry active molecules due to its amorphous state at room temperature (Lechanteur & Evrard 2020). Goula and Adamopoulos (2010) showed that lower Tg of spray dryer feed resulted from a higher moisture content of the spray products. Tonon et al. (2009) reported higher Tg of encapsulant tends to give more solid content of spray dryer product. In addition, other various factors also contribute to Tg, including the degree of polymerisation, molecular geometry, crystallinity, and molecular weight (Baik et al. 1997).

**MOISTURE CONTENT**

Figure 2(a) shows that increasing the viscosity of spray-dried encapsulant from 83 cP to 222 cP has reduced the moisture content of spray-dried powders up to 9.6%. In this work, hydrolysis using cellulase was applied to obtain different viscosities of glucomannan. During hydrolysis, lower molecular weights of glucomannan were released together with H\(_2\)O (Minowa, Zhen & Ogi 1997). The lower the viscosity of the hydrolysed glucomannan, the more H\(_2\)O found in the encapsulant. A high moisture content led to solid bridge formation between individual particles, which promotes agglomeration (Lechanteur & Evrard 2020). Since the spray dryer was set in the same condition for all the samples, the lower viscosity of glucomannan contained higher moisture in the spray-dried powders.

**BULK DENSITY**

Bulk density affects transportation and packaging costs and powder properties such as flowability (Ding et al. 2020). Figure 2(b) shows a slight decrease of bulk density from 0.67 to 0.58 g/mL as an effect of increasing viscosity. This lower density could be due to an increase in particle size with more particle volume as discussed in the next section. As described in the previous section, the lower moisture content of the higher viscosity sample decreased the powder’s mass, resulting in lower bulk density. Other than the viscosity of the feed, the molecular structure of the encapsulant also determines the density of resulted product, as reported by Yousefi, Emam-Djomeh and Mousavi (2011) in maltodextrin-pomegranate encapsulation.

**THE MEAN OF PARTICLE SIZE**

Particle size is one of the most important parameters of powder-type products. The particle size of the powder has a significant effect on its handling, stability, and storage properties. Encapsulant concentration, type of carrier, feed flow rate, atomization, and temperature of drying air intake are some factors that contribute to particle size (Tontul & Topuz 2017). Figure 2(c) shows the mean particle size range from 12,140 to 18,800 nm. An increase in glucomannan viscosity resulted in a bigger particle size due to a bigger drop size created during spraying. Ferrari, Germer and de Aguirre (2012) suggested that larger droplets are formed during the atomization process when using higher viscosity of feed solution resulting in larger particle size.

**ENCAPSULATION EFFICIENCY**

The higher viscosity of glucomannan performed better in encapsulation efficiency (EE) (Figure 2(d)). Increasing viscosity led to entrap more iron. However, further increases in the viscosity did not significantly
affect the efficiency. This can be due to the possibility that all the iron has been completely encapsulated at a specific viscosity so that the addition of glucomannan viscosity has no necessary effect. In this work, 222 cP of glucomannan solution could encapsulate 99.95% of the available iron. A similar trend has been reported by Kha, Nguyen and Roach (2011) on the drying of Gac fruit, where an increase in maltodextrin (10-30%) increases EE up to 85%. However, a further increase in maltodextrin does not significantly change the EE value. Although maltodextrin also included as a natural polysaccharide, its solution properties are very different from glucomannan. Maltodextrin has a much lower viscosity, i.e. 30.0 cP for 40% solution, hence lowered its ability to entrap and protect abilities the active agents (Churio & Valenzuela 2018).

**PARTICLE MORPHOLOGY**

The effect of glucomannan viscosity on particle morphology can be seen in Figure 3-left. Thicker encapsulants tended to produce a greater number of bigger particle sizes. These particles had more wrinkles on the surface resulting in a less spherical shape. This could be related to the trend of the moisture content of the particles, as shown in Figure 2. Although the particles were bigger, however, they contained less moisture because more water molecules pass through the matrix during the drying process (Churio & Valenzuela 2018). As a result, more hollow space and wrinkled surface were observed on spray-dried particles of a thicker matrix. On the other hand, some core materials have plasticizing effect that could reduce particle shrinkage (Churio & Valenzuela 2018). FeSO₄·7H₂O, the iron...
FIGURE 3. Particle morphology (left) at 3000 magnification and iron mapping from EDX determination of the encapsulated iron (right) at various viscosities of hydrolysed glucomannan: (a) 83, (b) 115, (c) 176, and (d) 222 cP
source in this research, contains hydrate compound which also vaporised during spray-drying and contributed on wrinkles formation. Other factors also contributed to the spray-dryer product form and stability, including the addition of stabilizers such as surfactants. Surfactants balance the surface strength of the viscous matrix in the drying droplets and allow the formation of fine spherical surfaces in dry particles (Arpagaus et al. 2017).

