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The Effect of Carboxymethyl Glucomannan Concentration on The Properties of Glucomannan-Chitosan Hydrogel for *Lactobacillus acidophilus* FNCC 0051 Encapsulation

(Efek Konsentrasi Glukomanan Karboksimetil terhadap Sifat Hidrogel Glukomanan-Kitosan dalam Mengenkapsulasi *Lactobacillus acidophilus* FNCC 0051)

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Abstract

Probiotic cell survival during the process, storage, and consumption is important in the development of probiotic-containing food products. Hydrogel generated from the interaction of negatively charged carboxymethyl glucomannan and positively charged chitosan may be developed for probiotic cell encapsulation. This research aimed to study the effect of glucomannan concentration on the properties of hydrogel for the encapsulation of *Lactobacillus acidophilus* FNCC 0051. Hydrogel was prepared by

extruding chitosan in acetic acid solution to different concentrations of carboxymethyl glucomannan. The properties of hydrogel such as morphology, particle size, polydispersity index, zeta potential, FTIR (Fourier-transform infrared spectroscopy) spectra, swelling ratio, encapsulation efficiency, and survivability of cells encapsulated in hydrogel were measured. This study showed that by increasing carboxymethyl glucomannan concentration, the particle became bigger and the zeta potential values became higher. However, it did not have the impact on polydispersity indexes. The concentration of 0.5% glucomannan in producing hydrogel reached the highest encapsulation efficiency and easy to swell-deswell in different pH environment. The cells were also well protected during heat treatment and cold storage. The good permeability of hydrogel can be functioned as the exchange surface of the nutrients, gases, and metabolites, therefore it is possible to be developed as cell encapsulant.
Keywords: chitosan; encapsulation; glucomannan; hydrogel; probiotic; properties

Abstrak

Kelangsungan hidup sel probiotik selama proses, penyimpanan, dan konsumsi penting dalam pengembangan produk-produk pangan probiotik. Hidrogel yang dihasilkan dari reaksi interaksi antara glukomanan karboksimetil yang bermuatan negatif dengan kitosan yang bermuatan positif dapat dikembangkan untuk enkapsulasi sel probiotik. Riset ini bertujuan untuk mempelajari efek konsentrasi glukomanan terhadap sifat hidrogel dalam mengenkapsulasi Lactobacillus acidophilus FNCC 0051. Hidrogel dibuat dengan mengekstrusi kitosan dalam larutan asam asetat ke larutan glukomanan karboksimetil yang berbeda konsentrasinya. Sifat hidrogel diukur, yaitu: morfologi, ukuran partikel, indeks polidispersitas, zeta potensial, spectra FTIR (Fourier-transform

infrared spectroscopy), rasio swelling, efisiensi enkapsulasi, dan kelangsungan hidup sel yang dienkapsulasi. Studi ini menunjukkan bahwa dengan meningkatnya konsentrasi glukomanan karboksimetil, ukuran partikel menjadi lebih besar dan zeta potensial menjadi lebih tinggi. Walaupun demikian, peningkatan konsentrasi ini tidak berpengaruh terhadap indeks polidispersitas. Hidrogel yang diproduksi dari glukomanan dengan konsentrasi 0,5% dapat mencapai efisiensi enkapsulasi tertinggi dan mudah untuk mengalami swell-deswell pada lingkungan pH yang berbeda. Sel juga dapat dilindungi selama perlakuan panas dan penyimpanan dingin. Permeabilitas permukaan hidrogel yang baik dapat dimanfaatkan sebagai penukar nutrisi, gas, dan metabolit, sehingga memungkinkan untuk dikembangkan sebagai enkapsulan.

Keywords: kitosan; enkapsulasi; glukomanan; hidrogel; probiotik; sifat

Introduction

The survival of probiotic cells during the process, storage, and consumption is important in developing probiotic products. Encapsulation is one effort to protect the cells from that harsh environment (Bosnea & Moschakis 2014; Halim et al. 2017; Rather et al. 2017; Xu et al. 2016). To make good encapsulant, there are important factors to be considered, such as survival of cell, mild processing, sturdy layer encapsulant, and absence of alteration in the mouth when consumed (Priya, Vijayalakshmi & Raichur 2011). Therefore, many different encapsulants were developed, such as microsphere from alginate, carrageenan-locust bean gum, aluminium carboxymethyl cellulose-rice bran, gellan gum, xanthan gum, etc (Banyuaji, Rahayu & Utami 2009; Chitprasert, Sudsai & Rodklongtan 2012; Florenza 2014; Lakkis 2007; Priya, Vijayalakshmi & Raichur 2011; Sathyabama et al. 2014; Shi et al. 2013; Trabelsi et al. 2013).

