Judul Artikel : Hydrogel Derived from Glucomannan-Chitosan to Improve the Survival of Lactobacillus acidophilus FNCC 0051 in Simulated Gastrointestinal Fluid
Nama Jurnal : The Scientific World Journal
ISSN : 1537-744X
Tahun Terbit 2022
Penerbit : Hindawi
No. Halaman : 1 s.d. 10
Url Cek Plagiasi :
http://reposister.almaata.ac.id/id/eprint/3421/1/gabungan%20plagiasi%20dan%20beda%20artikel%20dengan%20disertasi.pdf

Url Index : https://www.scopus.com/sourceid/24219
Url Jurnal : https://doi.org/10.1155/2022/7362077

REKAPITULASI KORESPONDENSI JURNAL

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1	Submit	8 Desember 2021
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3	Pertanyaan melalui Email Terkait Perubahan Status dan Pengusulan Reviewer (20 Januari 2022)	20 Januari 2022
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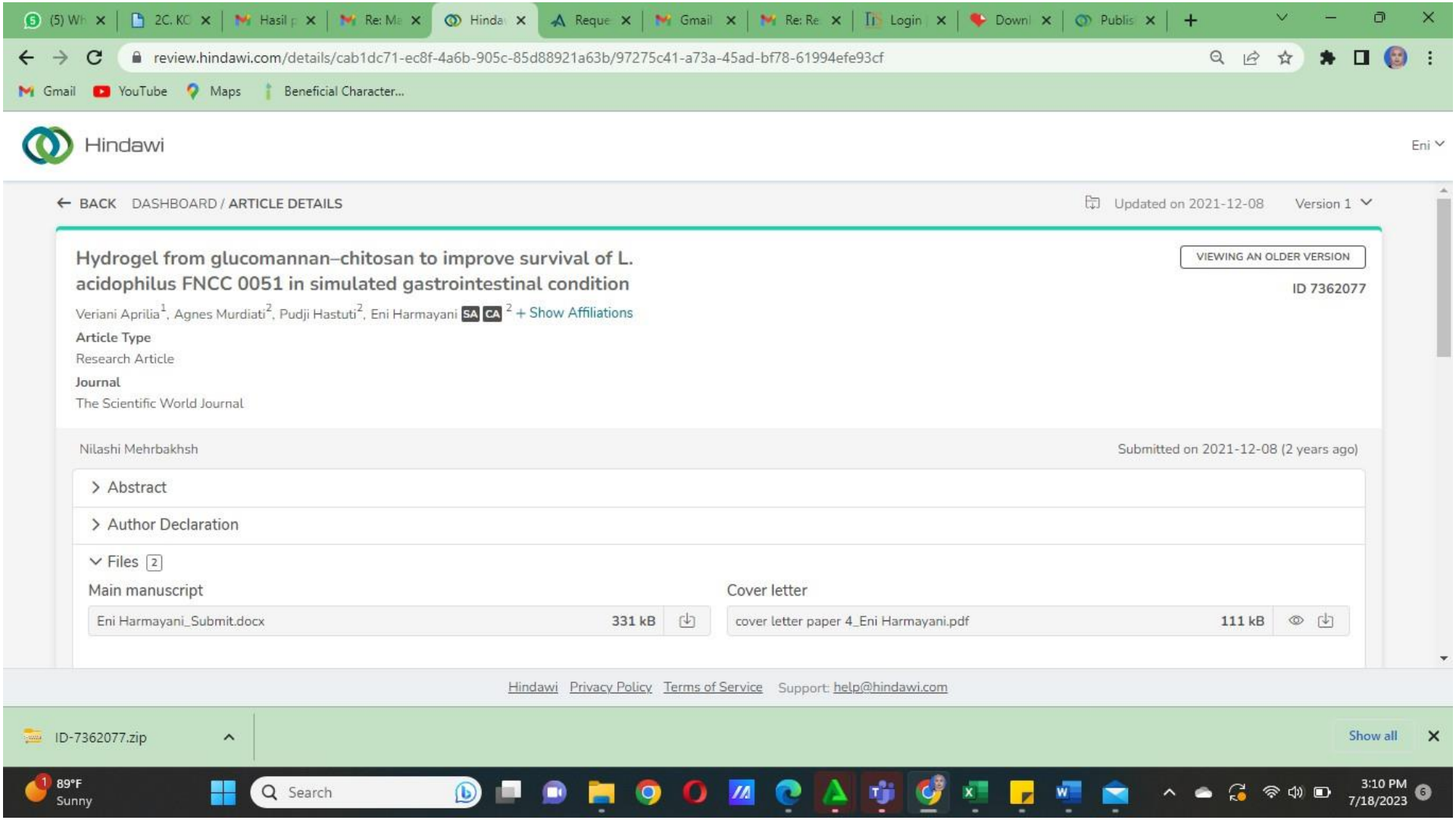
19	Published	15 Desember 2022

1. **Submit Artikel: 8 Desember 2021**



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The manuscript titled "Hydrogel from glucomannan–chitosan to improve survival of L. acidophilus FNCC 0051 in simulated gastrointestinal condition" has been submitted to The Scientific World Journal by Eni Harmayani.

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Dear Editor in Chief of The Scientific World Journal,

Please accept an original manuscript entitled, "**Hydrogel from glucomannan–chitosan to improve survival of *L. acidophilus* FNCC 0051 in simulated gastrointestinal condition***"* for consideration for publication in The Scientific World Journal.

This is an original work, and has not published elsewhere, nor it is being considered for publication elsewhere.

The paper presents the effect of initial *L. acidophilus* cell concentration in production of hydrogel on the properties of hydrogel and cell viability during gastrointestinal condition. To my knowledge, this is the first publication of the effect of initial cell concentration on the properties of glucomannan-chitosan hydrogel. Glucomannan was also sourced from porang tuber, local harvest from Indonesia that was different from famous konjac tuber. This publication will have the impact on the raise utilization of local product.

Please contact me if you have any question or concern regarding the manuscript. I look forward to receiving the results of the review.

Sincerely

Prof. Dr. Ir. Eni Harmayani, M.Sc

Hydrogel from glucomannan–chitosan to improve survival of *L. acidophilus* FNCC 0051 in simulated gastrointestinal condition

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Abstract

Glucomannan extracted from porang (*Amorphophallus oncophyllus)* has been successfully studied to interact with chitosan to form hydrogel. The hydrogel may be used as encapsulant of probiotic. However, its role in the survival of probiotic during gastrointestinal fluid exposure has not been studied. This study aimed to evaluate the effect of initial concentration of *L. acidophilus* FNCC 0051 probiotic on the properties of glucomannan–chitosan hydrogel and cell viability during simulated gastrointestinal exposure. Hydrogel was formed by complex coacervation method. It was analyzed for the encapsulation efficiency and physical properties like particle size, polydispersity index, and zeta potential. The survival of cells was analyzed during exposure of simulated gastrointestinal conditions in vitro for 120 min and the appearance of hydrogel was also observed. The result of study showed that the increase of initial cell concentration during encapsulation generated sensorially acceptable hydrogel properties with larger hydrogel diameter between 2 to 3 µm with a higher polydispersity index (1.23-1.65). The higher initial cell concentration generated higher zeta potential and electropositivity. The cells had good viability during exposure to gastric juice, either in the free form or encapsulated in the hydrogel, but they did not significantly different. In intestinal condition, cell viability (100%) of encapsulated cells was higher than that of free cells (86%). This viability was also comparable either with alginate hydrogel that has been widely used commercially or konjac glucomannan hydrogel as the comparison ingredient. In short, hydrogel have good prospective in food application but need to be developed. The in vivo study is also needed to prove the viability in actual condition.

Key words: hydrogel, viability, glucomannan, chitosan, gastrointestinal

Introduction

Glucomannan is a functional polysaccharide that can be extracted from *Amorphophallus* tuber. In addition to the popular and commercially used of glucomannan from *Amorphophallus konjac,* several studies are currently being conducted of this polymer from other variety sources. In Indonesia, *Amorphophallus oncophyllus* is a local source of glucomannan that is usually called porang (1,2). It has specific characteristics that differ from konjac, including mannose/glucose molar ratio, degree of polymerization, and degree of acetylation, leading to different solubility, viscosity, water holding capacity, and gelation properties (1,2). Thus, the application may also differently depend on the function.

Hydrogel is one of the technologically glucomannan products that take the advantage of gelation properties. It may be formed by the interaction between glucomannan and other polymers to form a three-dimensional polymeric network (3). This character has a potential to be used as encapsulant. A previous

study relating to this was hydrogel from the crosslinking of konjac glucomannan and chitosan, which have many advantages, which include being naturally formed without crosslinker, self-assembly formation, and responsible in different pH, and had been proven for the encapsulation of drug, protein, and enzyme (4,5). A modified study of hydrogel formation from the interaction between porang glucomannan and chitosan has successfully been conducted, which began from the production of basic material of carboxymethyl glucomannan, the compatibility of substitution degree of carboxymethyl glucomannan in hydrogel formation, the effect of polymer concentration on the glucomannan properties, to its application in encapsulation of probiotics (6–8).

Probiotic is a functional food in the form of living cells, which when consumed in sufficient quantities can have a health effect on the host (9). Probiotic is sensitive, and its growth highly relies on the environment. Therefore, glucomannan–chitosan hydrogel is expected to protect probiotics from manufacture and storage until consumption so that the number of cells can meet the criteria ($>10^6$–10^7 CFU/mL) in the human body. So far, the application of porang glucomannan–chitosan hydrogel in the encapsulation of probiotic has just reached its role in protection of cells during pasteurization and cold storage (8); however, its role during digestion has not been studied yet.

The study about the effect of concentration of cells on the properties of hydrogel needs to be developed. This is mainly intended to its suitability in food application, so that it can be sensorially acceptable. Besides its shape, size, and uniformity, encapsulation efficiency of hydrogel in encapsulation of cells should be specified. Encapsulation efficiency is a way to determine the effective process of hydrogel to reach the optimum number of cells that could be encapsulated. This may be calculated by dividing the encapsulated cells with the initial cells in the beginning of the encapsulation process (7,10). Several factors that influenced the encapsulation efficiency include steps in hydrogel production, concentration ratio of glucomannan to chitosan, and the number of cores added (4,5). In relation to this, the steps in the production of hydrogel have been studied, and a 1:1 ratio of porang glucomannan and chitosan could reach optimal encapsulation efficiency (8). However, the number of cells that should be added has not been studied yet.

This study aimed to determine the effect of the initial of cells on the physical properties (particle size, zeta potential, uniformity, and efficiency) of hydrogel and cell viability during simulated gastrointestinal exposure.

Materials and methods

Materials

The main material of this study was glucomannan from porang tuber (*Amorphophallus oncophyllus*), which was obtained from the Faculty of Agricultural Technology, Universitas Gadjah Mada. Carboxymethylation was applied to the glucomannan by using sodium chloroacetate (7). The chitosan that has a degree of 85%–89% deacetylation and fulfills the food qualifications was purchased from PT Biotech Surindo, Cirebon, West-Java, Indonesia.

Preparation of *Lactobacillus acidophilus* FNCC 0051 cells

Lactobacillus acidophilus FNCC 0051 was obtained from the stock culture collection of Food and Nutrition Culture Collection (FNCC), Laboratory of Food Microbiology, Center for Food and Nutrition Studies, Universitas Gadjah Mada. Cells in skim milk–glycerol suspension stocks were reactivated in de Man, Rogosa, and Sharpe (MRS) broth at 37 °C overnight. They were grown twice successively. The cell biomass was then collected by centrifugation at 2400 g for 9 min at 4 °C (11). The cells in saline solution were applied to the encapsulation process, after washing it twice with saline solution.

Encapsulation of probiotic in hydrogel

The hydrogel was formed by the complex coacervation method (7). 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). All the materials have been sterilized before treatment. The cells were mixed with polymer before coacervation. The hydrogel was then analyzed for morphology, particle size, polydispersity index, zeta potential as described below. The glucomannan concentration that generated the highest encapsulation efficiency was then analyzed for its viability during heating (pasteurization) at 65-°C for 30 min and storage at 5-°C for 2 months.

Particle size, polydispersity index, zeta potential, and encapsulation efficiency of hydrogel

The size of particles was estimated as the diameter of hydrogel and measured simultaneously with polydispersity index using a particle size analyzer (Horiba SZ-100 series, Japan). The zeta potential of hydrogel was measured by Zetasizer (Nano ZS Ver 6.20, Malvern Instruments Ltd, Malvern, UK). The appearances of hydrogel during exposure to simulated gastrointestinal conditions were observed by an optical microscope (Olympus BX51, Olympus Corp., Japan) assembled with OptiLab pro digital camera (Miconos, Indonesia).

To evaluate the encapsulation efficiency, the cells in hydrogel must be released from hydrogels by immersing in the buffer solution of pH 8 for 24 h at 37°C (7). They were then counted on MRS agar after 48 h of incubation. The number of released cells was then divided with the number of initial cells to determine the efficiency of encapsulations (10).

Survival of *L. acidophilus* FNCC 0051 during exposure of simulated gastrointestinal conditions in vitro

Approximately 7 mL of pepsin in hydrochloric acid, 2 g of sodium chloride, and 1 M of sodium hydroxide were used to formulate gastric juice, while 1% pancreatic powder, 6.8 g of potassium dihydrogen phosphate, and 77 mL of sodium hydroxide 0.2 N were prepared for intestinal juice as described before (12). Either 1 g of free or encapsulated cells (in the hydrogel of porang glucomannan–chitosan, konjac glucomannan–chitosan, and calcium alginate) was mixed with 9 mL of simulated gastrointestinal juices and incubated for 120 min at 37-°C. The samples were withdrawn at the interval of 0, 30, 60, and 120 min for gastric juice digestion and 0, 60, 90, and 120 min for intestinal juice digestion (13). The hydrogel was then rinsed twice with acetate buffer. The cells were then enumerated using the pour plate technique with MRS agar after 48 h of incubation. The number of viable cells after exposure was divided by the initial number of cells to determine the survival rate of the cell during exposure to simulated gastrointestinal conditions (12). Appearance of hydrogel during exposure to simulated gastrointestinal condition was also observed by optical microscope (Olympus BX51, Olympus Corp., Japan) equipped with optilab pro digital camera (Miconos, Indonesia).

Results and discussion

Properties of hydrogel in different concentrations of cells

The size of hydrogels that encapsulated *L. acidophilus* was detected by the instrument in the range of 0.7–9 µm and mostly distributed in the diameter of 2–3 µm (Table 1). They were classified as microgel because their particle size is mostly <100 µm. This small size did not result in a coarse texture in food (14). They also did not diminish cell's viability because the size was much smaller than 300–500 µm, allowing effective nutrition transport from the outside of the hydrogel to the cells (15). As presented in Table 1, there was a positive relationship between initial cell concentration and its particle size ($p < 0.05$), indicating that this study was in line with previous reports (16).

Table 1. Particle size, polydispersity index, zeta potential of hydrogel in different concentrations of cells

Initial cell concentration (Log CFU/mL)	Particle size (µm)	Polydispersity index	Zeta potential (mV)
8	2.23±0.11[a]	1.23±0.17[a]	24.40±0.75[a]
9	2.79±0.19[b]	1.39±0.04[ab]	32.28±0.80[b]
10	3.41±0.14[c]	1.65±0.27[b]	14.58±0.97[c]

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

The polydispersity indexes of hydrogel encapsulated cells were above 1 (Table 1), indicating a wide particle distribution or several particles of various sizes. These values began to change when the initial cell concentration added was 10 log CFU/mL. The higher initial cell number added, the higher the polydispersity index of hydrogels. A previous study reported that the concentration of glucomannan did not influence the polydispersity index of hydrogel (8).

Zeta potentials of the hydrogel became more electropositive as the cell concentration increased from 8 to 9 log CFU/mL, but decreased at 10 log CFU/mL (Table 1). An increase in the number of cells should result in a reduction of hydrogel charge. This was influenced by the reverse charges of hydrogel and cells, which were positive for empty hydrogel (8) and negative for *L. acidophilus* (17). This difference result may be due to the measurement of zeta potential that was detected only from the surface of hydrogel and affected by the surrounding environment (18).

Encapsulation efficiency of hydrogel in different concentrations of cells

As presented in Table 2, the concentration of encapsulated cells in the hydrogel was aligned with the number of initial cells added during the encapsulation process ($p < 0.05$). The highest encapsulated cell concentration of 7.94 log CFU/g was obtained from the addition of 10 log CFU/mL cells. This number met the criteria for probiotic products from FAO that was >6–7 log CFU/mL (Priya et al., 2011). Previous studies used the initial concentration of around 10–11 log CFU/mL to obtain 11 log CFU of *L. acidophilus* entrapment in calcium alginate beads or 10 log CFU of *L. paracasei* and *L. paraplantarum* entrapment in whey protein isolate–gum Arabic hydrogel (10,19).

Table 2. The concentration of encapsulated cell and encapsulation efficiency of hydrogel in different initial cell concentration

Initial cell concentration (log CFU/mL)	Concentration of encapsulated cell (log CFU/g)	Encapsulation efficiency (%)
8	4.47±0.18[a]	44.37±1.91[a]
9	6.60±0.13[b]	65.83±1.37[b]
10	7.94±0.21[c]	85.03±0.63[c]

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

Adding more initial cells resulted in more efficient encapsulation (Table 2). The same result was also observed in a previous study with the same encapsulant but different core type. The encapsulation efficiency would be steady at the certain number of core added because there was maximum capacity of core entrapment in an encapsulant (5,20).

Survival of cells during exposure to simulated gastrointestinal conditions in vitro

Survival of cells during exposure to gastric juice

Lactobacillus acidophilus had good viability during exposure to gastric juice of pH 2, either in the free form or encapsulated in the hydrogel (Figure 1). Generally, the growth of lactic acid bacteria is optimum at

pH 6–7 (closed to neutral pH). Some metabolic reaction changes when pH is below 5 or 4.4. Indeed, some minerals will be lost at pH 2 or below, so that storage at low pH for a long time will increase the risk of cell death (21,22). A previous study reported that several deaths of *Lactobacillus* occurred for 4 h during gastric exposure (23). This study only represented the actual condition in the human gastrointestinal tract for liquid food that has a transit period of 1.5–2.5 h in the stomach; however, further study is warranted to determine the effect for solid food with a transit period of 3–4 h (24). In addition to the shorter time of exposure in the stomach, the ability of cells in maintaining homeostasis between internal pH and external pH may influence this good viability result in this study.

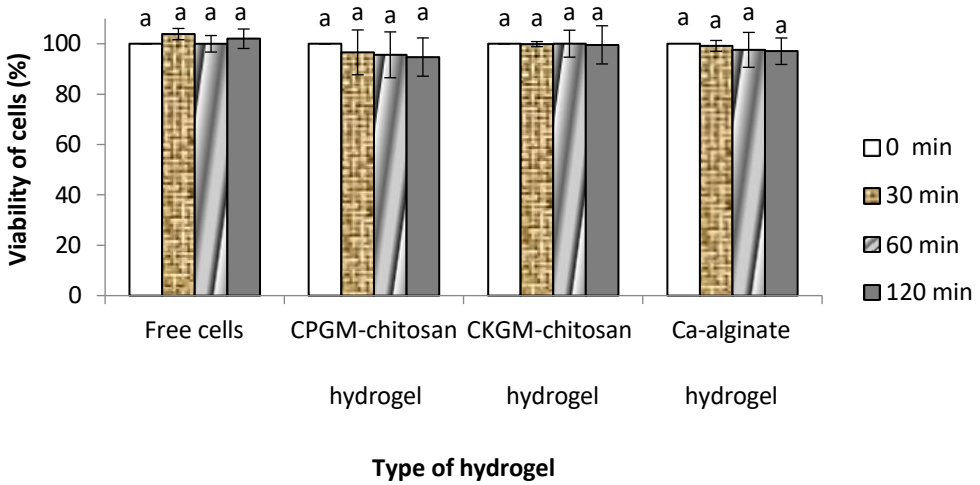

Figure 1. Viability of *L. acidophillus* FNCC 0051 during exposure to gastric juice for 120 min. Different letters in the same type of hydrogel indicates significantly different results at $p < 0.05$. CPGM (carboxymethyl porang glucomannan), CKGM (carboxymethyl konjac glucomannan).