As a semi-quantitative observation, the element mapping shows the distribution of some elements on the particle surface. The distribution of iron on the surface particles is presented in Figure 3-right. Increased glucomannan viscosity could affect the iron distribution on the particle surface. This was confirmed by the data in Table 1, which shows that lower viscosity decreased the ability of glucomannan to cover iron on the particle surface which led to expose more iron on the surface, a condition that led to oxidation. This result suggested that high-viscosity glucomannan could better in protecting iron from being exposed to the surface. However, the viscosity of the encapsulant is limited to blocking concern of the nozzle during spraying.

COLOUR
Colour is one of the physical appearances of the product that could attract consumers. Hence, it is very important to keep product colour attractive. The increase of $L^*$, $a^*$, $b^*$, and $\Delta E$ values describes the scale of darkness to lightness, greenness to redness, blueness to yellowness, and overall colour difference of the samples, respectively (Wardhani et al. 2020b). Encapsulant viscosity did not significantly influence the change of $a^*$, $b^*$, and $\Delta E$ (Figure 4) of the product.

### TABLE 1. Profile of iron on the surface particles

<table>
<thead>
<tr>
<th>Glucomannan viscosity (cP)</th>
<th>C (%)</th>
<th>O (%)</th>
<th>S (%)</th>
<th>K (%)</th>
<th>Fe (%)</th>
<th>Cu (%)</th>
<th>Zn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>48.42</td>
<td>48.26</td>
<td>0.88</td>
<td>0.29</td>
<td>1.47</td>
<td>0.38</td>
<td>0.29</td>
</tr>
<tr>
<td>115</td>
<td>56.13</td>
<td>42.50</td>
<td>0.31</td>
<td>0.13</td>
<td>0.53</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>176</td>
<td>54.81</td>
<td>43.79</td>
<td>0.34</td>
<td>0.22</td>
<td>0.46</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>222</td>
<td>52.41</td>
<td>45.72</td>
<td>0.35</td>
<td>0.24</td>
<td>0.5</td>
<td>0.45</td>
<td>0.33</td>
</tr>
</tbody>
</table>

FIGURE 4. Effect of glucomannan viscosity on the colour of the spray-dried iron
encapsulated particles. However, a significant difference in the L* value, which indicated a lighter colour of the encapsulated product, was observed in the higher viscosity one. This could be contributed by the light colour of the native glucomannan powder, which tended to give more solid lightness when used in high viscosity. Other than encapsulant colour, the colour of spray-dried product is also significantly affected by temperature process, exposure duration, and storage condition (Mishra, Mishra & Mahanta 2014), which in this work they were maintained similar.

SOLUBILITY AND SWELLING

The solubility and swelling analysis of spray-dried products were carried out at two pH solutions to represent acidity conditions of the nonenzymatic gastrointestinal tract, i.e., pH 1.2 and 6.8. Figure 5-top shows the effect of glucomannan viscosity on solubility and swelling of the spray-dried product. The trend of solubility for each pH solution was similar as the case for the swelling determination. Insignificant values were observed in solubility determination at both pHs. However, both determinations showed lower values in
pH 1.2 than those on pH 6.8. This could be due to high H⁺ in the low pH solution, which helped maintain the glucomannan structure intact and prevent water permeation (Wang et al. 2014). This result suggests that the hydrolysed glucomannan has the potency to protect the iron from degradation in an acidic environment such as in the stomach, pass this environment and deliver it to a more neutral condition such as in the intestine as the absorption site.

IRON STABILITY
After 10 months of storage, the iron content degraded around 64.89-72.67% (Figure 5-bottom). In this study, the iron encapsulated in the matrix-encapsulation type in which the iron distributed entire the particle. The iron on the surface of this encapsulation is potentially degraded as it directly exposed to the environment. However, higher glucomannan viscosity tends to show an insignificant effect on the iron degradation. The stability of microencapsulated powder was influenced by its moisture content, hygroscopicity, and thermal properties of wall material (Ramakrishnan et al. 2018). In this study, higher viscosity of spray-dryer’s feed reduced the glass transition temperature (Tg) of iron particle. Lower Tg increased the particle hygroscopicity, which produced less stable particle (Bhandari & Howes 1999). However, lower iron was presented on the particle surface as explained in the previous section. Duration of determination also affected the degradation level. Hence, all the factors contributed on the worse of degradation.

CONCLUSION
The viscosity of spray-dried feed influenced the properties of the particle product. Increasing spray dryer feed’s viscosity led to improve encapsulation efficiency, mean particle diameter, and lighten particle colour but reduced moisture content and bulk density. The highest encapsulation efficiency (99.95%) was obtained in using 222 cP of encapsulant. The thicker the encapsulant resulted in more numbers of larger and wrinkle particles. This encapsulant was better in protecting the iron from exposure to oxygen on the particles surface. Solubility and swelling of the particles were sensitive to pH solution. In both cases, the values for pH 6.8 were higher than those of pH 1.2, suggesting a protection encapsulation potential of iron using hydrolysed glucomannan in acid condition. Although higher viscosity of spray dryer’s feed is preferable for better iron protection, however, it has the possibility to block the spray process. Hence, the feed viscosity has to be determined sensibly considering many factors including the characteristic of the encapsulant, the aim of encapsulation, and the process condition itself.

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