In the recent years, hydrogel became popular in the pharmaceutical, biomedical, and nutraceutical field, because of its potential as delivery carrier of bioactive compounds (Li 2011). Hydrogel is a crosslinked polymeric material that can absorb a lot of water. It can be made from natural polysaccharide like carboxymethyl porang (*Amorphophallus oncophylus*) glucomannan and chitosan that are both having the opposite charges. The previous study showed the application of hydrogel from konjac glucomannan-chitosan for the encapsulation of drug and enzyme (Du et al. 2005; Korkiatithaweechai et al. 2011). Even though, it was sensitive to pH and may be potential used as probiotic carrier in the gastrointestinal tract (Aprilia et al., 2017, Annan et al., 2008; Gbassi and Vandamme, 2012; Priya et al., 2011; Valero-Cases and Frutos, 2015; Vidhyalakshmi et al., 2009; Xu et al., 2016). However, there was no data about the detail characters of this hydrogel, especially in encapsulating cells, like the encapsulation efficiency that determine the carried cells, the ability of hydrogel to protect the cells, particle size and its uniformity (polydispersity index) that is important in sensory parameters, and the impact of glucomannan-chitosan interaction to the cells that may be observed from its zeta potential. Those characters could be influenced by many factors, such as the ratio of polymers, encapsulation process, and the core (cell) concentration (Chitprasert et al., 2012, Sathyabama, Ranjith, Bruntha, Vijayabharathi, & Brindha, 2014)

This research aimed to study the effect of glucomannan-chitosan concentration on the properties of hydrogel for the encapsulation of *Lactobacillus acidophilus* FNCC 0051, like hydrogel morphology, particle size, polydispersity index, and zeta potential of hydrogel, encapsulation efficiency, and cell surviveability test.

Materials and Methods

Materials

The main materials were glucomannan extracted from porang tuber (*Amorphophallus oncophyllus*) and chitosan. Glucomannan was obtained from Faculty of Agricultural Technology, Universitas Gadjah Mada. Carboxymethyl glucomannan was produced with Na-chloroacetate (Aprilia et al. 2017). Food-grade chitosan with 85%–89% degree of deacetylation was purchased from PT Biotech Surindo, Cirebon, West-Java, Indonesia.

Probiotic

Lactobacillus acidophilus FNCC 0051 cells were used as the core. They were obtained from stock culture collection of *Food and Nutrition Culture Collection* (FNCC), Laboratory of Food Microbiology, Center for Food and Nutrition Studies, Universitas Gadjah Mada. Cells were reactivated from the working stocks in skim milk-glycerol suspension by growing twice successively in de Man, Rogosa, and Sharpe (MRS) Broth at 37°C overnight. Centrifugation at 2400 g for 9 minutes at 4°C were done to collect the cell biomass (Okuro et al. 2013). It was then washed twice with sterile saline solution and resuspended in saline solution before they were used in the encapsulation process.

Encapsulation of Probiotic in Hydrogel

Hydrogel was formed by complex coacervation method (Aprilia et al. 2017). The concentration of chitosan was 0.5% (w/v) in acetic acid solution, while the concentration of glucomannan varied between 0.3, 0.5, 0.7, and 0.9% (w/v). Before treatment, all the materials have been sterilized. The cells were mixed to the polymer before coacervation.

Hydrogel was then analyzed for the morphology, particle size, polydispersity index, zeta potential, FTIR (Fourier-transform infrared spectroscopy) spectra, and swelling ratio as described below. The concentration of glucomannan that generated the highest encapsulation efficiency was then analyzed for its viability during heating at 65◦C for 30 minutes and storage at 5◦C for 2 months.

Hydrogel Morphology

The morphology of hydrogel was observed by optical microscope (Olympus BX51, Olympus Corp., Japan) equipped with OptiLab pro digital camera (Miconos, Indonesia). To observe the surface by scanning electron microscope/SEM (Inspect S50, EDAX-AMETEK, USA), hydrogel was then dried and put in sample holder using carbon double-sided tape. Gold coating was done with sputter coater (Emitech SC7620, UK).