This study also found that porang glucomannan–chitosan hydrogel might have a similar capability in protecting the cells with konjac glucomannan–chitosan hydrogel and calcium alginate hydrogel from the gastric environment ($p > 0.05$). This study was in accordance with the ability of locus bean gum–carrageenan coated with milk in protecting *L. bulgaricus* during exposure to gastric juice (14). Alginate protected *L. acidophilus* from this harsh environment for 3 h of exposure (16), as well as *L. plantarum* (13).

As shown in Figure 2, the hydrogel was well kept in simulated gastric juice for 120 min of exposure. Associated with the swelling ratio study in the previous report (8), the hydrogel ran to deswell at the pH under 5. Deswelling caused the hydrogel to become smaller, which was formerly presumed to lead to the release of cells from the hydrogel. However, Figure 2 proved that the cells were still entrapped in the hydrogel. This may be influenced by the stronger electrostatic interaction between the carbonyl group of glucomannan and the amine group of chitosan when it was in an acid environment (8). The cells in hydrogel as the core maintained this interaction; thus, the deswelling could not be maximized leading to only a few released cells from the hydrogel. There is a possibility that some empty hydrogels will shrink optimally, so that some small hydrogels were no longer visible at 60 min of exposure. These results were in line with other studies that used hydrogels made from oxidized glucomannan and chitosan in entrapping of diclofenac drugs. During exposure to simulated gastric fluid at pH 1.2, not more than 1% of the drug was released from the matrix (5). This proved that the cores in the hydrogel were not released when the hydrogel was exposed to low pH conditions.

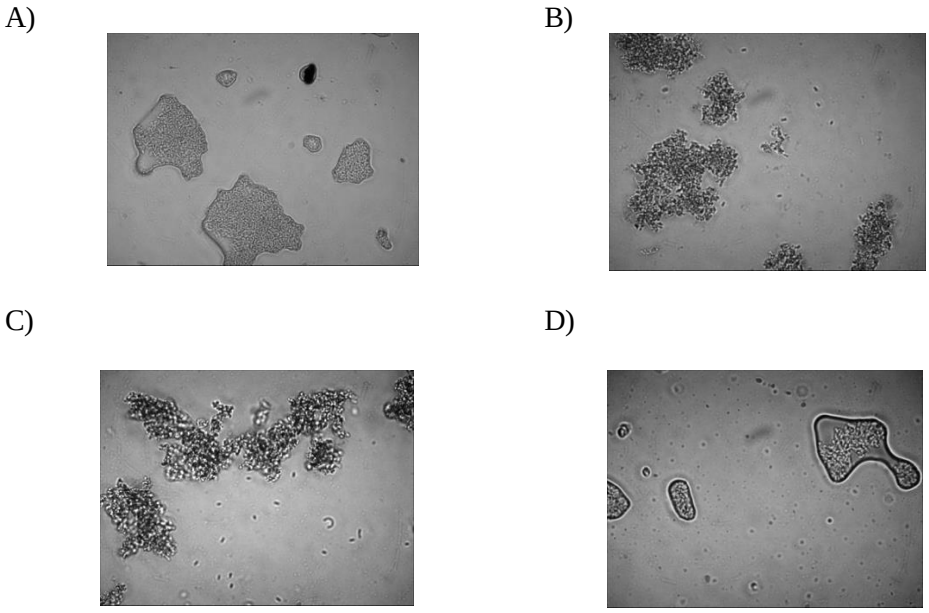

Figure 2. Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (magnification of 1.300x) during exposure to gastric juice for A) 0 min, B) 30 min, C) 60 min, D) 120 min.

Survival of cells during exposure to intestinal juice

As shown in Figure 3, the viability of free cells decreased during exposure to intestinal juice ($p < 0.05$), which was observed at the 60th min of exposure. Otherwise, the viability of cells encapsulated in hydrogel could be maintained during 120 min of exposure, indicating that encapsulation had a role in increasing the viability of *L. acidophilus*. The decrease in the number of free cells may be caused by cell death, which was not only due to the pH of the medium. Priya et al (17) reported that at pH 6.8, bacteria experienced good growth, but the presence of the pancreatin, consisting of amylase, trypsin, lipase, ribonuclease, and protease, damaged the encapsulation wall, resulting in cell death.

Figure 3 also described that porang glucomannan hydrogel had the same good protective effect as the hydrogel of konjac–chitosan glucomannan and calcium alginate. In this study, the alginate-based hydrogel was used as a comparison because it is widely used as an encapsulant in many studies for its cheap price, biocompatibility, and nontoxicity (25). Probiotic encapsulation using alginate in previous studies showed an increase in viability compared to free cells during exposure to intestinal juice (26). Therefore, the hydrogel of porang–chitosan glucomannan has the potential to be developed as a bacterial encapsulation.

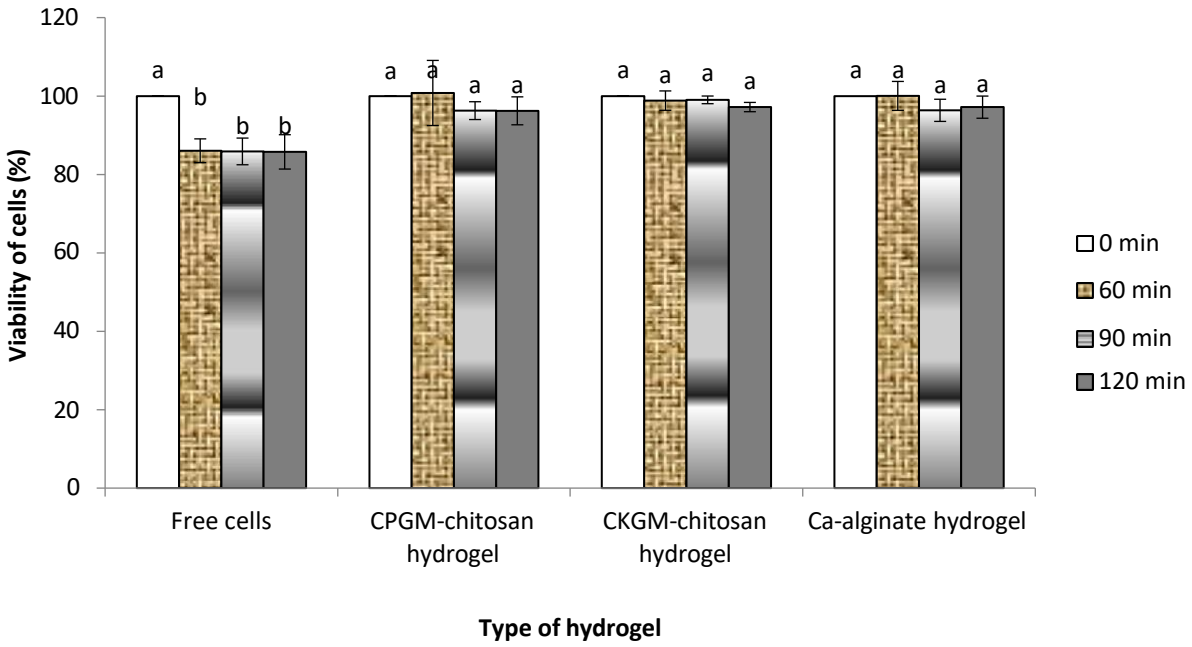

Figure 3. Viability of *L. acidophillus* FNCC 0051 during exposure to intestinal juice for 120 min. Different letters in the same type of hydrogel indicates significantly different results at $p < 0.05$. CPGM (carboxymethyl porang glucomannan), CKGM (carboxymethyl konjac glucomannan).

The hydrogel's microscopic appearance was used to clarify the cell viability data. Hydrogel from porang glucomannan–chitosan was stable for up to 2 h in the intestinal fluid. Hydrogel became larger at 61 min compared with that of at 0 min (Figure 4). This may be due to the swelling behavior of hydrogel at a pH of 6.8. Our previous study proved that porang glucomannan–chitosan hydrogel began to swell at pH > 5 (8). The swelling of hydrogel could be seen until 90 min of exposure. After 120 min of exposure, there were many small hydrogels and cells in the solution. The swelling made the interaction in hydrogels weaker, leading to some parts of the hydrogel being dissolved, leaving small hydrogels, and to the release of cells from the hydrogel. Another study also had a similar result. Exposing the hydrogel of konjac glucomannan carboxymethyl chitosan with bovine serum albumin core into pH 7.4 buffer showed a greater release of core than that at medium pH 5. This was caused by swelling, which resulted in enlarged pores (4). The completion of core release also occurred when the hydrogel of chitosan-oxidizing glucomannan was exposed to simulated intestine fluid for 2–8 h (5).

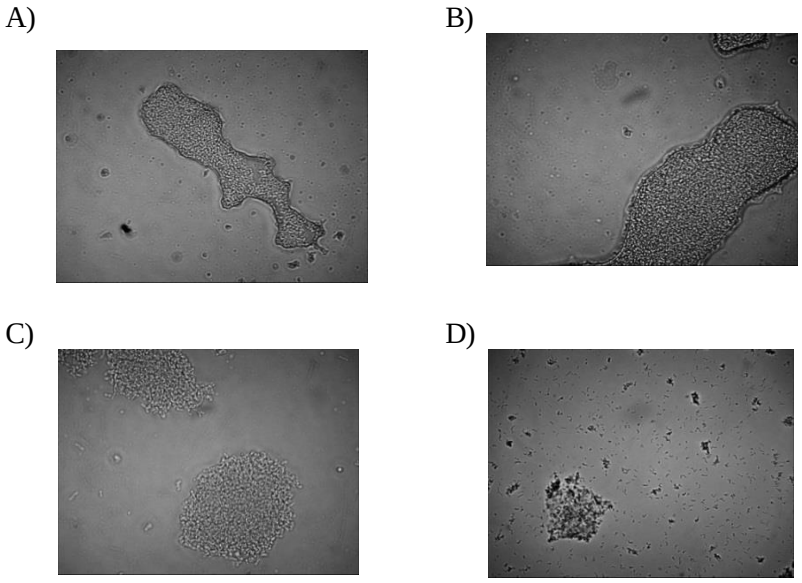

Figure 4. Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (magnification of 1.300x) during exposure to intestinal juice for A) 0 min, B) 30 min, C) 60 min, D) 120 min.

Conclusion

This current research proved that the initial concentration of *L. acidophilus* affected the properties of glucomannan–chitosan hydrogel. The increase of initial cell concentration during encapsulation yielded larger particle diameter between 2 and 3 µm with a higher polydispersity index, indicating many particles of various sizes. The zeta potential of particles also presented higher electropositivity. Encapsulation ensured the cell viability during exposure to simulated gastrointestinal condition. This viability of cells in porang glucomannan-chitosan hydrogel was as good as alginate hydrogel that has been widely used commercially or konjac glucomannan hydrogel as the comparison ingredient. This study proved that hydrogel may be used as the alternative encapsulant to protect probiotic or other functional food ingredients.

Data availability

The data used to support the findings of this study are included within the article.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was supported by the RISPRO Project of Lembaga Pengelola Dana Pendidikan (LPDP) (Indonesia Endowment Fund for Education) for 2016–2017 and Research Directorate and Reputation Team towards World Class University–Quality Assurance Office of Universitas Gadjah Mada according to Assignment Letter Number: 6144/UN1.P.III/DIT-LIT/PT/2021 dated September 27, 2021.

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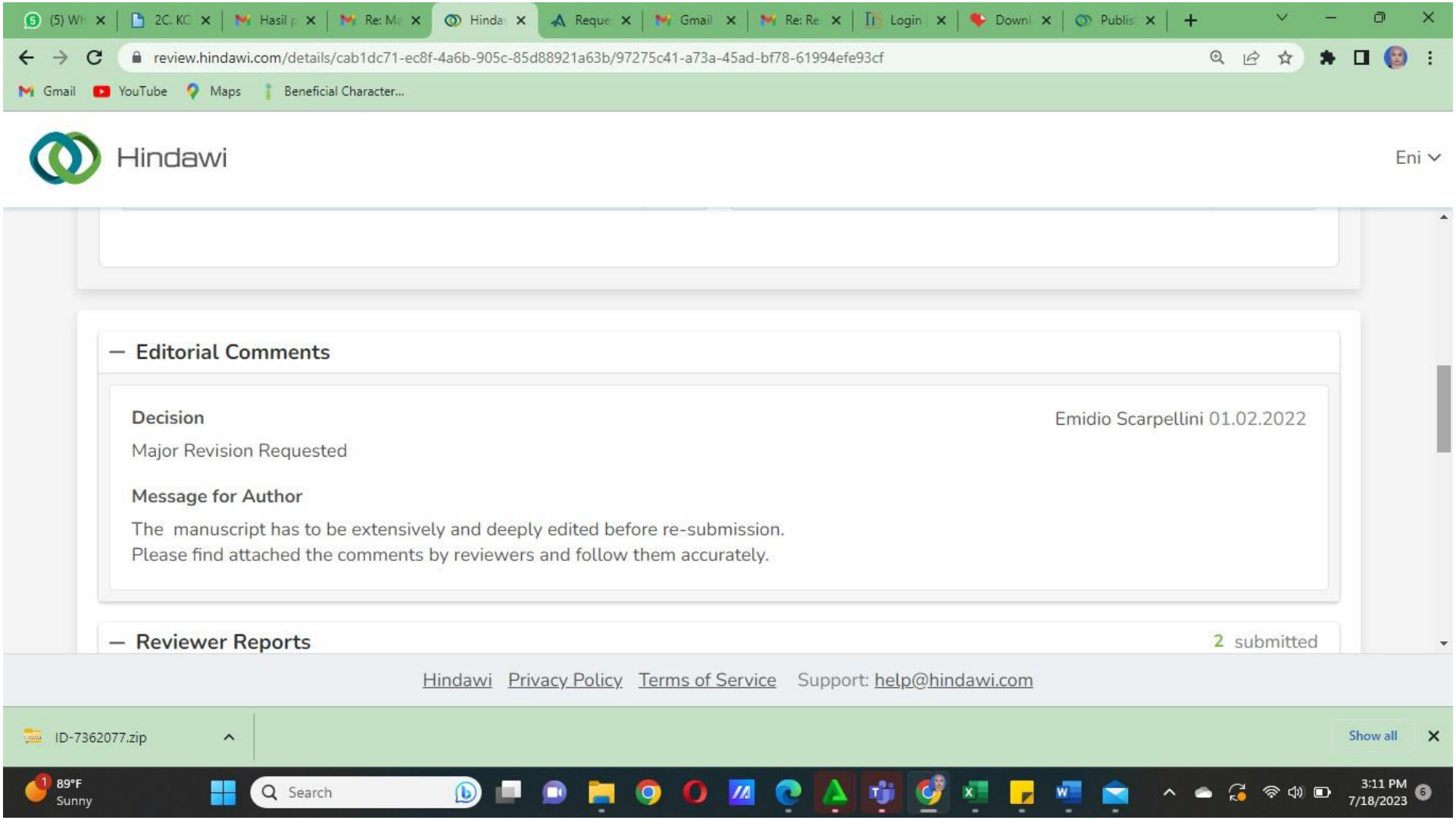
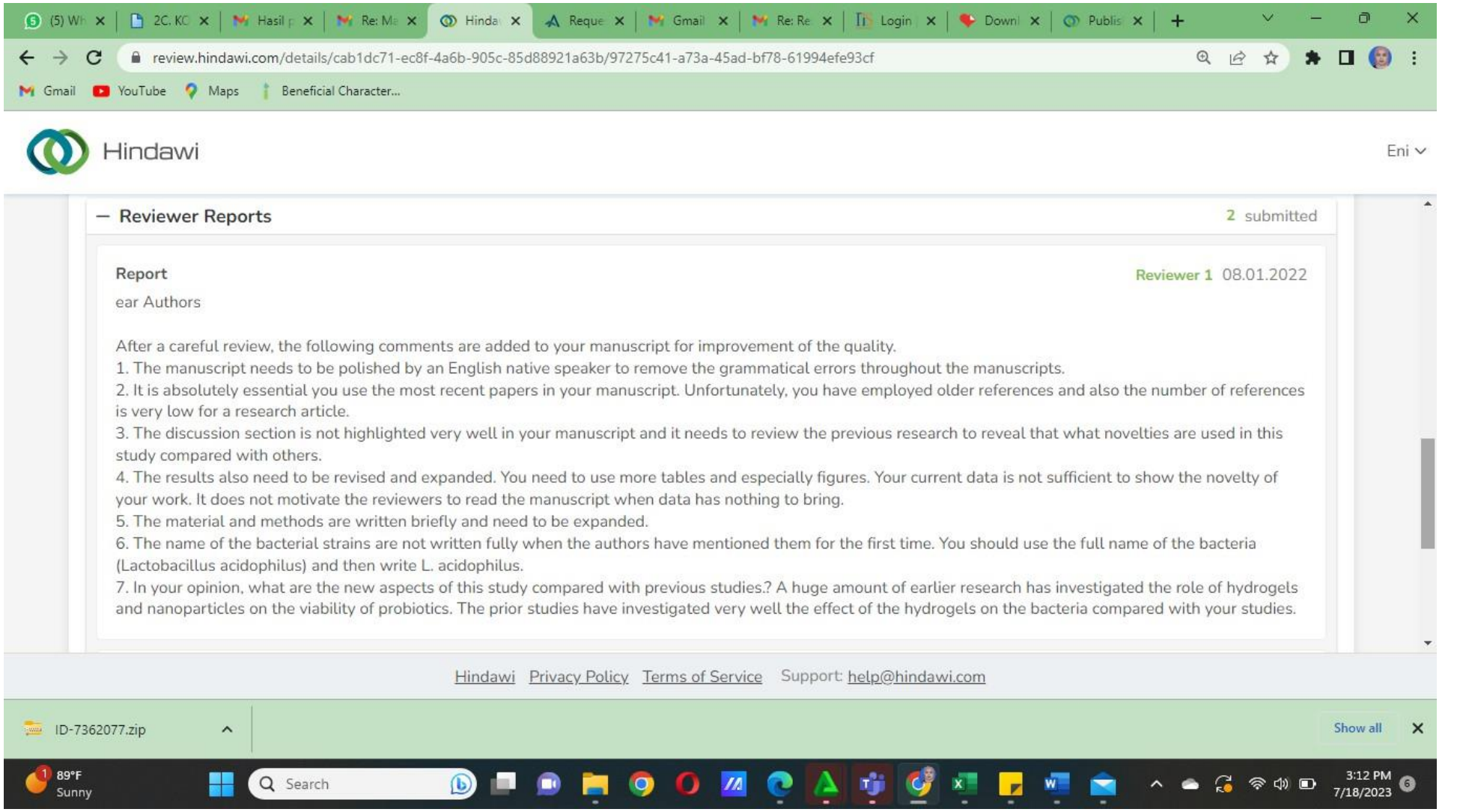
2. **Reviu Artikel: 9 Desember-1 Februari 2022**

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Reviewer Reports
2 submitted
Report
Reviewer 1 08.01.2022
ear Authors
After a careful review, the following comments are added to your manuscript for improvement of the quality.
1. The manuscript needs to be polished by an English native speaker to remove the grammatical errors throughout the manuscripts.
2. It is absolutely essential you use the most recent papers in your manuscript. Unfortunately, you have employed older references and also the number of references is very low for a research article.
3. The discussion section is not highlighted very well in your manuscript and it needs to review the previous research to reveal that what novelties are used in this study compared with others.
4. The results also need to be revised and expanded. You need to use more tables and especially figures. Your current data is not sufficient to show the novelty of your work. It does not motivate the reviewers to read the manuscript when data has nothing to bring.
5. The material and methods are written briefly and need to be expanded.
6. The name of the bacterial strains are not written fully when the authors have mentioned them for the first time. You should use the full name of the bacteria (Lactobacillus acidophilus) and then write L. acidophilus.
7. In your opinion, what are the new aspects of this study compared with previous studies.? A huge amount of earlier research has investigated the role of hydrogels and nanoparticles on the viability of probiotics. The prior studies have investigated very well the effect of the hydrogels on the bacteria compared with your studies.
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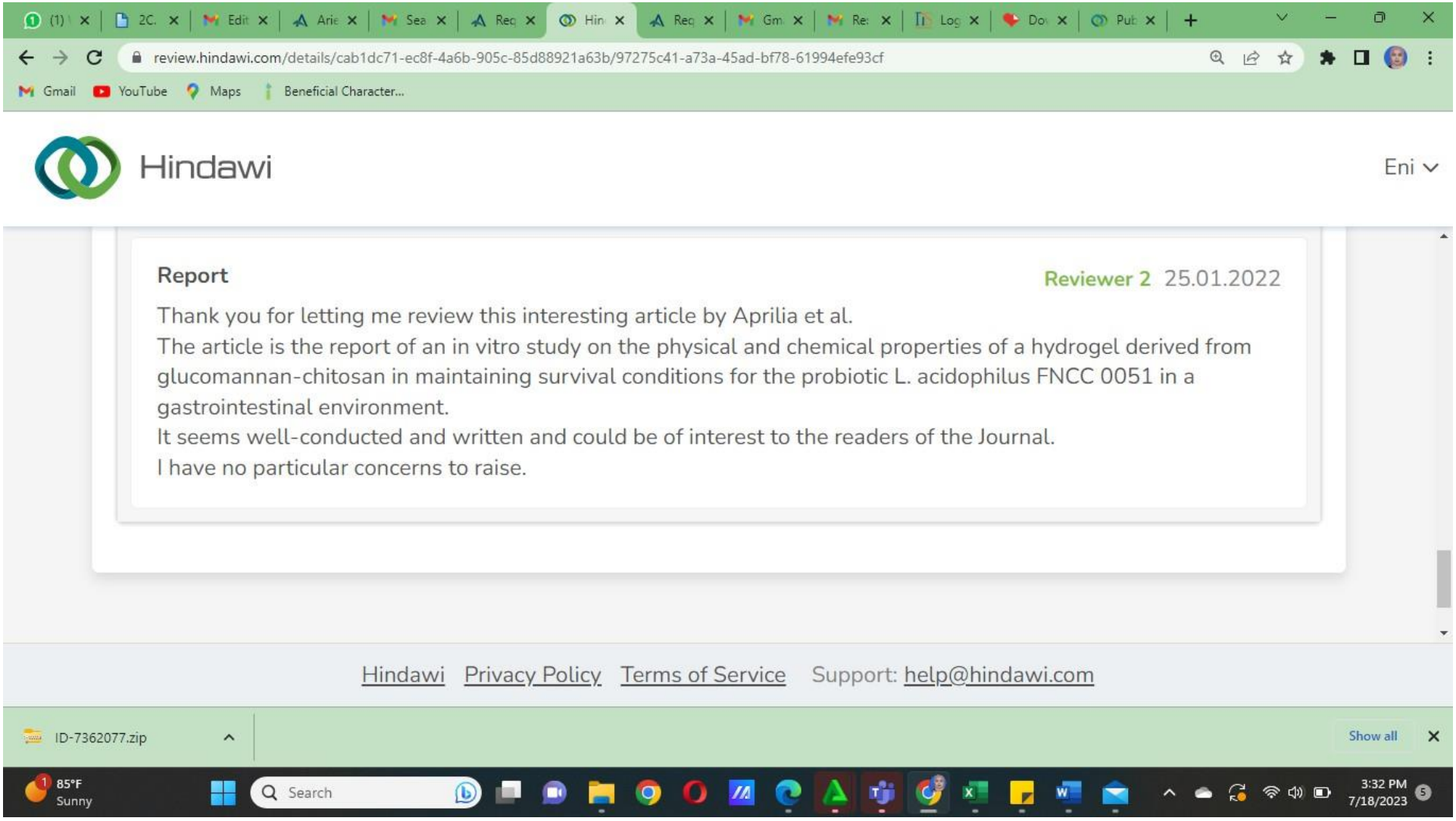
review.hindawi.com/details/cab1dc71-ec8f-4a6b-905c-85d88921a63b/97275c41-a73a-45ad-bf78-61994efe93cf
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Eni
Report
Reviewer 2 25.01.2022
Thank you for letting me review this interesting article by Aprilia et al.
The article is the report of an in vitro study on the physical and chemical properties of a hydrogel derived from glucomannan-chitosan in maintaining survival conditions for the probiotic L. acidophilus FNCC 0051 in a gastrointestinal environment.
It seems well-conducted and written and could be of interest to the readers of the Journal.
I have no particular concerns to raise.
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3:32 PM 7/18/2023

3. Pertanyaan melalui Email Terkait Perubahan Status dan Pengusulan Reviewer (20 Januari 2022)

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id>

progress paper of ID 7362077

5 pesan

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 20 Januari 2022 pukul 15.10
Kepada: polen.ilagan@hindawi.com, eniharmayani@ugm.ac.id

Dear Dr. Polen Ilagan

I am Veriani Aprilia, represents my corresponding author, Prof. Eni Harmayani for the manuscript ID 7362077 with the title " Hydrogel from glucomannan-chitosan to improve survival of L. acidophilus FNCC 0051 in simulated gastrointestinal condition".

We noticed in our account that the paper has been pending for approval. Could I know the reason for this status? thank you for your information

Regards,
Veriani Aprilia

Polen Ilagan <polen.ilagan@hindawi.com> 20 Januari 2022 pukul 15.16
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: verianiaprilia@almaata.ac.id

Dear Dr. Aprilia,

Thank you for contacting us. It means that an Editor has accepted our invitation to oversee your manuscript and he/she has currently assigning potential reviewers for your paper. Once a reviewer(s) accepts the invitation and submits review reports(s), the Editor will be able to make a decision.

We will notify you once the decision is finalized.

If I can be of any further assistance, please do let me know.

In this unprecedented time, Hindawi remains open and 'to publishing peer-reviewed academic work as normal. However, we realise that due to the current pandemic you may require more time to respond to us, or may even be unable to carry on with your normal academic activities. We are here to help and so if you are either unable to carry on or need more time, please reply to this email and we will work with you to find a solution.

Best Regards,

Polen

Polen Ilagan
Editorial Assistance

e. polen.ilagan@hindawi.com

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[Kutipan teks disembunyikan]

---

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 20 Januari 2022 pukul 15.44
Kepada: eniharmayani@ugm.ac.id

Assalamualaikum WrWb.
Ibu, nyuwun sewu, berikut ini balasan dari pihak Hindawi.
maturnuwun Ibu

Wassalamualaikum WrWb
Veriani Aprilia
[Kutipan teks disembunyikan]

---

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 25 Januari 2022 pukul 15.33
Kepada: Polen Ilagan <polen.ilagan@hindawi.com>

Dear Dr. Polen Ilagan,

Thank you for your information about the publication process.
Could we propose the reviewers who may be potential to review this paper?
If probable, these are the potential reviewer:

1. Dr. Satrijo Saloko, University of Mataram (expert in encapsulation)
2. Prof. Dr. Endang Sutriswati R., Universitas Gadjah Mada (expert food technology and microbiology)
3. Dr. Lily Arsanti L., Universitas Gadjah Mada (expert in food technology and microbiology)
4. Dr. Nani Ratnaningsih, Universitas Negeri Yogyakarta (expert in functional food)
5. Dr. Nanik Suhartatik, Universitas Slamet Riyadi (expert in functional food and microbiology)

Thank you,

Regards
Veriani Aprilia
[Kutipan teks disembunyikan]

Polen Ilagan <polen.ilagan@hindawi.com> 25 Januari 2022 pukul 17.07
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: verianiaprilia@almaata.ac.id

Dear Dr. Aprilia,

Thank you for contacting us.

Your handling Editor is still currently assigning potential reviewers for your manuscript.

Hence, authors are not allowed to give suggested reviewers as per our policy.

We will notify you once the decision is finalized.

In this unprecedented time, Hindawi remains open and 'to publishing peer-reviewed academic work as normal. However, we realise that due to the current pandemic you may require more time to respond to us, or may even be unable to carry on with your normal academic activities. We are here to help and so if you are either unable to carry on or need more time, please reply to this email and we will work with you to find a solution.

Best Regards,

Polen

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[Kutipan teks disembunyikan]

4. Reminding Email dari Jurnal mengenai Batas Pengumpulan Artikel

4.

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id>

7362077: Overdue revised manuscript

3 pesan

Polen Ilagan <polen.ilagan@hindawi.com> 3 Mei 2022 pukul 10.16
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: eniharmayani@yahoo.com
Cc: verianiaprilia@almaata.ac.id, amurdiati@ugm.ac.id, hastutipudji@yahoo.com

Dear Dr. Eni Harmayani,

This is to inform you that the revised version of your manuscript 7362077 titled "Hydrogel from glucomannan–chitosan to improve survival of L. acidophilus FNCC 0051 in simulated gastrointestinal condition," to The Scientific World Journal is overdue, as it has been over 3 months since you received your decision email.

To submit the revised manuscript please log into your review.hindawi account and upload your revised files. The revised files can only be uploaded through the account of the submitting author. When submitting your revision, please ensure to upload the revised manuscript file by replacing the file in the ‘Main Manuscript’ section. Additionally, please ensure to upload a clear and detailed “Response to Editor/Reviewer comments” document in the ‘Response to Revision Request’ section, which outlines in a point-by-point fashion how you have addressed the previous review comments.

Please note, should your manuscript be accepted, we will require editable versions of your figure files. Therefore, if you are able to upload your editable figure files at this stage it may save time at the production stages should your paper reach publication.

If you require additional time for submitting your revised manuscript, please let me know as soon as possible. Unfortunately, if we do not hear from you, or receive your revised manuscript within 2 weeks, we will be withdrawing your manuscript.

I look forward to receiving your response.

Best Regards,

Polen

Polen Ilagan
Editorial Assistance

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carry on or need more time, please reply to this email and we will work with you to find a solution.

Polen Ilagan <polen.ilagan@hindawi.com> 12 Mei 2022 pukul 05.12
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: eniharmayani@yahoo.com
Cc: verianiaprilia@almaata.ac.id, amurdiati@ugm.ac.id, hastutipudji@yahoo.com

Ticket #5508705} raised by Eni Harmayani (eniharmayani@yahoo.com).

Dear Dr. Harmayani,

Please confirm the receipt of my previous email, and provide your response at your earliest convenience.

Your assistance is appreciated.

If you require additional time for submitting your revised manuscript, please let me know as soon as possible.

Unfortunately, if we do not hear from you, or receive your revised manuscript within 1 week, we will be withdrawing your manuscript.

Best Regards,

Polen

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e. polen.ilagan@hindawi.com

[Kutipan teks disembunyikan]

5. Jawaban Permohonan Penundaan Pengumpulan Artikel

---

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 12 Mei 2022 pukul 11.49
Kepada: Polen Ilagan <polen.ilagan@hindawi.com>

Dear Dr. Polen Ilagan

Regarding the progress of our manuscript, we still doing work laboratory to give additional data to our manuscript. Therefore, we need additional time to revise our manuscript "Hydrogel from glucomannan to improve survival of L. acidophilus FNCC 0051 in simulated gastrointestinal condition".

Thank you for your understanding

Regards
Veriani Aprilia
[Kutipan teks disembunyikan]

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id>

Re: 7362077: Overdue revised manuscript- Reminder 1

3 pesan

Polen Ilagan <polen.ilagan@hindawi.com> 12 Mei 2022 pukul 12.46
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: eniharmayani@yahoo.com
Cc: verianiaprilia@almaata.ac.id, amurdiati@ugm.ac.id, hastutipudji@yahoo.com

Dear Dr. Aprilia,

Thank you for your response.

In order to grant you an extension, kindly provide us an **exact date** when will we expect your revision to be uploaded on the system.

I look forward to hearing from you.

Best Regards,

Polen

Polen Ilagan
Editorial Assistance

e. polen.ilagan@hindawi.com

> On Tue, 3 May at 4:16 AM , Polen Ilagan <polen.ilagan@hindawi.com> wrote:
> Dear Dr. Eni Harmayani,
>
> This is to inform you that the revised version of your manuscript 7362077 titled "Hydrogel from glucomannan–chitosan to improve survival of L. acidophilus FNCC 0051 in simulated gastrointestinal condition," to The Scientific World Journal is overdue, as it has been over 3 months since you received your decision email.
>
> To submit the revised manuscript please log into your review.hindawi account and upload your revised files. The revised files can only be uploaded through the account of the submitting author. When submitting your revision, please ensure to upload the revised manuscript file by replacing the file in the ‘Main Manuscript’ section. Additionally, please ensure to upload a clear and detailed “Response to Editor/Reviewer comments” document in the ‘Response to Revision Request’ section,

which outlines in a point-by-point fashion how you have addressed the previous review comments.

Please note, should your manuscript be accepted, we will require editable versions of your figure files. Therefore, if you are able to upload your editable figure files at this stage it may save time at the production stages should your paper reach publication.

If you require additional time for submitting your revised manuscript, please let me know as soon as possible. Unfortunately, if we do not hear from you, or receive your revised manuscript within 2 weeks, we will be withdrawing your manuscript.

I look forward to receiving your response.

Best Regards,

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---

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 14 Mei 2022 pukul 06.23
Kepada: Polen Ilagan <polen.ilagan@hindawi.com>

Dear Dr. Ilagan

We hope that we can submit our revised articles on August 13, 2022.
Thank you for your understanding.

Regards
Veriani Aprilia
[Kutipan teks disembunyikan]

Polen Ilagan <polen.ilagan@hindawi.com> 16 Mei 2022 pukul 11.50
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: verianiaprilia@almaata.ac.id
Cc: eniharmayani@yahoo.com, amurdiati@ugm.ac.id, hastutipudji@yahoo.com

Ticket #5508705} raised by Eni Harmayani (eniharmayani@yahoo.com).

Dear Dr. Aprilia,

Thank you for your reply.

This has been noted.

We look forward to hearing from you soon.

Best Regards,

Polen

Polen Ilagan
Editorial Assistance

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> On Thu, 12 May at 6:46 AM , Polen Ilagan <polen.ilagan@hindawi.com> wrote:
> Dear Dr. Aprilia,
>
> Thank you for your response.
>
> In order to grant you an extension, kindly provide us an **exact date** when will we expect your revision to be uploaded on the system.
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> I look forward to hearing from you.
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> Best Regards,
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> Polen
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EDITORIAL CERTIFICATE

Authors:	Document title:	Date Issued:
Veriani Aprilia, Agnes Murdiati, Pudji Hastuti, Eni Harmayani	**Hydrogel from glucomannan-chitosan to improve survival of Lactobacillus acidophilus FNCC 0051 in simulated gastrointestinal fluid**	**12 Aug 2022**

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Order No. 166-94-63
11 August 2022

Editor's Report

Thank you for the opportunity to edit your manuscript. It was a pleasure to review your work, which was engaging and persuasively argued. I have focused on correcting the grammar and improving the flow and tone of the work throughout and have applied US English conventions, as requested.

As specified in the order notes, I have also focused on the alignment of the document to the new submission criteria for research articles in the guide to authors for The Scientific World Journal. Please carefully read through my edits and in-text comments—which provide further detail and suggestions for improvements—before accepting or rejecting any changes. In addition, please review the table below for an assessment of your manuscript and an overview of key points that have been addressed.

I noticed that some of the text was highlighted in yellow, and I have not changed it as I was unsure whether this was deliberate. Please check to ensure the highlighting of the text reflects your intent throughout.

Please note that continuous line numbering has been applied in line with your target journal's guidelines.

Please also note that the Harvard reference style requires references with more than two authors to be reported in the text as "(Author1 et al., Year)." For example, "(Harmayani et al., 2014)". I have not made these changes anywhere in the document since you are using a reference manager to manage your citations. Please update the reference format before submission.

Finally, I noticed that you requested an editing certificate in the order notes. Please note that you can generate a certificate for each of your completed orders on the Cambridge Proofreading website. Logging into the client area on the website allows you to view a list of your completed orders. Selecting the order in question will then give you the option to

generate an editing certificate. Should you have any trouble, please reach out to George (at info@cambridgeproofreading.com), who will assist you.

I wish you the best of luck with your submission, and I look forward to working with you again soon.

Sincerely,

T. Pemberton, PhD

Summary	Section
Abstract	I have edited the abstract for clarity, conciseness, consistency, flow, and tone. After editing, the abstract is 132 words, below the journal's 300-word maximum. Overall, I found it to adequately summarize the findings of your study in an engaging and compelling manner.
Introduction	I have edited the introduction for clarity, conciseness, consistency, flow, and tone. Overall, I found it to provide adequate background to understand your study and place it within the wider context of the field.
Materials and Methods	I have edited the methods for clarity, conciseness, consistency, flow, and tone. Overall, I found it to explain the methods used in your study in sufficient detail to be understandable and repeatable by the reader in their own study.
Results and Discussion	I have edited the results and discussion for clarity, conciseness, consistency, flow, and tone. Overall, I found them to clearly present the findings of your study and place them in the wider context of the field in an engaging and compelling manner.
Conclusions	I have edited the conclusions for clarity, conciseness, consistency, flow, and tone. Overall, I found them to adequately summarize your findings and identify how they will benefit future studies.
References	Since your target journal does not have a specific referencing style, the references were edited for internal consistency only. I noticed that the reference list was created using reference management software. Consequently, these edits will be lost if you refresh your reference list using your reference manager. I recommend that you incorporate these edits into the relevant records in your reference database to ensure they are preserved.

Hydrogel from glucomannan–chitosan to improve survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid

Abstract

The probiotic encapsulating hydrogel made from the interaction between porang (*Amorphophallus oncophyllus*) glucomannan and chitosan washas been investigated for its encapsulation efficiency, physical properties, prebiotic activity, and survival under simulated gastrointestinal conditions. The eEncapsulation efficiency was improved by varying the concentration of *Lactobacillus acidophilus* FNCC 0051, whichthat has also increasedaffected in the raise of diameter (2–3 mm), polydispersity index (1.23–1.65), positively zeta potential, the whiteness, and brightness of the hydrogel. The hydrogel's prebiotic activity score of hydrogels was found higher than that of inulin after 24 h of incubation, reflecting . It attributed to its role as a cell encapsulant of cells, particularlyespecially in maintaining cellsthe cells during exposure to simulated gastrointestinal fluid. CellThe viability mainly increasedraised from 86% to 100% when immersed init was applied to intestinal juice, and showed the comparable result towith alginate and konjac glucomannan hydrogel. Future animal studies are neededmay be carried out to animal experiments to determine cellthe viability in actual gastrointestinal conditions andor the health effects of the hydrogel.