FTIR Spectroscopic Analysis

FTIR was performed to compare the interaction between glucomannan and chitosan in different concentrations of glucomannan. The FTIR spectra were recorded on a Shimadzu 8201 PC spectrophotometer in the region between 4000 cm^{-1} and 400 cm^{-1}. Freeze-dried hydrogel was mixed with KBr and pressed to a plate for measurement.

Particle size, Polydispersity Index, and Zeta Potential of Hydrogel

The size and polydispersity index of hydrogels were measured by using particle size analyzer (Horiba SZ-100 series, Japan). Zeta potential was measured by Zetasizer (Nano ZS Ver 6.20, Malvern Instruments Ltd, Malver, UK).

Encapsulation Efficiency of Hydrogel

Encapsulation efficiencies were calculated by dividing the number of cells entrapped in hydrogel with the number of cells added in polymer (Bosnea & Moschakis 2014). Cells in hydrogel were released with buffer solution of pH 8 (Aprilia et al. 2017). The hydrogels were then incubated for 24 hours at 37°C. They were then serially diluted in saline solution before plated on MRS agar. Encapsulation efficiencies of hydrogels in several probiotics were also determined.

Swelling Ratio of Hydrogel

Hydrogel was determined for its swelling ratio in different pH solutions and salt concentrations (Du et al. 2006). The solutions for swelling studies were buffer-produced from HCl-KCl (pH 1 and 2), citrate (pH 3), acetate (pH 4 and 5), phosphate (pH 6, 7, and 8), carbonate (pH 9). The concentrations of salt solution were 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1%. The swelling ratios were then calculated by using the formula based on Du et al. (2006).

Cell Survivability Test

Cell survivability test was conducted to know the properties of hydrogel in protecting the cells during heat and storage treatment. The stability of cells was compared between free cells, encapsulated cells in hydrogel of porang glucomannan-chitosan, konjac glucomannan-chitosan, and Ca-alginate. In stability test, 1 gram of hydrogel was mixed with 9 mL of milk. It was then pasteurized at 65°C for 30 minutes (Charalampopoulos & Rastall 2009). For storage stability test, it was stored in a cold room

with temperature of 5°C for 56 days. The cells were enumerated at the day of 1st, 7th, 14th, 28th, and 56th.

Before enumerating, cells in hydrogel were released by mixing 1 gram of hydrogel in 9 mL of phosphate buffer of pH 8 and incubated overnight at 37°C (Aprilia et al. 2017). One mL of solution was then serially diluted in 0.85% salt solution and pour-plated in MRS agar. Cells were enumerated after 48 hours of incubation. The survival rate was calculated by dividing the number of viable cells within the hydrogel after treatment with the initial number of cells (Xu et al. 2016).

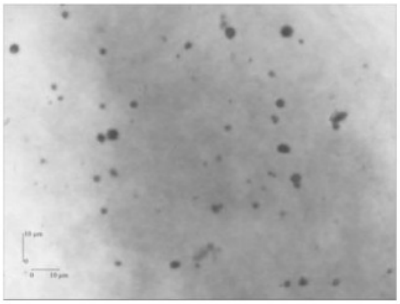
Statistical analysis

Data were reported as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA). Multiple comparisons were performed using Duncan's multiple range test (DMRT) at $p<0.05$. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results and Discussion

Spherical shape hydrogel (Figure 1A) was successfully produced by interacting porang glucomannan and chitosan. The surface of blank hydrogels was smooth. It became rough after addition of cells (Figure 1B and 1C). It proved that hydrogel could encapsulate the cells.

A



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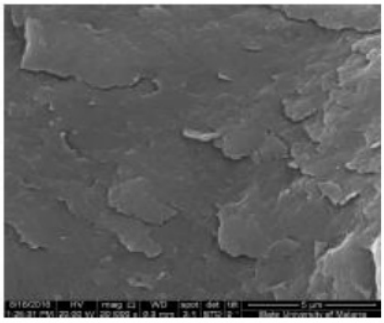