Key words: hydrogel; viability; glucomannan; chitosan; gastrointestinal.

Introduction

Glucomannan is a functional polysaccharide that can be extracted from *Amorphophallus* tubers. While In addition to the popular and commercially used of glucomannan from *Amorphophallus konjac* has popular and commercial uses, several studies are currently investigating glucomannan being conducted of this polymer from other variety sources. In Indonesia, *Amorphophallus oncophyllus* is a local source of glucomannan source in Indonesia, that is commonly known asusually called porang (Harmayani, Aprilia, and Marsono, 2014) (Yanuriati *et al.*, 2017). It has specific characteristics that differ from konjac, including mannose/glucose molar ratio, degree of polymerization, and degree of acetylation, leading to different solubility, viscosity, water holding capacity, and gelation properties (Harmayani, Aprilia, and Marsono, 2014; Yanuriati *et al.*, 2017). Thereforeus, the its applications may also differdifferently depending on the function.

Hydrogels areis one of the technologically glucomannan products that leveragestake the advantage of gelation properties. TheyIt canmay be formed throughby the interactions between glucomannan and other polymers to form a three-dimensional polymeric network (Li, 2011). This characteristic has a potential to be used as an encapsulant. A previous study used relating to this was hydrogel created by from the crosslinking of konjac glucomannan and chitosan, which hasve many advantages, which includinge being naturally formed without a crosslinker, self-assembly formation, tolerance to and responsible in different pH, and its demonstrated abilities in had been proven for the encapsulatingon of drugs, proteins, and enzymes (Du *et al.*, 2006; Korkiatithaweechai *et al.*, 2011). A similar modified study onf hydrogel formed byation from the interaction ofbetween porang glucomannan and chitosan has successfully considered been conducted which began from the production of the primary basic material of carboxymethyl glucomannan material, the compatibility of substitution degree of carboxymethyl glucomannan in hydrogel formation, the effect of polymer concentration on the glucomannan properties, and to its application in probiotic encapsulation of probiotics (Aprilia *et al.*, 2017a, 2017b, 2021). Its key innovation was the use of The invention was emphasized in the use of porang, which has different characteristics from the other source of glucomannan sourcesthat had different characteristics, such as solubility, viscosity, water holding capacity, degree of polymerization, degree of acetylation, purity, and also X-ray diffraction (XRD) pattern (Harmayani, Aprilia and Marsono, 2014; Yanuriati *et al.*, 2017). The other Other differences include were the type of modification that used (carboxymethylation) and its use applicated as a probiotic the encapsulant of probiotics,. In contrast, while the previous study used oxidation (Korkiatithaweechai *et al.*, 2011) and used as encapsulatedant of drugs, proteins, and enzymes (Du *et al.*, 2006; Korkiatithaweechai *et al.*, 2011).

Commented [TP1]: Please remember to include full author names and their complete institutional mailing addresses and email addresses before submission, as requested by the journal.

Commented [TP2]: After editing, the abstract is 132 words, below the journal's 300-word maximum.

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Commented [TP3]: The Harvard reference style requires references with more than two authors to be reported in the text as "(Author1 et al., Year)." For example, this in-text citation should be "Harmayani et al., 2014". I have not made this change anywhere in the document since you are using a reference manager to manage your citations. Please update the reference format before submission.

Commented [TP4]: Please combine these in-text citations using your reference manager. For example, "(Harmayani et al., 2014; Yanuriati et al., 2017)." I have not made this change since you are using a reference manager to manage your citations. Please make sure to do this before submission.

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The role of this new hydrogel to encapsulate probiotics needs to be further studied since the living cells has theis new hydrogel's role in encapsulating probiotics needs to be further studied since the living cells have different characteristics towith other inanimate compoundsobjects. The new capsulesencapsulate should ensure the survival of probiotics during food processing, and storageing, and fulfilling the sufficient delivery quantities ($>10^6$–10^7 colony forming units [CFU]/mL) when consumed. Furthermore, it also needs to reach the achieve lower gastrointestinal tracts in order to have a beneficial effect onfor humans., therefore Therefore, its survival during gastrointestinal digestion and its also the capability to increase probiotictthe growth of probiotics in the colon is important. As we know before, the carbohydrates Carbohydrates known to that is able to stimulate probiotic the growth are called of probiotics can be defined as prebiotics. We previously Our previous study has been conducted for the optimatizedon of probiotic encapsulation efficiency by varying the glucomannan concentration of glucomannan and also studied for its role in protectingon of cells during pasteurization and cold storage (Aprilia *et al.*, 2021). However, itIts role in protecting of probiotic cells during digestion and its potentialpossibility as a prebiotic remain unexploredhas not been studied yet.

This study aimed to improve the probiotic encapsulation efficiency and properties of the e studied of hydrogel formed by from glucomannan and chitosan still wished to be improved. In this recent study, the probiotic encapsulation efficiency of hydrogel by varying the cell concentration of cells to increase the achieve more number of probiotic carried, and examines the its effects on its the physical properties of hydrogel, the prebiotic activity score, and also analyzed is viability during simulated gastrointestinal exposure.

Materials and Mmethods

Materials

The primarymain material used inof this study was glucomannan from porang tuber (*Amorphophallus A. oncophyllus*), which was obtained from the Faculty of Agricultural Technology, Universitas Gadjah Mada (Yogyakarta, Indonesia). Carboxymethylation of was applied to the glucomannan used by using sodium chloroacetate as previously described (Aprilia *et al.*, 2017b). The chitosan with that has a degree of 85%–89% deacetylation and that meets fulfills the food quality criteriaqualifications was obtained purchased from PT Biotech Surindo (, Cirebon, West- Java, Indonesia).

Preparation of *Lactobacillus acidophilus* FNCC 0051 cells

Lactobacillus L. acidophilus FNCC 0051 cells werewas obtained taken from the stock culture collection of Food and Nutrition Culture Collection (FNCC), Laboratory of Food Microbiology, Center for Food and Nutrition Studies, Universitas Gadjah Mada. Cells in skim milk–glycerol suspension stocks were rejuvenated in de Man, Rogosa, and Sharpe (MRS) broth at 37°C overnight and grown twice successively. The cell biomass was then harvested by centrifugation at 2400 g for 9 min at 4°C (Okuro *et al.*, 2013) and rinsed with saline solution.

Hydrogel Encapsulation encapsulation of probiotics in hydrogel and determination of its encapsulation efficiency

The hydrogel was createdgenerated from by the interaction ofbetween porang glucomannan and with 0.5% chitosan using with concentration of 0.5% by the complex coacervation method. The hHydrogels were prepared withby three different variations of cells concentrations of , those were 8, 9, and 10 log CFU/mL. The cells were mixedblended to with glucomannan before the coacervation process (Aprilia *et al.*, 2021). The encapsulation efficiency was determined by dividing the number of viable cells entrapped in the hydrogel (after post-encapsulation) bywith the number of cells blended into the pre- encapsulation solution (before encapsulation) (Zeashan *et al.*, 2020). The cells entrapped in the hydrogel were released by submersing it the hydrogel in a buffer solution of at pH 8 for 24 h andat 37°C for 24 h (Aprilia *et al.*, 2017b).

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Hydrogel ~~Properties~~ properties~~of hydrogel~~

Particle size, polydispersity index, zeta potential

~~The size of particles~~ Particle size was estimated based on~~as~~ the hydrogel's diameter ~~of hydrogel~~ and measured simultaneously with the polydispersity index using a particle size analyzer (Horiba SZ-100 series~~,~~ ; Kyoto, Japan). The hydrogel's zeta potential ~~of hydrogel~~ was measured ~~by~~ with a Nano ZS Zetasizer (~~Nano ZS Ver~~ v.6.20~~,~~ ; Malvern Instruments Ltd~~,~~ ; Malvern, UK).

Color

The hydrogel was freeze-dried and groundgrinded before the color measurement ~~of the color~~. ~~The value~~ Values ~~of~~ redness (a*), yellowness (b*), and light~~n~~ness (L*) were determined with~~by~~ a CR200 chromameter ~~CR200~~ (Minolta~~,~~ ; Osaka, Japan). The whiteness index was ~~also~~ calculated as previously described ~~study~~ (Akgün, Ova Özcan, and Övez, 2022).

~~*X-ray diffraction (XRD)*~~

The XRD ~~X-ray pattern~~ of hydrogels was~~ere~~ determined with a~~measured by~~ Shimadzu Lab X XRD-6000 ~~Shimadzu~~ (Kyoto, Japan) equipped with a Cu Kα target at 40 kV and~~,~~ 30 mA with a scanning rate of 4°/min. The pattern was collected in the 2θ range between 3.02 ~~to~~ and 90°. Crystallinity percentage (%) was calculated by dividing the area under the peaks by the ~~with~~ total curve area (Wang *et al.*, 2015).

Prebiotic activity scores

The prebiotic activity score was calculated~~done based on previous study~~ by subtracting the ~~value of~~ ratio ~~increase~~ of probiotic cells growth ~~in~~ with an assessed prebiotics and glucose from ~~with~~ the ~~value of~~ ratio ~~increase~~ of enteric cells growth~~-~~ ~~in~~ with an assessed prebiotics and glucose as previously described (Huebner, Wehling, and Hutkins, 2007). The probiotic used was *L. acidophilus* FNCC 0051, while the enteric cells used were *Escherichia coli* FNCC 0091 ~~was used as enteric cells~~. The test was performed~~done~~ by adding 1% (vol/vol) of probiotic cells into MRS broth containing 2% (w~~t~~/v~~ol~~) glucose or prebiotic and 1% (v/v) of enteric cells into M9 broth containing 2% (w~~t~~/v~~ol~~) glucose or prebiotic. The cells were incubated at 37°C for 0, 24, and 48 h with and enumerated by the plate count method using MRS ~~agar~~ and nutrient agar. Each test was replicated three times.

~~Survival of~~ *L. acidophilus* FNCC 0051 survival during exposure to~~of~~ simulated gastrointestinal conditions ~~in vitro~~

Simulated ~~Approximately 7 mL of pepsin in hydrochloric acid, 2 g of sodium chloride, and 1 M of sodium hydroxide were used to formulate gastric juice, while 1% pancreatic powder, 6.8 g of potassium dihydrogen phosphate, and 77 mL of sodium hydroxide~~gastric and intestinal juices ~~0.2 N~~ were prepared ~~for intestinal juice~~ as described by Xu et al. (2016). Gastric juice was prepared by mixing 7 mL of pepsin in hydrochloric acid, 2 g of sodium chloride, and 1 M of sodium hydroxide. Intestinal juice was prepared by mixing 1% pancreatic powder, 6.8 g of potassium dihydrogen phosphate, and 77 mL of 0.2 N sodium hydroxide. Either 1 g of free or encapsulated cells (in the hydrogel of porang glucomannan~~-~~–chitosan, konjac glucomannan~~-~~–chitosan, and calcium alginate) was mixed with 9 mL of simulated gastrointestinal juices and incubated ~~for 120 min~~ at 37°C for 120 min. The samples were withdrawn at ~~at the~~ intervals of 0, 30, 60, and 120 min for gastric juice digestion and 0, 60, 90, and 120 min for intestinal juice digestion (Rather *et al.*, 2017). The hydrogel was then rinsed twice with acetate buffer. The cells were ~~then~~ enumerated using the pour plate technique with MRS agar after 48 h of incubation. The number of viable cells after exposure was divided by the initial number of cells to determine their survival rate ~~of the cell~~ during exposure to simulated gastrointestinal conditions (Zeashan *et al.*, 2020). The hydrogel's

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appearances of hydrogel during exposure to simulated gastrointestinal conditions wasere observed withby an optical BX51 microscope (Olympus BX51, Olympus Corp., .; Tokyo, Japan) and assembled with OptiLab pro digital camera (Miconos, Indonesia).

Results and dDiscussion

Encapsulation efficiency of hydrogel in different concentrations of cells

As presented in Table 1, the The concentration of encapsulated cells was lower than that of the initial cell concentration (**Table 1**), , indicating It indicated that not all of the cells werecould be encapsulated in the hydrogel and , It affectinged on the calculated encapsulation efficiency. Indeed, The trend was that the higher concentration of initial cells added, the higher the encapsulation efficiency. For this study, the The highest encapsulated cell concentration of 7.94 log CFU/g was obtainedachieved with when log 10 CFU/mL of cells was added, that was 7.94 log CFU/g. This number exceeds had met the Food and Agricultural Organization of the United Nations (FAO) criteria for probiotic products from FAO that was minimum of >6–7 log CFU/mL (Priya, Vijayalakshmi, and Raichur, 2011).

Previous studies using that used the different encapsulants obtainedyielded different encapsulation efficienciesy. For As the example, was the encapsulation of *L. acidophilus* in the hydrogel formedgenerated from sodium alginate and soy protein isolate could achieved 95–98% of encapsulation efficiency, while the encapsulation of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* in emulsion could achieved 97–99% efficiency (Zeashan *et al.*, 2020; Mahmoodi Pour, Marhamatizadeh, and Fattahi, 2022). The difference Differences in value of encapsulation efficiency might reflect may be influenced by the type of encapsulant type and the encapsulation the method used used for encapsulation (Zeashan *et al.*, 2020). We Our previously showed study also proved that the same ratio of glucomannan and chitosan affected encapsulation efficiency due to since it was needed for the chemical bonding of both polymers and the difference in electrostatic values between the core and polymer also influencinged cell the entrapment of cells (Aprilia *et al.*, 2021).

Table 1. The concentration of encapsulated cell concentration and hydrogel encapsulation efficiency of hydrogel in with different initial cell concentrations.

Hydrogels within different cell concentrations of cells (log CFU/mL)	Cell Cconcentration of cells before encapsulation (log CFU/mL)	Cell Concentration concentration of cells after encapsulation (log CFU/g)	Encapsulation efficiency (%)
8	9.39 ± 0.00	4.47 ± 0.18	44.37 ± 1.91*a
9	9.56 ± 0.00	6.60 ± 0.13	65.83 ± 1.37*b
10	10.10 ± 0.00	7.94 ± 0.21	85.03 ± 0.63*c

Values represent mean ± standard deviation (SD). Key: *, *Different superscript letters in the same column indicate significant different results at* $p < 0.05$.

Properties of hydrogel in different concentrations of cells

The size of hydrogels encapsulatinged *L. acidophilus* was measureddetected by the instrument in the range of of 0.7–9 µm, with and mostly having a distributed in the diameter of 2–3 µm (**Table 2**). The size of hydrogel Hydrogels that was mostly <100 µm in diameter are was classified the particle as microgels. The concentration of cells significantly influenced the hydrogel particle size of hydrogel ($p < 0.05$). The more cells encapsulated in the hydrogel, the greater its more diameter of hydrogels that were measured. It was also correlatedaligned with the value of encapsulation efficiency in (**Table 1**) since more as the prediction of the greater number of cores can be that could be entrapped in larger hydrogel particles. The other factors that influencinge the particle size were the concentration and viscosity of the solution (Zeashan *et al.*, 2020; Aprilia *et al.*, 2021)

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Table 2. Hydrogel particle size, polydispersity index, and zeta potential with different initial cell concentrations.

Initial cell concentration (log CFU/mL)	Particle size (µm)	Polydispersity index	Zeta potential (mV)
8	2.23 ± 0.11*[a]	1.23 ± 0.17*[a]	24.40 ± 0.75*[a]
9	2.79 ± 0.19*[b]	1.39 ± 0.04*[ab]	32.28 ± 0.80*[b]
10	3.41 ± 0.14*[c]	1.65 ± 0.27*[b]	14.58 ± 0.97*[c]

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

The polydispersity indexes of hydrogel encapsulated cells were above >1 (**Table 2**), indicating a broadwide particle distribution or several particles of various sizes. The indexThese values began to change when the initial cell concentration added was 10 log CFU/mL. The greater themore initial cell concentration added, the higher the polydispersity index of hydrogels. This result contrasts with A a previous study reported that found that the concentration of glucomannan concentration did did not influence the polydispersity index of hydrogel (Aprilia *et al.*, 2021).

Hydrogel Zeta zeta potentials of the hydrogel became more electropositive as the cell concentration increased from 8 to 9 log CFU/mL but decreased withat 10 log CFU/mL (**Table 2**). An increase in cell the number of cells should cause result in a reduction inof hydrogel charge due to the . This was influenced by the reverse charges of hydrogel and cells, which were positive charge for of empty hydrogel and the negative charge of cells (Aprilia *et al.*, 2021), including and negative for *L. acidophilus* (Priya, Vijayalakshmi and Raichur, 2011). The observed pattern mightis difference result may be due to the measurement of zeta potential being measured on the hydrogel's that was detected only from the surface, which can be of hydrogel and affected by the surrounding environment (Raei et al., 2015).

Table 3. Hydrogel Color color value withof hydrogel in different initial cell concentrations. of cells

Initial cell concentration (log CFU/mL)	L*	a*	b*	Whiteness
control	65.06 ± 0.12*[a]	7.02 ± 0.09*[a]	12.50 ± 0.08*[a]	62.24 ± 0.15*[a]
8	76.97 ± 0.32*[b]	5.42 ± 0.01*[b]	14.24 ± 0.11*[b]	72.38 ± 0.21*[b]
9	79.48 ± 0.33*[c]	5.61 ± 0.07*[b]	15.14 ± 0.01*[c]	73.89 ± 0.25*[c]
10	77.39 ± 0.23*[b]	4.22 ± 0.23*[c]	13.24 ± 0.13*[d]	73.46 ± 0.30[c]*

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

The lighteness (L**) and whiteness values of the hydrogel increased after addingwith the addition of cells, while the a* redness value, reflected by a* decreased, and the . The inconsistent value was shown by b* value varied inconsistently value as the yellowness indicator (**Table 3**). The instrument determines these values works based on the reflection bounce of cells after of a direct light beam of light from a chromameter by the cells. Therefore, the more cells encapsulated in the hydrogel, the greater the reflection more bounce that happened (Theodore, 2005), with the The other study showed that they would be the chromatic change differing amongcolor of food- containing cells (Vaikousi, Biliaderis, and Koutsoumanis, 2008).

XRDX-ray diffraction spectraums represents the interaction between diffraction the intensity of diffraction and angle (**Figure 1**). The A crystalline state was indicated by the sharp diffraction peak, while the amorphous and solid state was indicated by described from the declivous peak (Yanuriati *et al.*, 2017). The pattern of X-ray diffractogram patterns of all hydrogels showed a very broad band in Figure 1 at 2θ were between 5–90°. In addition, It illustrates a very broad band. Aall hydrogelsof samples hadalso showed almost the same highest high peaks with the strongest peak at around 2θ 7.06–10.46, 7.62–11.00, 7.48–10.94, and 7.16–11.20° for hydrogels without the cells, and with the cells in at concentrations of

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log 8, 9, 10 CFU/mL, respectively. These differ from were different compared to porang glucomannan, which that had its highest peaks at around 19–20° and 35° (Yanuriati *et al.*, 2017). However, there were found a small peak in all samples at around 2θ 10.5° which indicated as a small peak in all samples at around 2θ 10.5°, indicating the existence of chitosan (Yu, Lu, and Xiao, 2007). This observation suggests that the mixture between glucomannan, hydrogel, and the cells strengthened made their stronger chemical interaction, consistent with which also confirmed from previous FTIR (Fourier-transform infrared spectroscopy (FTIR) findingsstudy (Aprilia *et al.*, 2021), and that there were still some chitosan had that did not interacted with glucomannan. The A previous study reported that the Schiff's crosslinking between glucomannan aldehyde groups of glucomannan and chitosan amino groups of chitosan could suppress chitosan's the crystallineity state, of chitosan that usually strengthened by a hydrogen bond between amino groups and hydroxyl groups (Yu, Lu, and Xiao, 2007). We also find The low of crystallinity, with values of degree also indicated in this study. Those were 26%, 25%, 17%, and 21%, respectively for hydrogels without cells and with cells in at concentrations of 8, 9, and 10 log CFU/mL, respectively. The addition of *L. acidophilus*, appeared to have seemed had no effect on the diffraction peak, indicating which means that the entrapment of microbes in hydrogel did not affect the interaction between glucomannan and chitosan.