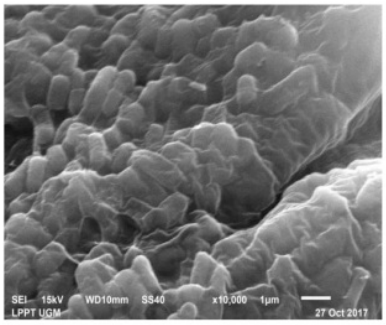


Figure 1. Porang glucomannan-chitosan hydrogel observed by optical microscope (magnification 1300x) (A) and scanning electron microscope (SEM) (magnification 10000x) for blank hydrogel (B) and hydrogels encapsulating cells (C)

FTIR Spectroscopic Analysis

FTIR spectra of hydrogels in different concentrations of glucomannan were performed as shown in Figure 2. For the IR spectrum of chitosan, the characteristic absorptions appeared at 1597 cm^{-1} (protonated amide I), 1658 cm^{-1} (amide I, vibration from C=O and C=N), and amide III (1381 cm^{-1} and 1419 cm^{-1}). The absorption peaks at 810 cm^{-1} (mannose), while 1627 cm^{-1} (symmetric carbonyl) and 3418 (OH) for carboxylic acid were characterized for glucomannan. The interaction between glucomannan and chitosan was indicated from the stronger intensity at 2924 cm^{-1} compared to chitosan's, but it was weaker compared to glucomannan's. At the peak of 2337 cm^{-1}, there was a stronger

intensity compared to both polymers. Among all hydrogels, different concentration of glucomannan gave impact on the absorption peak between 1026 and 1087 cm^{-1}. Those peaks were attributed to bending vibration of C-O-C groups (Du et al. 2004) that came from glucomannan.

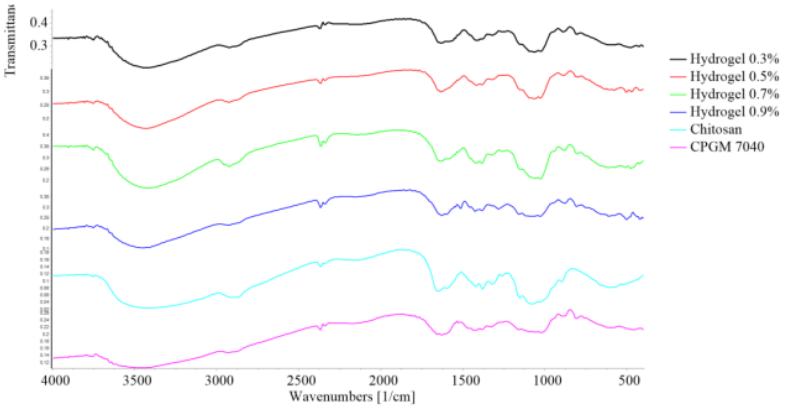


Figure 2. FTIR spectra of hydrogel formed in different concentrations of glucomannan

Particle Size, Polydispersity Index, and Zeta Potential of Hydrogel

The impact of glucomannan concentration on the properties of particle was also studied (Figure 3). Particle sizes increased smoothly in the higher concentration of glucomannan (slope 1.505). It may be because the higher concentration of glucomannan and the more glucomannan dissolved in the solution yielded higher viscosity that gives impact on the bigger particles. Particle size may be influenced by the size of nozzle that was used in coacervation process; the type, concentration, and the temperature of polymer; the distance between nozzle and polymer; and the condition of environment like pH and salt concentration (Brun-graeppi et al. 2011; Du et al. 2005; Gaudio et al. 2005; Shewan & Stokes 2013).This result was also confirmed by previous study in konjac glucomannan-

chitosan hydrogel. The bigger particle size was due to the increased number of molecule units at higher polymer concentrations (Du et al. 2004).

Polydispersity index is a parameter to measure the uniformity of particle. As shown in Figure 3, polydispersity index was almost no change in the increase of glucomannan concentration (slope was very low, 0.225). It may be due to the control of spinning rate during the coacervation process (Shewan & Stokes 2013). The polydispersity indexes of hydrogel in this research were between 0.4-0.5 that were higher compared to other studies that used konjac glucomannan-chitosan as the hydrogel materials (Du et al. 2004).

The increase of glucomannan concentration gave the impact on the lower value of hydrogel zeta potentials. It was shown by negative slope value (Figure 3). The higher the glucomannan concentration, the lower the positive charge of hydrogel. It may be caused by the more glucomannan proportion in particles, the more negative charge from carbonyl groups leading to lower resultant charge between glucomannan and chitosan. Zeta potential of particles were influenced by total charge of particles with the microbes entrapped inside them (Priya, Vijayalakshmi & Raichur 2011).