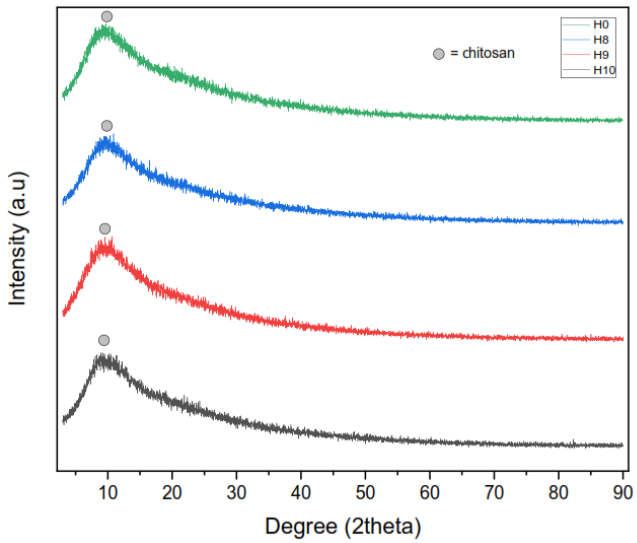

Figure 1. X-ray diffractogram for H0 (hydrogel without *L. acidophilus*);), H8, H9, and H10 (hydrogels with *L. acidophilus*, in at concentrations of 8, 9, and 10 log CFU/mL).

Hydrogen Prebiotic prebiotic activity of hydrogel

Table 4 shows the increase of *L. acidophilus* and *E. coli*, cell density increased during 0, 24, and 48 hours of incubation in the presence with addition of carbohydrates, such as glucose, inulin, and hydrogel (**Table 4**). Both bacteriaof cells showed no did not show the significant increase in with almost all carbohydrates, except *L. acidophilus* within inulin and *E. coli* in with glucose. These data suggest From this data it can be known that only inulin canthat could specifically stimulate the growth of good bacteria and

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suppressed the growth of enteric bacteriacells., consistent with its well-known use as aAs we know, inulin is the famous commercially prebiotic that had been widely used in the worldwide.

Table 4. The Cell density density of *Lactobacillus L. acidophillus* FNCC 0051 cells in log10 (CFU/mL) aftert 0, 24, and 48 hours of incubation, with prebiotics reported as log10 (CFU/mL) in inulin, hydrogel, and glucose.

Prebiotic	*L. acidophilus*			*E. coli*		
	h 0 h	h 24 h	h 48 h	h 0 h	h 24 h	h 48 h
Glucose	6.94 ± 1.32^{a*}	8.35 ± 0.81^{*a}	9.17 ± 0.01^{*a}	6.65 ± 0.92^{*a}	8.54 ± 0.09^{*ab}	9.29 ± 0.49^{*b}
Inulin	6.59 ± 0.19^{a*}	7.33 ± 0.49^{*ab}	8.48 ± 0.88^{*b}	9.53 ± 0.09^{*a}	7.59 ± 0.32^{*a}	8.47 ± 0.75^{a*}
Hydrogel	9.37 ± 0.10^{a*}	9.58 ± 0.46^{a*}	10.15 ± 0.21^{*a}	8.80 ± 1.13^{*a}	8.17 ± 0.86^{a*}	9.02 ± 2.18^{a*}

Values represent mean ± SD. Key: *, *Different superscript letters in the same row indicate significant different results at* $p < 0.05$.

The prebiotic activity scores result in Figure 2 was used in this study to know the The prebiotic potentialpotency of the hydrogel was compared as prebiotic by comparing with inulin using prebiotic activity scores (**Figure 2**). The prebiotic activity score of Hydrogel hydrogel was showed higher prebiotic activity score than inulin afterin 24 h of incubation, but become became lower than inulin after 48 h, of incubation. It suggestings that hydrogel was a preferred energy source easier to be available as food for cells. This result is consistent with the It relates to XRD findings study that confirmed the amorphous hydrogel state of hydrogel., which This state has no long-range order, that makinge it easier possible to digest, easily and the amount of carbohydrates will decrease within the longer time. Meanwhile, known prebiotic inulin that has been proved to have prebiotic activity (Kamel *et al.*, 2021) needed a longer time to be available for bacteria since it has long polymeric carbon chains (n = 2–60)) with ((2→1) linked β-d-fructosyl residues) (Mensink *et al.*, 2015).

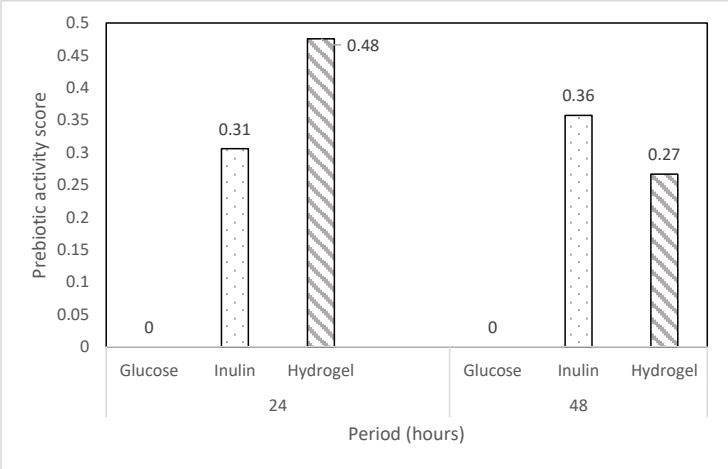

Figure 2. Prebiotic activity scores of *Lactobacillus L. acidophilus* FNCC 0051 on glucose, inulin, and hydrogel.

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~~Survival of cells~~ Cell survival during exposure to simulated gastrointestinal conditions ~~in vitro~~

~~Survival of cells~~ Cell survival during exposure to gastric juice

L. acidophilus showed~~had~~ good viability during exposure to gastric juice ~~of~~ at pH 2, either in ~~the~~ its free form or when encapsulated in ~~the~~ hydrogel (**Figure 3**). ~~Generally, the~~ The growth of lactic acid bacteria is generally optimum at pH 6–7 (close~~d~~ to neutral pH). Some metabolic reactions change~~s~~ when pH is ~~below~~ ≤5 or ≤4.4. Indeed, some minerals will be lost at pH ≤2 ~~or below~~, and~~so~~ prolonged ~~that~~ storage at low pH ~~for a long time~~ will increase the risk of cell death (Hayek dan Ibrahim, 2013). Our results are consistent with a~~A~~ previous study ~~reported the same result with this study (Zeashan *et al.*, 2020), (Zeashan *et al.*, 2020). However, an~~~~but there was also~~ other study showed~~proved~~ that ~~several deaths of~~ *Lactobacillus* death occurred ~~for~~ during 4 h of~~during~~ gastric exposure (Tokatl et al., 2015). This study only considered ~~represented~~ the actual conditions in the human gastrointestinal tract for liquid food, which ~~that~~ has a transit time~~period~~ of 1.5–2.5 h in the stomach~~;~~. ~~however~~However, further studies are needed~~y is warranted~~ to determine the effect on~~for~~ solid or solid enriched macronutrient foods with a longer transit time~~period~~ (Müller, Canfora, and Blaak, 2018). In addition, ~~to the~~a shorter exposure time ~~of exposure~~ in the stomach enables ~~, the ability of~~ cells to~~in~~ maintain~~ing~~ homeostasis between internal ~~pH~~ and external pH, potentially ~~may~~ influenc~~inge~~ the~~is~~ good viability shown~~result~~ in this study.

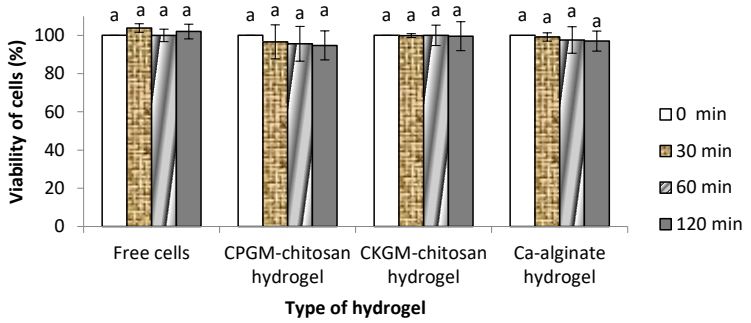

Figure 3. ~~Viability of~~ *L. acidophi~~l~~lus* FNCC 0051 viability during exposure to gastric juice for 120 min. Key: a, ~~Different letters in the same type of hydrogel indicates significantly different results at~~ $p < 0.05$~~.~~ ~~;~~ CPGM, ~~(~~carboxymethyl porang glucomannan~~),~~; CKGM, ~~(~~carboxymethyl konjac glucomannan~~)~~.

This study also found that porang glucomannan~~-~~–chitosan hydrogel might have a similar cell protecting capability from the gastric environment as ~~in protecting the cells with~~ konjac glucomannan–chitosan hydrogel and calcium alginate hydrogel ~~from the gastric environment~~ ($p > 0.05$). This finding accords~~study was in accordance~~ with the ability of locus bean gum~~-~~–carrageenan coated with milk to~~in~~ protect~~ing~~ L*actobacillus*~~.~~ *bulgaricus* during exposure to gastric juice (Shi et al., 2013). Similarly, ~~Alginate~~ alginate also protected *L. acidophilus* ~~from this harsh environment for 3 h of exposure~~ (Chandramouli et al., 2004)~~,~~ and ~~as well as~~ *L. plantarum* (Rather et al., 2017) from this harsh environment for 3 h of exposure.

~~As shown in Figure 4, t~~The hydrogel was stable~~well kept~~ in simulated gastric juice for 120 min of exposure (**Figure 4**)~~.~~ consistent ~~Associated~~ with a previous ~~the~~ swelling ratio study ~~in the previous report~~ (Aprilia *et al.*, 2021)~~,~~ that found the hydrogel did not ~~ran to~~ de-swell at the pH ~~under~~ ≤5. De-swelling cause~~sd~~ the hydrogel to become smaller, which was previously thought ~~formerly presumed~~ to ~~lead to the~~ release ~~of~~ cells from the hydrogel. However, ~~Figure 4 proved that the~~ cells are ~~were~~ still entrapped in the hydrogel (**Figure 4**~~.~~), perhaps reflecting ~~This may be influenced by~~ the stronger electrostatic interaction between the glucomannan carbonyl group ~~of glucomannan~~ and the chitosan amine group ~~of chitosan when it was~~ in an

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acid environment (Aprilia *et al.*, 2021). ~~The cells~~ Cells remain in the hydrogel because~~as~~ this interaction maintains the core ~~maintained this interaction;~~. Therefore~~thus~~, ~~the~~ de-swelling could not be maximized, leading to only a small number of ~~few released~~ cells being released from the hydrogel. There is a possibility that some empty hydrogels will shrink to the extent that they ~~optimally, so that some small hydrogels~~ are~~were~~ no longer visible at 60 min of exposure. These results ~~were~~ are consistent ~~in line~~ with other studies using~~that used~~ hydrogels made from oxidized glucomannan and chitosan ~~in~~ to entrap~~ping~~ ~~of~~ diclofenac drugs that found ~~. During exposure to simulated gastric fluid at pH 1.2, not more than~~ ≤1% ~~of the drug~~ was released ~~from the matrix~~ during exposure to simulated gastric fluid at pH 1.2 (Korkiatithaweechai et al., 2011). This result shows ~~proved~~ that hydrogel ~~the~~ cores ~~in the hydrogel~~ were not released when it ~~the hydrogel~~ was exposed to low pH conditions.

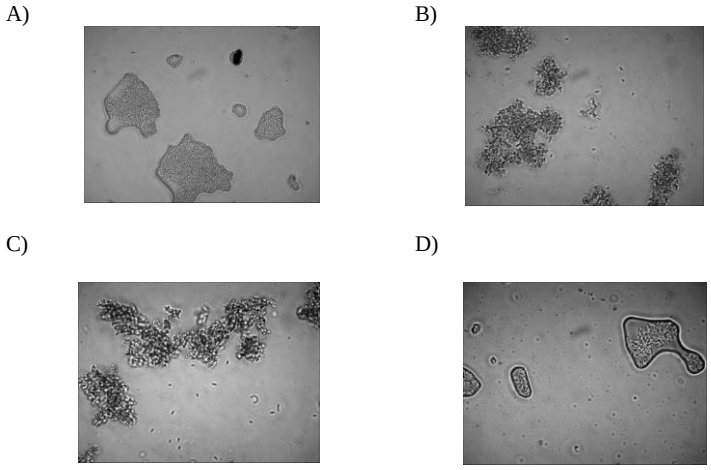

Figure 4. Microscopic appearance of hydrogel~~s~~ containing *L. acidophilus* FNCC 0051 (~~magnification of~~ 1~~.~~300~~×~~ magnification~~×~~) during exposure to gastric juice for (**A**) 0 ~~min~~, (**B**) 30 ~~min~~, (**C**) 60 ~~min~~, and (**D**) 120 min.

~~Survival of cells~~ Cell survival during exposure to intestinal juice

~~As shown in Figure 5, t~~The viability of free cells decreased significantly during exposure to intestinal juice for 60 min (**Figure 5**; $p < 0.05$)~~, which was observed at the 60th min of exposure~~. Indeed~~O~~therwise, the viability of cells encapsulated in hydrogel could be maintained over~~during~~ 120 min of exposure, indicating that encapsulation ~~had a role in~~ increase~~sing~~ ~~the viability of~~ *L. acidophilus* viability. ~~The~~ A decrease in the number of free cells may reflect ~~be caused by~~ cell death, which can be caused by factors other than ~~was not only due to~~ the pH of the medium. Priya et al. (2011) reported that while ~~at pH 6.8,~~ bacteria showed~~experienced~~ good growth at pH 6.8, ~~but~~ the presence of ~~the~~ pancreatin, comprising ~~consisting of~~ amylase, trypsin, lipase, ribonuclease, and protease, damaged the encapsulation wall, causing ~~resulting in~~ cell death.

Figure 5 indicates ~~also described~~ that porang glucomannan hydrogel has~~d~~ the same good protective effect as ~~the hydrogel of~~ konjac~~-~~chitosan glucomannan and calcium alginate hydrogels. In this study, the alginate-based hydrogel was used for ~~as a~~ comparison since~~because~~ it is widely used as an encapsulant due to ~~in many studies for~~ its low~~cheap~~ price, good biocompatibility, and nontoxicity (Sathyabama *et al.*,

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2014). A previous study showed that ~~Probiotic~~ probiotic encapsulation using alginate ~~in previous studies showed an~~ increase~~d~~ entrapped cell ~~in~~ viability compared to free cells during exposure to intestinal juice (Trabelsi *et al.*, 2013). Therefore, the ~~hydrogel of~~ porang-chitosan glucomannan hydrogel has ~~the~~ potential ~~to be developed~~ as a bacterial encapsula~~n~~tion.

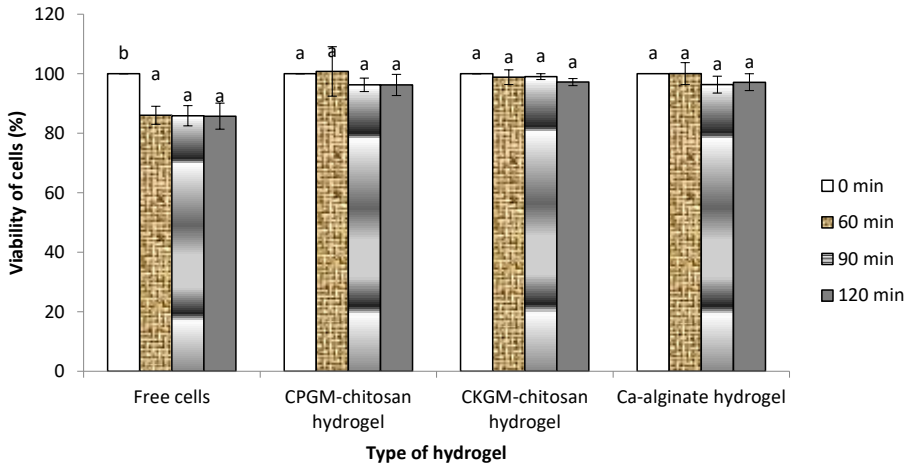

Figure 5. ~~Viability of~~ *L. acidophi~~l~~lus* FNCC 0051 cell viability during exposure to intestinal juice for 120 min. Key: a or b, ~~*Different letters in the same type of hydrogel indicates significantly different results at* $p < 0.05$.~~ ; CPGM, ~~(~~carboxymethyl porang glucomannan~~).~~; CKGM, ~~(~~carboxymethyl konjac glucomannan~~)~~.

The hydrogel's microscopic appearance was used to confirm~~clarify~~ the cell viability data. ~~Hydrogel from p~~Porang glucomannan-chitosan hydrogel was stable for up to 2 h in the intestinal fluid. However, it was~~Hydrogel became~~ larger ~~at~~ after 60~~1~~ min than~~compared with that of at~~ 0 min exposure (**Figure 6**~~).~~), potentially reflecting ~~This may be due to the~~its swelling behavior ~~of hydrogel~~ at ~~a~~ pH ~~of~~ 6.8. We ~~Our~~ previous~~ly showed study proved~~ that porang glucomannan-chitosan hydrogel begins~~began~~ to swell at pH >~~5~~ (Aprilia *et al.*, 2021). The swelling of the hydrogel was evident~~could be seen~~ until 90 min of exposure. ~~After 120 min of exposure, there were m~~Many small hydrogels and cells were visible in the solution after 120 min of exposure. The swelling ~~made the interaction in hydrogels weaker, leading to some parts of the hydrogel being dissolved, leaving small hydrogels, and to the release of~~weakened the interaction in hydrogels, leading to some parts of the hydrogel being dissolved, resulting in smaller hydrogels and the release of cells from the hydrogel. This result is consistent with ~~Another~~ another study that ~~also had a similar result. found~~~~Exposing~~ ~~the hydrogel of~~ konjac glucomannan carboxymethyl chitosan hydrogel with a bovine serum albumin core ~~into pH 7.4 buffer~~ showed ~~a~~ greater core release at pH 7.4 ~~of core~~ than ~~that~~ at ~~medium~~ pH 5 due to ~~. This was caused by~~ swelling~~, which resulted in~~ enlarg~~ed~~ its pores (Du et al., 2006). This~~e completion of~~ core release also occurred when ~~a~~the ~~hydrogel of~~ chitosan-oxidizing glucomannan hydrogel was exposed to simulated intestine fluid for 2–8 h (Korkiatithaweechai et al., 2011).