Zeta potentials were measured as the total charge in particles. The data of this study showed that all hydrogels had positive charges. It indicated the domination of positive charge in the surface of hydrogels although they were produced from the opposite charge polymers. Du et al. (2004) explained that chitosan has cationic charge. The cationic charge becomes higher when the deacetylation degree was increased.

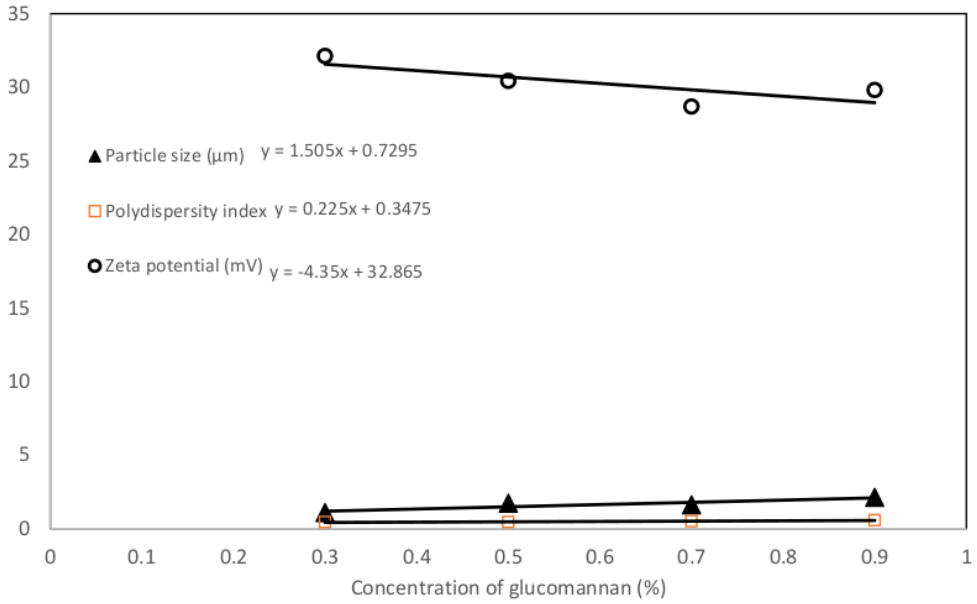


Figure 3. Effect of glucomannan concentration on the particle size, zeta potential, and polydispersity index of hydrogel

Encapsulation Efficiency of Hydrogel

Encapsulation efficiencies of hydrogel were almost the same when different concentrations of glucomannan were conducted, except at the concentration of 0.5% glucomannan (Table 1). This was in line with previous study that used the same polymers with L-asparaginase as the core. The same ratio concentration of glucomannan and chitosan was needed not only for the electrostatic interaction but also for chemical bonding (Wang et al. 2008). The difference charge between hydrogel and the core also influenced the entrapment of cells. It served as the substrate for the adsorption of polycation as the first-layer polymer encapsulant (Priya, Vijayalakshmi & Raichur 2011).

Table 1. Encapsulation efficiency of hydrogel in different concentrations of glucomannan

Concentration of glucomannan (%w/v)	Encapsulation efficiency (%)
0.3	51.20±5.74[a]
0.5	65.83±1.37[b]
0.7	51.59±3.39[a]
0.9	56.27±4.12[a]

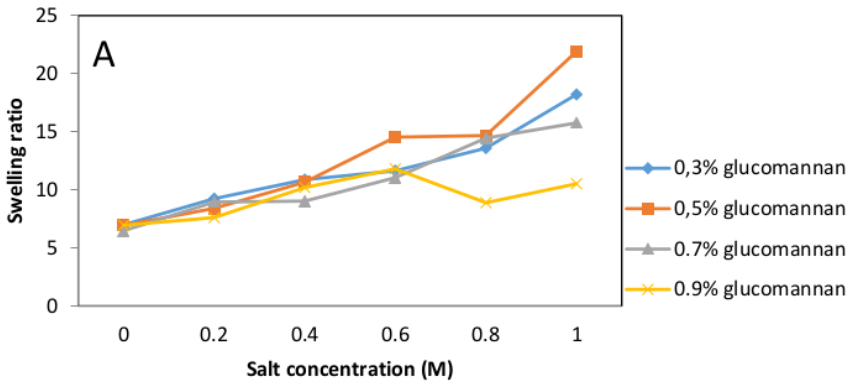
Values represent mean ± SD. Different superscript letters in the same column indicate significant different results at p<0.05