A) B)

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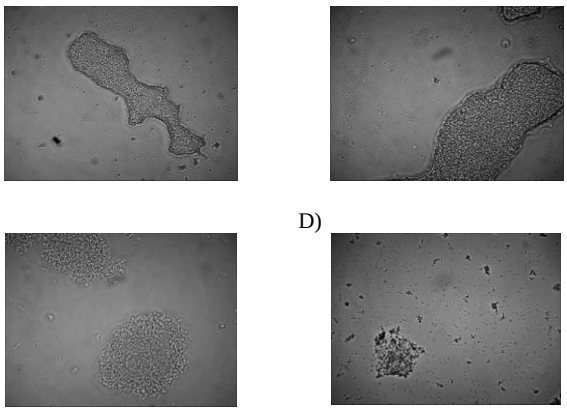

Figure 6. Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (1300× magnification ~~of 1.300x~~) during exposure to intestinal juice for (**A**) 0 ~~min~~, (**B**) 30 ~~min~~, (**C**) 60 ~~min~~, and (**D**) 120 min.

Conclusions

The encapsulation of *L. acidophilus* in hydrogel made from glucomannan and chitosan was improved by varying the concentration of cells added. Higher concentrations showed greater ~~These were presented by the higher~~ encapsulation efficiency, ~~the raise of~~ diameter (2–3 mm), polydispersity index (1.23–1.65), positive~~ly~~ zeta potential, ~~the~~ whiteness, and brightness ~~of hydrogel~~. In addition, ~~The~~ the hydrogel ~~also~~ showed potential ~~the potency~~ as a prebiotic ~~that has been shown by its score of prebiotic activity~~, particularly ~~especially~~ after 24 h of incubation. Moreover, the hydrogel protected ~~It also attributed to its role as~~ encapsulated~~nt of~~ cells, ~~especially in~~ maintaining them ~~the cells~~ during exposure to simulated gastrointestinal fluid. Furthermore, cell ~~The~~ viability ~~of bacteria~~ increased ~~mainly raised~~ from 86% to 100% when it was exposed ~~applied~~ to intestinal juice, ~~and showed the~~ comparable to ~~result with~~ alginate and konjac glucomannan hydrogel. ~~-~~ Further~~Future~~ animal studies are needed ~~may be carried out to animal experiments~~ to determine ~~the~~ cell viability in actual gastrointestinal conditions and the ~~or~~ health effects of the hydrogel.

Data Aavailability

The data used to support the findings of this study are included ~~with~~in the article.

Conflict of Iinterest

The authors declare ~~that they have~~ no conflicts of interest.

Acknowledgments

This research was supported by the Riset Inovatif Produktif ~~RISPRO~~ pProject ~~of~~ Lembaga Pengelola Dana Pendidikan ~~(LPDP)~~ (Indonesia Endowment Fund for Education) for 2016–2017 and the Research Directorate and Reputation Team towards World Class University ~~-~~ Quality Assurance Office of Universitas Gadjah Mada ~~(according to A~~assignment ~~Letter~~ letter ~~Number~~number~~:~~ 6144/UN1.P.III/DIT-LIT/PT/2021

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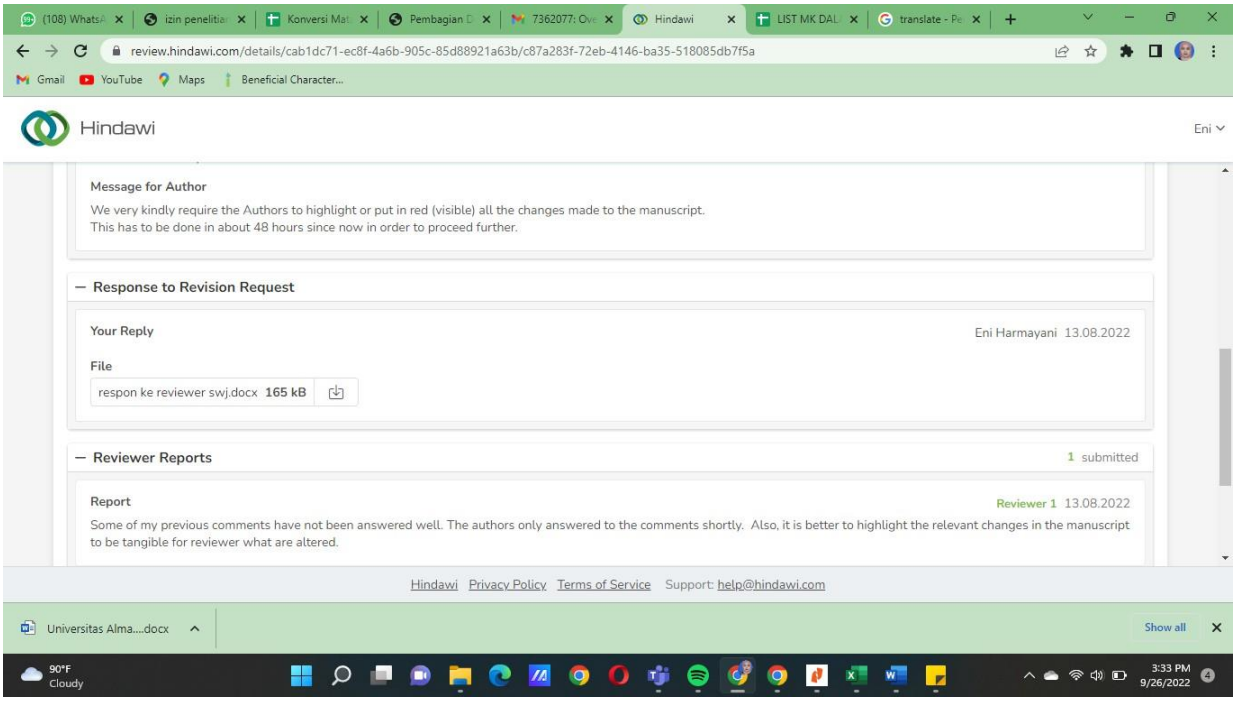

August 13, 2022

Dear Editor in Chief of The Scientific World Journal,

Please accept our revision entitled, "**Hydrogel from glucomannan-chitosan to improve survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid***"* for consideration for publication in The Scientific World Journal.

We had revised all of the suggestion and correction from the reviewer. Please contact me if you have any question or concern regarding the manuscript. I look forward to receiving the results of the review.

Sincerely

Prof. Dr. Ir. Eni Harmayani, M.Sc

Responses the reviewer's comments

Manuscript ID:
Title: Hydrogel from glucomannan–chitosan to improve survival of *L. acidophilus* FNCC 0051 in simulated gastrointestinal fluid

Reviewer 2

No.	Reviewer's Comments	**Responses** (for author)
1.	It seems well-conducted and written and could be of interest to the readers of the Journal. I have no particular concerns to raise	There was no revision request.

Reviewer B

No.	Reviewer's Comments	**Responses** (for author)
1	The manuscript needs to be polished by an English native speaker to remove the grammatical errors throughout the manuscripts	We have proofread it by The Cambridge Proofreading LLC. The certificate is attached below.
2	It is absolutely essential you use the most recent papers in your manuscript. Unfortunately, you have employed older references and also the number of references is very low for a research article	We have revised it and now 21 of 26 papers are included in research paper, while the new references are 16 of 26 papers (>50%).
3	The discussion section is not highlighted very well in your manuscript and it needs to review the previous research to reveal that what novelties are used in this study compared with others.	We have revised it in all of discussion section (123-304)
4	The results also need to be revised and expanded. You need to use more tables and especially figures. Your current data is not sufficient to show the novelty of your work. It does not motivate the reviewers to read the manuscript when data has nothing to bring.	We have added the data presented in Table 3, Table 4, Figure 1, Figure 2, Figure 3.
5	The material and methods are written briefly and need to be expanded.	We have been revised (line 57-121).
6	The name of the bacterial strains are not written fully when the authors have mentioned them for the first time. You should use the full name of the bacteria (Lactobacillus acidophilus) and then write L. acidophilus	Has been revised (in almost all paragraph)
7	In your opinion, what are the new aspects of this study compared with previous studies.? A huge amount of earlier research has investigated the role of hydrogels and nanoparticles on the viability of probiotics. The prior studies have investigated very well the effect of the hydrogels on the bacteria compared with your studies.	Our study investigated the hydrogel from porang glucomannan and chitosan that was applicated as bacterial encapsulant. The findings are: 1. We used porang glucomannan that has different character

		with konjac glucomannan (Line 36-39) 2. We applicated the hydrogel as probiotic encapsulant that has different character with the inanimate objects (line 39-51). We had to ensure that probiotic is still life during processing and in gastrointestinal tract.

Editorial Certificate

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Authors:	Document title:	Date Issued:
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Hydrogel from glucomannan-chitosan to improve survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid

Abstract

The probiotic encapsulating hydrogel made from porang (*Amorphophallus oncophyllus*) glucomannan and chitosan was investigated for its encapsulation efficiency, physical properties, prebiotic activity, and survival under simulated gastrointestinal conditions. Encapsulation efficiency was improved by varying the concentration of *Lactobacillus acidophilus* FNCC 0051, which also increased the diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness of the hydrogel. The hydrogel's prebiotic activity score was higher than inulin after 24 h of incubation, reflecting its role as a cell encapsulant, particularly in maintaining cells during exposure to simulated gastrointestinal fluid. Cell viability increased from 86% to 100% when immersed in intestinal juice, comparable to alginate and konjac glucomannan hydrogel. Future animal studies are needed to determine cell viability in actual gastrointestinal conditions and the health effects of the hydrogel.

Keywords: hydrogel; viability; glucomannan; chitosan; gastrointestinal.

Introduction

Glucomannan is a functional polysaccharide that can be extracted from *Amorphophallus* tubers. While glucomannan from *Amorphophallus konjac* has popular and commercial uses, several studies are currently investigating glucomannan from other sources. *Amorphophallus oncophyllus* is a local glucomannan source in Indonesia, commonly known as porang (Harmayani, Aprilia, and Marsono, 2014)(Yanuriati *et al.*, 2017). It has specific characteristics that differ from konjac, including mannose/glucose molar ratio, degree of polymerization, and degree of acetylation, leading to different solubility, viscosity, water holding capacity, and gelation properties (Harmayani, Aprilia, and Marsono, 2014; Yanuriati *et al.*, 2017). Therefore, its applications may also differ depending on the function.

Hydrogels are one technological glucomannan product that leverages gelation properties. They can form through interactions between glucomannan and other polymers to form a three-dimensional polymeric network (Stasiak-Różańska *et al.*, 2021). This characteristic has potential as an encapsulant. A previous study used hydrogel created by crosslinking konjac glucomannan and chitosan, which has many advantages, including being naturally formed without a crosslinker, self-assembly, tolerance to different pH, and its demonstrated abilities in encapsulating drugs, proteins, and enzymes (Du *et al.*, 2006; Korkiatithaweechai *et al.*, 2011). A similar study on hydrogel formed by the interaction of porang glucomannan and chitosan considered the production of the primary carboxymethyl glucomannan material, compatibility of substitution degree of carboxymethyl glucomannan in hydrogel formation, effect of polymer concentration on the glucomannan properties, and its application in probiotic encapsulation (Aprilia *et al.*, 2017a, 2017b, 2021). Its key innovation was the use of porang, which has different characteristics from other glucomannan sources, such as solubility, viscosity, water holding capacity, degree of polymerization, degree of acetylation, purity, and X-ray diffraction (XRD) pattern (Harmayani, Aprilia and Marsono, 2014; Yanuriati *et al.*, 2017). Other differences include the type of modification used (carboxymethylation) and its use as a probiotic encapsulant. In contrast, the previous study used oxidation (Korkiatithaweechai *et al.*, 2011) and encapsulated drugs, proteins, and enzymes (Du *et al.*, 2006; Korkiatithaweechai *et al.*, 2011).

This new hydrogel's role in encapsulating probiotics needs to be further studied since the living cells have different characteristics to inanimate compounds. The new capsules should ensure the survival of probiotics during food processing and storage and sufficient delivery (>10^6–10^7 colony forming units [CFU]/mL) when consumed. Furthermore, it also needs to reach the lower gastrointestinal tracts to have a beneficial effect on humans. Therefore, its survival during gastrointestinal digestion and its capability to increase probiotic growth in the colon is important. Carbohydrates known to stimulate probiotic growth are called prebiotics. We previously optimized probiotic encapsulation efficiency by varying the glucomannan

concentration and also studied its role in protecting cells during pasteurization and cold storage (Aprilia *et al.*, 2021). However, its role in protecting probiotic cells during digestion and its potential as a prebiotic remain unexplored.

This study aimed to improve the probiotic encapsulation efficiency and properties of the hydrogel formed by glucomannan and chitosan by varying the cell concentration to increase the number of carried and examines the effects on its physical properties, prebiotic activity score, and viability during simulated gastrointestinal exposure.

Materials and Methods

Materials

The primary material used in this study was glucomannan from porang tuber (*A. oncophyllus*) obtained from the Faculty of Agricultural Technology, Universitas Gadjah Mada (Yogyakarta, Indonesia). Carboxymethylation of glucomannan used sodium chloroacetate as previously described (Aprilia *et al.*, 2017b). The chitosan with 85–89% deacetylation that meets food quality criteria was obtained from PT Biotech Surindo (Cirebon, West Java, Indonesia).

Preparation of *Lactobacillus acidophilus* FNCC 0051 cells

L. acidophilus FNCC 0051 cells were obtained from the Food and Nutrition Culture Collection (FNCC), Laboratory of Food Microbiology, Center for Food and Nutrition Studies, Universitas Gadjah Mada. Cells in skim milk-glycerol suspension were rejuvenated in de Man, Rogosa, and Sharpe (MRS) broth at 37°C overnight and grown twice successively. The cell biomass was then harvested by centrifugation at 2400 g for 9 min at 4°C and rinsed with saline solution.

Hydrogel encapsulation of probiotics and determination of its efficiency

The hydrogel was created by the interaction of porang glucomannan with 0.5% chitosan using the complex coacervation method. Hydrogels were prepared with three different cell concentrations of 8, 9, and 10 log CFU/mL. The cells were mixed with glucomannan before the coacervation process (Aprilia *et al.*, 2021). The encapsulation efficiency was determined by dividing the number of viable cells entrapped in the hydrogel (post-encapsulation) by the number of cells blended into the pre- encapsulation solution (Zeashan *et al.*, 2020). The cells entrapped in the hydrogel were released by submersing it in a buffer solution at pH 8 and 37°C for 24 h (Aprilia *et al.*, 2017b).

Hydrogel properties

Particle size, polydispersity index, zeta potential

Particle size was estimated based on the hydrogel's diameter and measured simultaneously with the polydispersity index using a particle size analyzer (Horiba SZ-100 series; Kyoto, Japan). The hydrogel's zeta potential was measured with a Nano ZS Zetasizer (v.6.20; Malvern Instruments Ltd; Malvern, UK).

Color

The hydrogel was freeze-dried and ground before the color measurement. Values redness (a*), yellowness (b*), and lightness (L*) were determined with a CR200 chromameter (Minolta; Osaka, Japan). The whiteness index was calculated as previously described (Akgün, Ova Özcan, and Övez, 2022).

XRD

The XRD of hydrogels was determined with a Shimadzu LabX XRD-6000 (Kyoto, Japan) equipped with a Cu Kα target at 40 kV and 30 mA with a scanning rate of 4°/min. The pattern was collected in the 2θ

range between 3.02 and 90°. Crystallinity percentage (%) was calculated by dividing the area under the peaks by the total curve area (Yazdani *et al.*, 2020).

Prebiotic activity scores

The prebiotic activity score was calculated by subtracting the ratio of probiotic cells growth with prebiotics and glucose from the ratio of enteric cells growth with prebiotics and glucose as previously described (Huebner, Wehling, and Hutkins, 2007). The probiotic used was *L. acidophilus* FNCC 0051, while the enteric cells used were *Escherichia coli* FNCC 0091. The test was performed by adding 1% (vol/vol) of probiotic cells into MRS broth containing 2% (w/v) glucose or prebiotic and 1% (v/v) of enteric cells into M9 broth containing 2% (w/v) glucose or prebiotic. The cells were incubated at 37°C for 0, 24, and 48 h with and enumerated by the plate count method using MRS and nutrient agar. Each test was replicated three times.

L. acidophilus FNCC 0051 survival during exposure to simulated gastrointestinal conditions

Simulated gastric and intestinal juices were prepared as described by Xu et al. (2016). Gastric juice was prepared by mixing 7 mL of pepsin in hydrochloric acid, 2 g of sodium chloride, and 1 M of sodium hydroxide. Intestinal juice was prepared by mixing 1% pancreatic powder, 6.8 g of potassium dihydrogen phosphate, and 77 mL of 0.2 N sodium hydroxide. Either 1 g of free or encapsulated cells (in the hydrogel of porang glucomannan-chitosan, konjac glucomannan-chitosan, and calcium alginate) was mixed with 9 mL of simulated gastrointestinal juices and incubated at 37°C for 120 min. The samples were withdrawn at intervals of 0, 30, 60, and 120 min for gastric juice digestion and 0, 60, 90, and 120 min for intestinal juice digestion (Rather *et al.*, 2017). The hydrogel was then rinsed twice with acetate buffer. The cells were enumerated using the pour plate technique with MRS agar after 48 h of incubation. The number of viable cells after exposure was divided by the initial number of cells to determine their survival rate during exposure to simulated gastrointestinal conditions (Zeashan *et al.*, 2020). The hydrogel's appearance during exposure to simulated gastrointestinal conditions was observed with an optical BX51 microscope (Olympus Corp.; Tokyo, Japan) and OptiLab pro digital camera (Miconos, Indonesia).

Results and Discussion

Encapsulation efficiency of hydrogel in different concentrations of cells

The concentration of encapsulated cells was lower than the initial cell concentration (**Table 1**), indicating that not all the cells were encapsulated in the hydrogel and affecting the calculated encapsulation efficiency. Indeed, the higher concentration of initial cells, the higher the encapsulation efficiency. The highest encapsulated cell concentration of 7.94 log CFU/g was obtained with log 10 CFU/mL of cells. This number exceeds the Food and Agricultural Organization of the United Nations (FAO) criteria for probiotic products of >6–7 log CFU/mL (Priya, Vijayalakshmi, and Raichur, 2011).

Previous studies using different encapsulants obtained different encapsulation efficiencies. For example, the encapsulation of *L. acidophilus* in the hydrogel formed from sodium alginate and soy protein isolate achieved 95–98% encapsulation efficiency, while the encapsulation of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* in emulsion achieved 97–99% efficiency (Zeashan *et al.*, 2020; Mahmoodi Pour, Marhamatizadeh, and Fattahi, 2022). Differences in encapsulation efficiency might reflect encapsulant type and the encapsulation method used (Zeashan *et al.*, 2020). We previously showed that the same ratio of glucomannan and chitosan affected encapsulation efficiency due to the chemical bonding of both polymers and the difference in electrostatic values between the core and polymer influencing cell entrapment (Aprilia *et al.*, 2021).

Table 1. The encapsulated cell concentration and hydrogel encapsulation efficiency with different initial cell concentrations.

Hydrogels with different cell concentrations (log CFU/mL)	Cell concentration before encapsulation (log CFU/mL)	Cell concentration after encapsulation (log CFU/g)	Encapsulation efficiency (%)
8	9.39 ± 0.00	4.47 ± 0.18	44.37±1.91[a]
9	9.56 ± 0.00	6.60 ± 0.13	65.83±1.37[b]
10	10.10 ± 0.00	7.94 ± 0.21	85.03±0.63[c]

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

Properties of hydrogel in different concentrations of cells

The appearance of hydrogel generated from glucomannan and chitosan containing *L. acidophilus* was shown in **Figure 1**. The polymer solution was clear before the encapsulation process and became turbid after the encapsulation process. It proved that there was the formation of particle that influenced the turbidity of solution. After drying process, the hydrogel shape looks like a white cotton. The particles and value of colors of hydrogel was explained in the next paragraph.

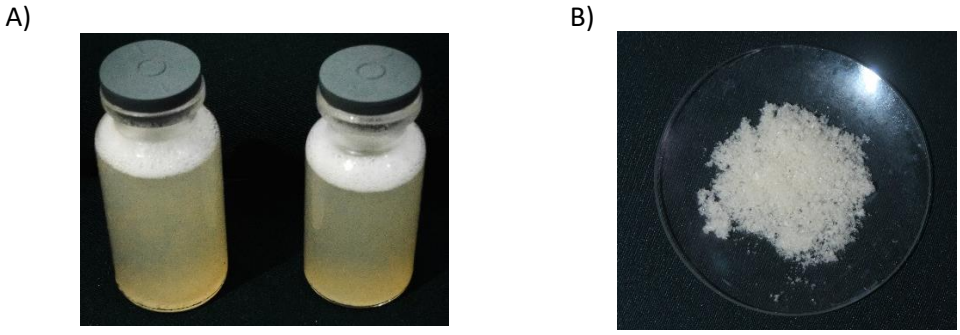

Figure 1. The appearance of hydrogel A) before drying and B) after drying process

The size of hydrogels encapsulating *L. acidophilus* was measured in the range of 0.7–9 µm, with most having a diameter of 2–3 µm (**Table 2**). Hydrogels <100 µm in diameter are classified as microgels. The concentration of cells significantly influenced hydrogel particle size ($p < 0.05$). The more cells encapsulated in the hydrogel, the greater its diameter. It was also correlated with encapsulation efficiency (**Table 1**) since more cores can be entrapped in larger hydrogel particles. The other factors influencing particle size were the concentration and viscosity of the solution (Zeashan *et al.*, 2020; Aprilia *et al.*, 2021)

Table 2. Hydrogel particle size, polydispersity index, and zeta potential with different initial cell concentrations.

Initial cell concentration (log CFU/mL)	Particle size (µm)	Polydispersity index	Zeta potential (mV)
8	2.23±0.11[a]	1.23±0.17[a]	24.40±0.75[a]
9	2.79±0.19[b]	1.39±0.04[ab]	32.28±0.80[b]
10	3.41±0.14[c]	1.65±0.27[b]	14.58±0.97[c]

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

The polydispersity indexes of hydrogel encapsulated cells were >1 (**Table 2**), indicating a broad particle distribution or particles of various sizes. The index began to change when the initial cell concentration was 10 log CFU/mL. The greater the initial cell concentration, the higher the polydispersity

index. This result contrasts with a previous study that found that glucomannan concentration did not influence the polydispersity index (Aprilia *et al.*, 2021).

Hydrogel zeta potentials became more electropositive as the cell concentration increased from 8 to 9 log CFU/mL but decreased with 10 log CFU/mL (**Table 2**). An increase in cell number should cause a reduction in hydrogel charge due to the positive charge of empty hydrogel and the negative charge of cells (Aprilia *et al.*, 2021), including *L. acidophilus* (Priya, Vijayalakshmi and Raichur, 2011). The observed pattern might be due to the zeta potential being measured on the hydrogel's surface, which can be affected by the pH of surrounding environment (Barbosa *et al.*, 2019).

The L*, b*, and whiteness values of the hydrogel increased after adding cells, while the a* value decreased (**Table 3**). The instrument determines these values based on the reflection of a direct light beam from a chromameter by the cells. Therefore, the more cells encapsulated in the hydrogel, the greater the reflection. Bacterial may also generate distinct shade of color like red. Based on the previous study, *Lactobacillus pluvialis* could reflect orange color from the pigment of canthaxanthin (Venil, Dufossé and Renuka Devi, 2020). This was in agreement with this result, especially the increase of b* value after the addition of *L. acidophilus.*

Table 3. Hydrogel color value with different initial cell concentrations.

Initial cell concentration (log CFU/mL)	L*	a*	b*	Whiteness
control	65.06±0.12^{a}	7.02±0.09^{a}	12.50±0.08^{a}	62.24±0.15^{a}
8	76.97±0.32^{b}	5.42±0.01^{b}	14.24±0.11^{b}	72.38±0.21^{b}
9	79.48±0.33^{c}	5.61±0.07^{b}	15.14±0.01^{c}	73.89±0.25^{c}
10	77.39±0.23^{b}	4.22±0.23^{c}	13.24±0.13^{d}	73.46±0.30^{c}

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

XRD spectra represent the interaction between diffraction intensity and angle (**Figure 2**). A crystalline state was indicated by the sharp diffraction peak, while the amorphous and solid state was indicated by the declivous peak (Yanuriati *et al.*, 2017). The X-ray diffractogram patterns of all hydrogels showed a very broad band at 2θ between 5–90°. In addition, all hydrogels had almost the same highest peak at around 2θ 7.06–10.46, 7.62–11.00, 7.48–10.94, and 7.16–11.20° for hydrogels without cells and with cells at concentrations of log 8, 9, 10 CFU/mL, respectively. These differ from porang glucomannan, which had its highest peaks at around 19–20° and 35° (Yanuriati *et al.*, 2017). However, there was a small peak in all samples at around 2θ 10.5°, indicating the existence of chitosan (Yu, Lu, and Xiao, 2007). This observation suggests that the mixture between glucomannan, hydrogel, and cells strengthened their chemical interaction, consistent with previous Fourier-transform infrared spectroscopy (FTIR) findings (Aprilia *et al.*, 2021), and that some chitosan had not interacted with glucomannan. A previous study reported that Schiff's crosslinking between glucomannan aldehyde groups and chitosan amino groups could suppress chitosan's crystalline state, usually strengthened by a hydrogen bond between amino and hydroxyl groups (Yu, Lu, and Xiao, 2007). We also find low crystallinity, with values of 26%, 25%, 17%, and 21% for hydrogels without cells and with cells at concentrations of 8, 9, and 10 log CFU/mL, respectively. The addition of *L. acidophilus* appeared to have no effect on the diffraction peak, indicating that the entrapment of microbes in hydrogel did not affect the interaction between glucomannan and chitosan.

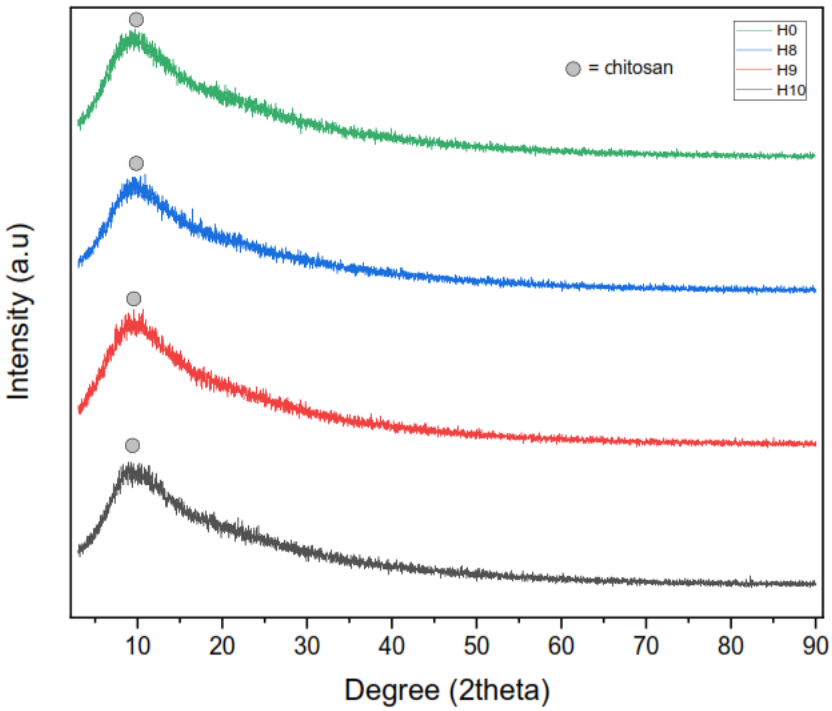

Figure 2. X-ray diffractogram for H0 (hydrogel without *L. acidophilus*), H8, H9, and H10 (hydrogels with *L. acidophilus* at concentrations of 8, 9, and 10 log CFU/mL).

Hydrogen prebiotic activity

L. acidophilus and *E. coli* cell density increased during 0, 24, and 48 hours of incubation in the presence of carbohydrates glucose, inulin, and hydrogel (**Table 4**). Both bacteria showed no significant increase with almost all carbohydrates, except *L. acidophilus* with inulin and *E. coli* with glucose. These data suggest that only inulin can specifically stimulate the growth of good bacteria and suppress the growth of enteric bacteria, consistent with its well-known use as a commercial prebiotic worldwide.

Table 4. The density of *L. acidophilus* FNCC 0051 cells in log10 (CFU/mL) after 0, 24, and 48 hours of incubation with prebiotics inulin, hydrogel, and glucose.

Prebiotic	*L. acidophilus*			*E. coli*		
	0 h	24 h	48 h	0 h	24 h	48 h
Glucose	6.94±1.32^{a}	8.35±0.81^{a}	9.17±0.01^{a}	6.65±0.92^{a}	8.54±0.09^{ab}	9.29±0.49^{b}
Inulin	6.59±0.19^{a}	7.33±0.49^{ab}	8.48±0.88^{b}	9.53±0.09^{a}	7.59±0.32^{a}	8.47±0.75^{a}
Hydrogel	9.37±0.10^{a}	9.58±0.46^{a}	10.15±0.21^{a}	8.80±1.13^{a}	8.17±0.86^{a}	9.02±2.18^{a}

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

The prebiotic potential of the hydrogel was compared with inulin using prebiotic activity scores (**Figure 3**). The prebiotic activity score of hydrogel was higher than inulin after 24 h of incubation but became lower after 48 h, suggesting that hydrogel was a preferred energy source for cells. This result is consistent with the XRD findings that confirmed the amorphous hydrogel state, which has no long-range order, making it easier to digest, and the amount of carbohydrates will decrease with time. Meanwhile, known prebiotic inulin (Kamel *et al.*, 2021) needed a longer time to be available for bacteria since it has long polymeric carbon chains, that is 2–60 molecules (Samolińska and Grela, 2017).

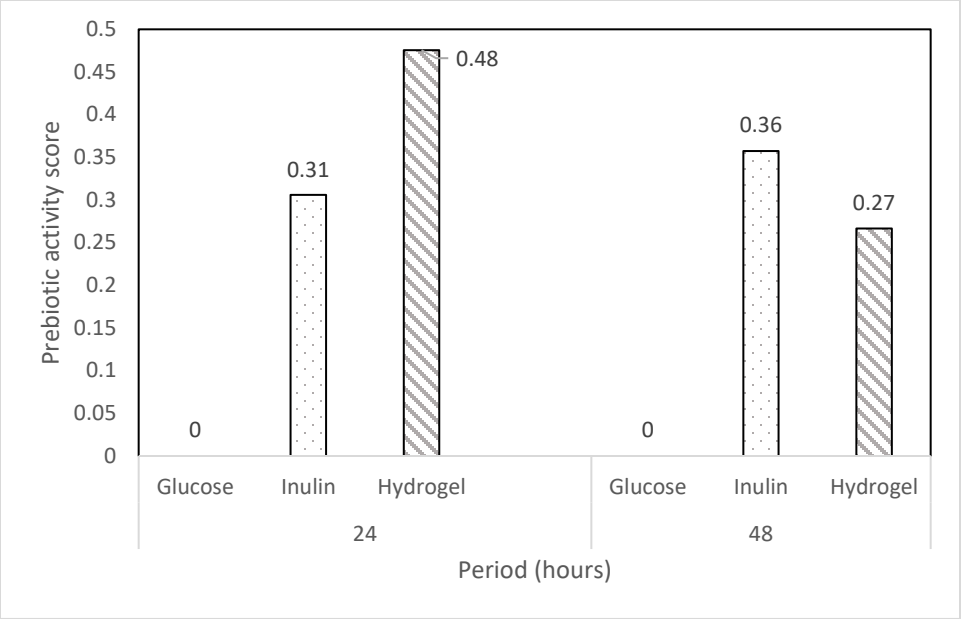

Figure 3. Prebiotic activity scores of *L. acidophilus* FNCC 0051 on glucose, inulin, and hydrogel.

Cell survival during exposure to simulated gastrointestinal conditions

Cell survival during exposure to gastric juice

L. acidophilus showed good viability during exposure to gastric juice at pH 2, either in its free form or when encapsulated in hydrogel (**Figure 4**). The growth of lactic acid bacteria is generally optimum at pH 6–7 (close to neutral pH). Some metabolic reactions change when pH is <5 or <4.4. Indeed, some minerals will be lost at pH ≤2, and prolonged storage at low pH will increase the risk of cell death (Hayek dan Ibrahim, 2013). Our results are consistent with a previous study (Zeashan *et al.*, 2020; Stasiak-Różańska *et al.*, 2021). A further studies are needed to determine the effect on solid or solid enriched macronutrient foods with a longer transit time (Müller, Canfora, and Blaak, 2018). In addition, a shorter exposure time in the stomach enables cells to maintain homeostasis between internal and external pH, potentially influencing the good viability shown in this study.

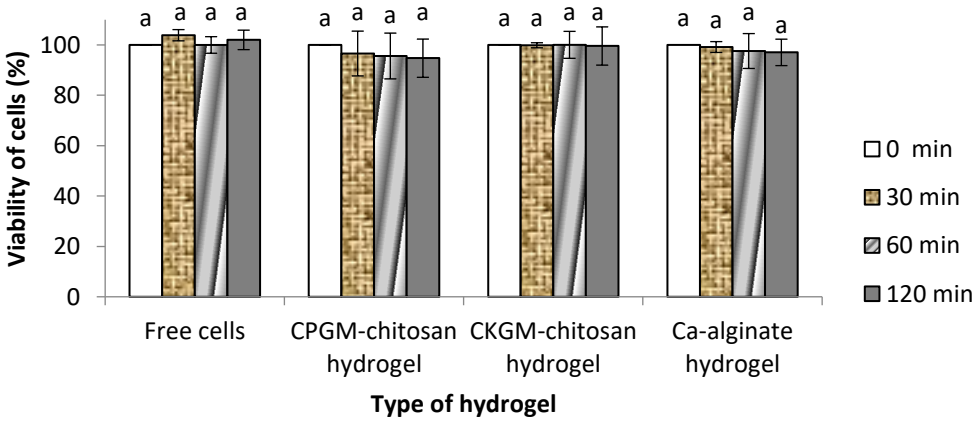

Figure 4. *L. acidophilus* FNCC 0051 viability during exposure to gastric juice for 120 min. Key: a, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

This study also found that porang glucomannan-chitosan hydrogel might have a similar cell protecting capability from the gastric environment as konjac glucomannan–chitosan hydrogel and calcium alginate hydrogel ($p > 0.05$). This finding accords with the ability of alginate also protected *L. plantarum* (Rather et al., 2017) and *Lactobacillus rhamnosus* from this harsh environment for 3 h of exposure (Oberoi *et al.*, 2021).

The hydrogel was stable in simulated gastric juice for 120 min of exposure (**Figure 5**), consistent with a previous swelling ratio study (Aprilia *et al.*, 2021) that found the hydrogel did not deswell at the pH <5. Deswelling causes the hydrogel to become smaller, which was previously thought to release cells from the hydrogel. However, cells are still entrapped in the hydrogel (**Figure 5**), perhaps reflecting the stronger electrostatic interaction between the glucomannan carbonyl group and the chitosan amine group in an acid environment (Aprilia *et al.*, 2021). Cells remain in the hydrogel because this interaction maintains the core. Therefore, deswelling could not be maximized, leading to only a small number of cells being released from the hydrogel. There is a possibility that some empty hydrogels will shrink to the extent that they are no longer visible at 60 min of exposure. These results are consistent with other studies using hydrogels made from oxidized glucomannan and chitosan to entrap diclofenac drugs that found <1% was released during exposure to simulated gastric fluid at pH 1.2 (Korkiatithaweechai et al., 2011). This result shows that hydrogel cores were not released when it was exposed to low pH conditions.

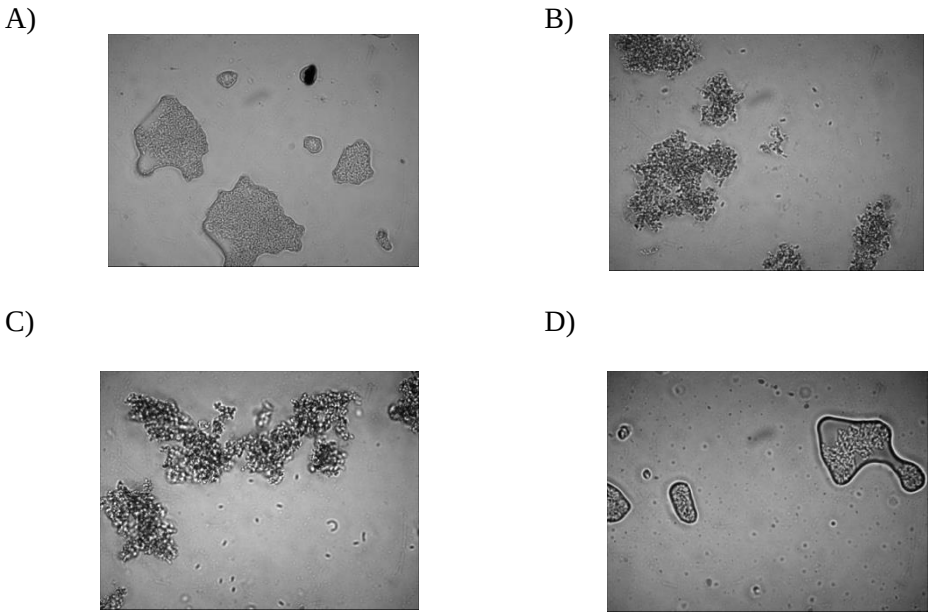

Figure 5. Microscopic appearance of hydrogels containing *L. acidophilus* FNCC 0051 (1300× magnification) during exposure to gastric juice for (**A**) 0, (**B**) 30, (**C**) 60, and (**D**) 120 min.

Cell survival during exposure to intestinal juice

The viability of free cells decreased significantly during exposure to intestinal juice for 60 min (**Figure 6**; $p < 0.05$). Indeed, the viability of cells encapsulated in hydrogel could be maintained over 120 min of exposure, indicating that encapsulation increases *L. acidophilus* viability. A decrease in the number of free cells may reflect cell death, which can be caused by factors other than the pH of the medium. Priya et al.

(2011) reported that while bacteria showed good growth at pH 6.8, the presence of pancreatin, comprising amylase, trypsin, lipase, ribonuclease, and protease, damaged the encapsulation wall, causing cell death.

Figure 6 indicates that porang glucomannan hydrogel has the same good protective effect as konjac-chitosan glucomannan and calcium alginate hydrogels. In this study, the alginate-based hydrogel was used for comparison since it is widely used as an encapsulant due to its low price, good biocompatibility, and nontoxicity. A previous study showed that probiotic encapsulation using alginate increased entrapped cell viability compared to free cells during exposure to simulated gastrointestinal condition (Stasiak-Różańska *et al.*, 2021). Therefore, the porang-chitosan glucomannan hydrogel has potential as a bacterial encapsulant.