Swelling Ratio of Hydrogel

Figure 4 showed swelling ratio of hydrogel at different media (concentration of salt and pH). Figure 4A showed the increase of swelling ratio in all hydrogels with the salt concentration up to 1 M. It due to hydrogel could not resist the external ionic strength from sodium chloride solution. The higher the salt concentration, the higher the ionic strength. It disturbed the ionic interaction in hydrogel. This condition made the water easier to enter the hydrogel, therefore increasing swelling ratio. It was supported by Du et al. (2005) who reported the increase of hydrogel size when salt concentration was increased. Egan et al. (2014) also proved that salt concentration could give the cationic competition and led to the release of core from microgel.

Figure 4B showed that swelling ratio of hydrogel began to increase at pH up to 5. Previous study reported that this was due to the difference in interaction strength at different pH. At pH < 4.5, there was ionic interaction between both polymers which leads to the lower swelling ratio, while at pH 4.5–6, positive charge from chitosan and ionic charge from glucomannan were almost the same which leads to lower swelling ratio. At pH above 6, both polymers had the same charge; therefore, there was repulsion between polymers which yielded higher swelling ratio (Du et al. 2006). The variation of

glucomannan concentration added to hydrogel processing influenced swelling ratio. When lower glucomannan concentrations (0.3 and 0.5%) were used, swelling began at pH>6, but they did at pH>8 when higher glucomannan concentrations (0.7 and 0.9%) were applicated. It was influenced by the more carboxymethyl groups in higher glucomannan concentration which led to the more interaction with amine group from chitosan that made hydrogel more stable. Yu, Lu, and Xiao (2007) reported the same result when producing hydrogel from oxidized glucomannan and chitosan. The sensitivity of hydrogel made from lower concentration of glucomannan may be used to control the release of entrapped core. In the delivery of bioactive substances in the digestive tract, it may protect the bioactives in the low pH of gastric juices but it may be released in neutral pH of intestinal juice (Alvarez-lorenzo et al. 2013).



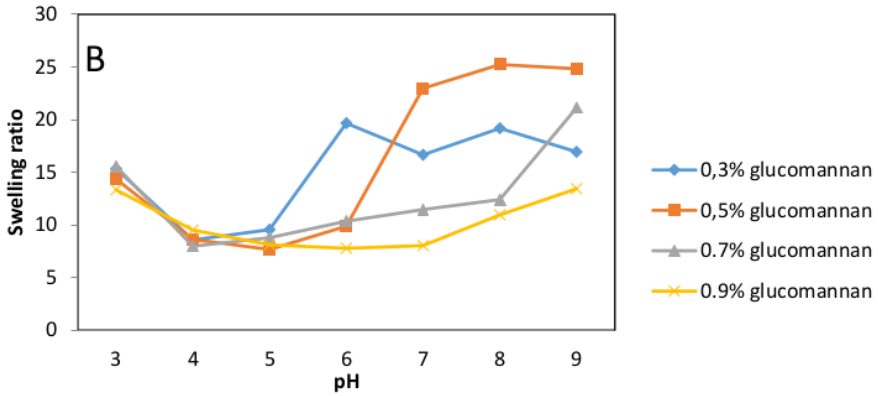


Figure 4. Swelling ratio of glucomannan-chitosan hydrogel in different salt concentrations (A) and pH medium (B)

Cell Survivability Test

Figure 5A showed that hydrogel made from porang glucomannan-chitosan had the same ability in protecting *L.* acidophilus from heat treatment with other popular hydrogels. The viability of free cells in this study was about 58.13±18.5%, and there were statistically no difference with cell encapsulated in other hydrogel tested. Jiang, Han, Li, Yang, and Liu (2015) reported that it may be the attenuation of interaction in hydrogel during heating because of polymer degradation.

Study on the impact of cold storage (Figure 5B) on cell viability showed that there was elevation of cells in porang glucomannan-chitosan hydrogel. The existence of hydrogel could protect the cells from inconvenient environment. In other research, the amount of free cells that were stored at 5◦C for 20 days in yogurt decreased to 1 log cycle (Mortazavian et al. 2007) and it much lower (achieved to 4 log cycles) when the storage at 4◦C for 21 days in non-milk media (concentrated juice) (Buriti, Komatsu & Saad 2007). The elevation of cells in hydrogel also proved that there were pores in the surface that lead to the insertion of milk (media) to the core in hydrogel. The milk may be consumed

by the microbes and used as growth media. Rathore, Desai, Liew, Chan, and Heng (2013) declared that permeability of encapsulant was needed to exchange the nutrients, gases, and metabolites; therefore, cell viability could be maintained.

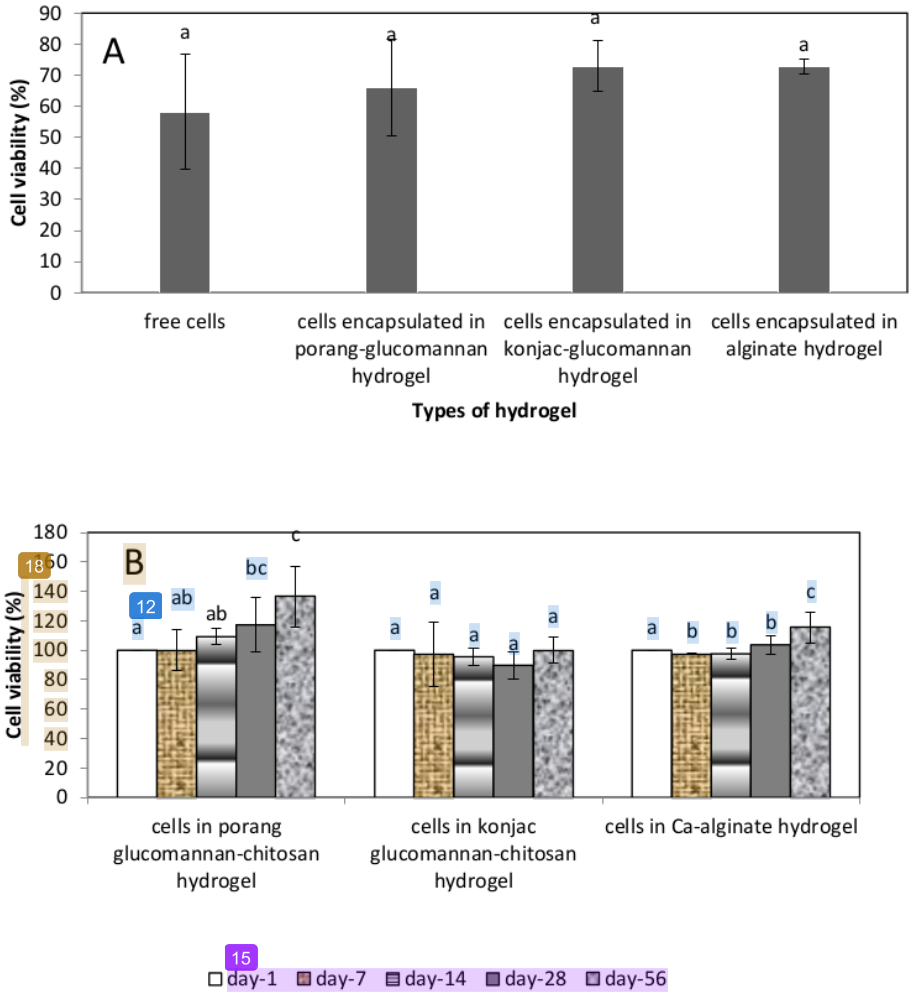


Figure 5. Survivability test of *L. acidophilus FNCC 0051* encapsulated in different types of hydrogels during heat treatment at 65◦C for 30 minutes (A) and during 56 days of cold storage at 5◦C (B)

Conclusion

Hydrogel for encapsulation of *L. acidophilus* FNCC 0051 can be efficiently prepared by combining 0.5% glucomannan and 0.5% chitosan. The increase of glucomannan concentration yielded bigger particles but lower zeta potential value. However, there was no impact on polydispersity indexes. The hydrogels were sensitive in different pH environments, which allows hydrogel to de-swell when it reached the stomach and swell in the intestinal colon. It is the possible way of hydrogel to encapsulate and release the cells in the desired area. The cells were also well protected during heat treatment and cold storage. The good permeability of hydrogel can be functioned as the exchange surface of the nutrients, gases, and metabolites.

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