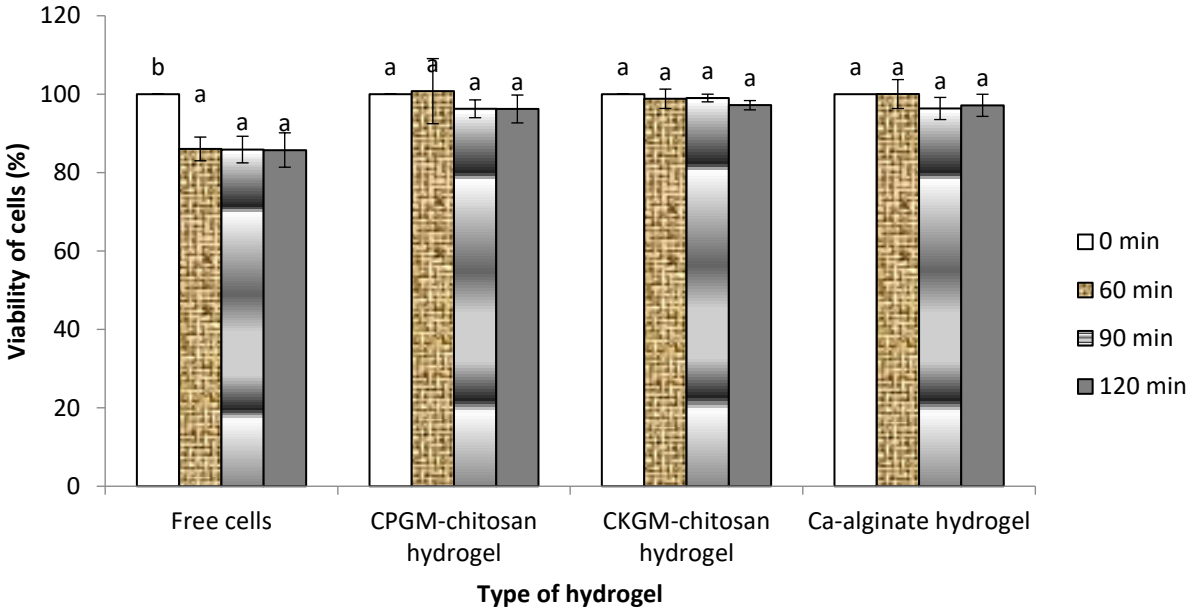

Figure 6. *L. acidophilus* FNCC 0051 cell viability during exposure to intestinal juice for 120 min. Key: a or b, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

The hydrogel's microscopic appearance was used to confirm the cell viability data. Porang glucomannan-chitosan hydrogel was stable for up to 2 h in the intestinal fluid. However, it was larger after 60 min than 0 min exposure (**Figure 7**), potentially reflecting its swelling behavior at pH 6.8. We previously showed that porang glucomannan-chitosan hydrogel begins to swell at pH >5 (Aprilia *et al.*, 2021). The swelling of the hydrogel was evident until 90 min of exposure. Many small hydrogels and cells were visible in the solution after 120 min of exposure. The swelling weakened the interaction in hydrogels, leading to some parts of the hydrogel being dissolved, resulting in smaller hydrogels and the release of cells from the hydrogel. This result is consistent with another study that found konjac glucomannan carboxymethyl chitosan hydrogel with a bovine serum albumin core showed greater core release at pH 7.4 than at pH 5 due to swelling enlarging its pores (Du et al., 2006). This core release also occurred when a chitosan-oxidizing glucomannan hydrogel was exposed to simulated intestine fluid for 2–8 h (Korkiatithaweechai et al., 2011).

A) B)

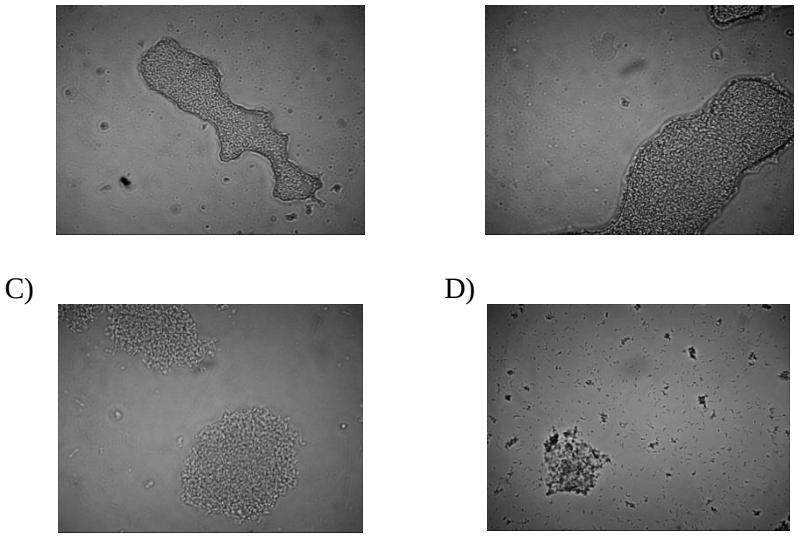

Figure 7. Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (1300× magnification) during exposure to intestinal juice for (**A**) 0, (**B**) 30, (**C**) 60, and (**D**) 120 min.

Conclusions

The encapsulation of *L. acidophilus* in hydrogel made from glucomannan and chitosan was improved by varying the concentration of cells added. Higher concentrations showed greater encapsulation efficiency, diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness. In addition, the hydrogel showed potential as a prebiotic, particularly after 24 h of incubation. Moreover, the hydrogel protected encapsulated cells, maintaining them during exposure to simulated gastrointestinal fluid. Furthermore, cell viability increased from 86% to 100% when it was exposed to intestinal juice, comparable to alginate and konjac glucomannan hydrogel. Further animal studies are needed to determine cell viability in actual gastrointestinal conditions and the health effects of the hydrogel.

Data Availability

The data used to support the findings of this study are included in the article.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgments

This research was supported by the Riset Inovatif Produktif project Lembaga Pengelola Dana Pendidikan (Indonesia Endowment Fund for Education) for 2016–2017 and the Research Directorate and Reputation Team towards World Class University-Quality Assurance Office of Universitas Gadjah Mada (assignment letter number 6144/UN1.P.III/DIT-LIT/PT/2021 dated September 27, 2021).

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8. Reminding Revisi dan Jawaban Permohonan Perpanjangan Revisi 2

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7362077: Overdue revised manuscript

3 pesan

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Cc: hastutipudji@yahoo.com, amurdiati@ugm.ac.id, verianiaprilia@almaata.ac.id

Dear Dr. Eni Harmayani,

This is to inform you that the revised version of your manuscript 7362077 titled "Hydrogel from glucomannan-chitosan to improve survival of Lactobacillus acidophilus FNCC 0051 in simulated gastrointestinal fluid," to The Scientific World Journal is overdue, as it has been over 1 month since you received your decision email.

To submit the revised manuscript please log into your review.hindawi account and upload your revised files. The revised files can only be uploaded through the account of the submitting author. When submitting your revision, please ensure to upload the revised manuscript file by replacing the file in the ‘Main Manuscript’ section. Additionally, please ensure to upload a clear and detailed “Response to Editor/Reviewer comments” document in the ‘Response to Revision Request’ section, which outlines in a point-by-point fashion how you have addressed the previous review comments.

Please note, should your manuscript be accepted, we will require editable versions of your figure files. Therefore, if you are able to upload your editable figure files at this stage it may save time at the production stages should your paper reach publication.

If you require additional time for submitting your revised manuscript, please let me know as soon as possible. Unfortunately, if we do not hear from you, or receive your revised manuscript within 2 weeks, we will be withdrawing your manuscript.

I look forward to receiving your response.
Best Regards,

Polen

Polen Ilagan
Editorial Assistance

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---

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 20 September 2022 pukul 08.07
Kepada: Polen Ilagan <help@hindawi.com>

Dear Polen Ilagan

We revised our manuscript and uploaded it to the system on August 13, 2022. and the status became under review.
Would you like to check it again?
Please tell us if your system did not record it.
Thank you in advance

Regards,
Veriani Aprilia
[Kutipan teks disembunyikan]

---

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 20 September 2022 pukul 08.11
Kepada: eniharmayani@ugm.ac.id

[Kutipan teks disembunyikan]

---

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 25 September 2022 pukul 08.43
Kepada: Polen Ilagan <help@hindawi.com>, eniharmayani@ugm.ac.id

Dear Polen Ilagan,

Regarding our manuscript revision (7362077 titled: “Hydrogel from glucomannan-chitosan to improve survival of Lactobacillus FNCC 0051 in simulated gastrointestinal fluid”), we informed you that it had been uploaded to the system on August 13, 2022. Now, we are trying to access the system to know the progress, but we cannot. The system seems not well working several times we tried. Please inform us whether you could receive our manuscript or not. thank you in advance

Regards,

Veriani Aprilia

Pada tanggal Sel, 20 Sep 2022 pukul 07.34 Polen Ilagan <help@hindawi.com> menulis:
[Kutipan teks disembunyikan]

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id>

7362077: Overdue revised manuscript

8 pesan

verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 25 September 2022 pukul 08.45
Kepada: Polen Ilagan <polen.ilagan@hindawi.com>

Dear Polen Ilagan,

Regarding our manuscript revision (7362077 titled: “Hydrogel from glucomannan-chitosan to improve survival of Lactobacillus FNCC 0051 in simulated gastrointestinal fluid”), we informed you that it had been uploaded to the system on August 13, 2022. Now, we are trying to access the system to know the progress, but we cannot. The system seems not well working several times we tried. Please inform us whether you could receive our manuscript or not. thank you in advance

Regards,

Veriani Aprilia

Polen Ilagan <polen.ilagan@hindawi.com> 26 September 2022 pukul 10.00
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: verianiaprilia@almaata.ac.id

Dear Dr. Aprilia,

Thank you for your response.

As we have explained recently, you have uploaded the revision as requested by your handling Editor last August 13, 2022.

He/she invited reviewers and received a review report on that day (August 13, 2022), and request a 'Minor Revision' last August 19, 2022. From his recent request, you have not yet uploaded your revision.

The revised files can only be uploaded through the account of the submitting author. When submitting your revision, please ensure to upload the revised manuscript file by replacing the file in the ‘Main Manuscript’ section. Additionally, please ensure to upload a clear and detailed “Response to Editor/Reviewer comments” document in the ‘Response to Revision Request’ section, which outlines in a point-by-point fashion how you have addressed the previous review comments.

Please note, should your manuscript be accepted, we will require editable versions of your figure files. Therefore, if you are able to upload your editable figure files at this stage it may save time at the production stages should your paper reach publication.

If you require additional time for submitting your revised manuscript, please let me know as soon as possible. Unfortunately, if we do not hear from you, or receive your revised manuscript within 1 week, we will be withdrawing your manuscript.

I look forward to receiving your response.

Polen

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[Kutipan teks disembunyikan]
, verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> wrote:
[Kutipan teks disembunyikan]

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verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 26 September 2022 pukul 14.29
Kepada: Polen Ilagan <polen.ilagan@hindawi.com>, eniharmayani@ugm.ac.id

Dear Polen Ilagan,

Thank you for your explanation. we miss that information.
We can finish it next week, but please help us. We can not access the system: https://mts.hindawi.com/author/.
How can we open the review result that has been submitted on August 19, 2022?

We attach the screenshot of the system that was shown on our computer.
thank you for your assistance

REgards
Veriani Aprilia
[Kutipan teks disembunyikan]

screenshot the system.docx
361K

Polen Ilagan <polen.ilagan@hindawi.com> 26 September 2022 pukul 15.07

Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: verianiaprilia@almaata.ac.id
Cc: eniharmayani@yahoo.com, amurdiati@ugm.ac.id, hastutipudji@yahoo.com

Dear Dr. Aprilia,

Thank you for your reply.

Kindly advise your submitting author to try logging in using his/her registered email account 'eniharmayani@yahoo.com' at review.hindawi.com

Or, to have the issue resolved, please access
URL: https://review.hindawi.com/ via Google incognito window using your account (eniharmayani@yahoo.com).

Please try to clear your browser's cache and cookies or use your other browser and/or incognito window using your account (eniharmayani@yahoo.com).

Kindly search only the manuscript ID and do not filter the status and order.

I hope this works for you this time, but please do get in touch again if you continue to experience any difficulties.

If this all suggestions did not work, providing a screenshot of the error is a great help for us to check the error further in using our Review system.

Hence I have also included here the Editor and reviewer's comments for your reference:

Editor's comment:

We very kindly require the Authors to highlight or put in red (visible) all the changes made to the manuscript. This has to be done in about 48 hours since now in order to proceed further.

Reviewer's report:

Some of my previous comments have not been answered well. The authors only answered to the comments shortly. Also, it is better to highlight the relevant changes in the manuscript to be tangible for reviewer what are altered.

Best Regards,

Polen

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[Kutipan teks disembunyikan]

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verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 28 September 2022 pukul 08.08
Kepada: Polen Ilagan <polen.ilagan@hindawi.com>

Dear Polen Ilagan,

Thank you for your assistance. Now, we can see the processing system.
As we discussed yesterday, we need time to fix the language (proofread again) once more.
Could we send back the revision 1 week from now? or should 1 week be calculated from Tuesday or Monday?
Thank you,

Regards,
Veriani Aprilia

[Kutipan teks disembunyikan]

Polen Ilagan <polen.ilagan@hindawi.com> 28 September 2022 pukul 13.10
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: verianiaprilia@almaata.ac.id
Cc: eniharmayani@yahoo.com, amurdiati@ugm.ac.id, hastutipudji@yahoo.com

Dear Dr. Aprilia,

Thank you for your reply.

In order to grant you an extension, please provide us an **exact date** when will we expect your revision to be uploaded to the system.

Looking forward to hearing from you.

Best Regards,

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[Kutipan teks disembunyikan]

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verianiaprilia verianiaprilia <verianiaprilia@almaata.ac.id> 28 September 2022 pukul 14.41
Kepada: Polen Ilagan <polen.ilagan@hindawi.com>

Dear Polen Ilagan

Thank you for the chance given to us.
We will be ready maximum on October 6, 2022.

Regards,
Veriani Aprilia

[Kutipan teks disembunyikan]

Polen Ilagan <polen.ilagan@hindawi.com> 28 September 2022 pukul 15.32
Balas Ke: Polen Ilagan <polen.ilagan@hindawi.com>
Kepada: verianiaprilia@almaata.ac.id
Cc: eniharmayani@yahoo.com, amurdiati@ugm.ac.id, hastutipudji@yahoo.com

Dear Dr. Aprilia,

Thank you for your response.

This has been noted.

Looking forward to receiving your revision until October 06, 2022.

Best Regards,

Polen

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Thursday, September 29, 2022

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~~Hydrogel~~ Use of hydrogel derived from glucomannan-chitosan to improve the survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid

Abstract

~~The~~ The probiotic encapsulating hydrogel ~~made~~ derived from porang (*Amorphophallus oncophyllus*) glucomannan~~,~~ and chitosan was investigated ~~for~~ with regard to its encapsulation efficiency, physical properties, prebiotic activity, and survival under simulated gastrointestinal conditions. ~~Encapsulation~~ The hydrogel's encapsulation efficiency was improved by varying the concentration of the *Lactobacillus acidophilus* FNCC 0051, which also ~~increased~~ served to increase the diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness of the hydrogel. ~~The~~ Moreover, the hydrogel's prebiotic activity score was higher than that of inulin after 24 h of incubation, reflecting its role as a cell encapsulant, particularly when it comes to~~in~~ maintaining cells during exposure to simulated gastrointestinal fluid. ~~Cell~~ The cell viability increased from 86% to 100% when immersed in intestinal juice, which is comparable to the increase achieved using alginate and konjac glucomannan hydrogels. Future animal studies are ~~needed~~ required to determine the cell viability in actual gastrointestinal conditions ~~a~~and assess~~nd~~ the health effects of the hydrogel.

Keywords: hydrogel; viability; glucomannan; chitosan; gastrointestinal.

Introduction

Glucomannan is a functional polysaccharide that can be extracted from *Amorphophallus* tubers. While the glucomannan obtained from *Amorphophallus konjac* has a number of popular and commercial uses, several ~~studies~~ research groups are currently investigating the potential of glucomannan derived from other sources. *Amorphophallus oncophyllus*, which is commonly known as porang, is a local glucomannan source in Indonesia~~,~~ ~~commonly known as porang~~ (Harmayani, Aprilia, ~~and~~ & Marsono, 2014~~;~~)(Yanuriati et al., 2017). It has ~~specific~~ several characteristics that differ from those of konjac, including the mannose/glucose molar ratio, degree of polymerization, and degree of acetylation, leading it to exhibit different solubility, viscosity, ~~water~~ water-holding capacity, and gelation properties (Harmayani, Aprilia, ~~and~~ & Marsono, 2014; Yanuriati et al., 2017). Therefore, the~~its~~ applications of porang may also differ depending on the function.

~~Hydrogels~~ A hydrogel ~~are~~ is ~~one~~ a kind of technological glucomannan product that leverages its gelation properties. ~~They~~ Hydrogels ~~can form~~are formed through interactions between glucomannan and other polymers ~~to form~~that lead to the formation of a three-dimensional polymeric network (Stasiak-Różańska et al., 2021). This characteristic results in hydrogels exhibiting~~has~~ potential as ~~an~~ encapsulants. A previous study used a hydrogel created by crosslinking konjac, glucomannan, and chitosan, which ~~has~~ was found to have many advantages, including ~~being naturally formed~~natural formation without the need for a crosslinker, self-assembly, tolerance to different pH levels, and ~~its demonstrated~~demonstrable ability ~~in encapsulating~~to encapsulate drugs, proteins, and enzymes (Du et al., 2006; Korkiatithaweechai et al., 2011). A similar study ~~on~~ involving hydrogels formed by means of the interaction ~~of~~ between porang glucomannan and chitosan ~~considered~~ investigated the production of the primary carboxymethyl glucomannan material, the compatibility of the substitution degree of the carboxymethyl glucomannan involved in the hydrogel formation, the effect of the polymer concentration on the glucomannan properties, and ~~its~~ the application in relation to probiotic encapsulation (Aprilia et al., 2017a, 2017b, 2021). ~~Its~~ The key innovation of the study was the use of porang, which has ~~different~~ characteristics that differ from those of other glucomannan sources, such as the solubility, viscosity, ~~water~~ water-holding capacity, degree of polymerization, degree of acetylation, purity, and X-ray diffraction (XRD) pattern (Harmayani, Aprilia, ~~&~~ and Marsono, 2014; Yanuriati et al., 2017). ~~Other~~ The other differences include the type of modification used (carboxymethylation) and ~~its~~ the use of the hydrogel as a probiotic encapsulant. ~~In~~ By contrast, ~~the previous study~~prior studies ~~used~~ made use of the oxidation method (Korkiatithaweechai et al., 2011) and encapsulated drugs, proteins, and enzymes (Du et al., 2006; Korkiatithaweechai et al., 2011).

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Style Definition: Comment Text

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Commented [.3]: The style guide of the Scientific World Journal requires that the abstract be no more than 300 words in length. The current abstract is an acceptable 154 words.

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~~This~~ However, given that living cells have different characteristics to inanimate compounds, the role of this new hydrogel~~'s role~~ in encapsulating probiotics needs to be further studied ~~since the living cells have different characteristics to inanimate compounds~~. ~~The~~ Indeed, the new capsules should ensure the survival of the probiotics during food processing and storage, in addition to ensuring ~~and~~ sufficient delivery when consumed (>10^6–10^7 colony forming units [CFU]/mL) ~~when consumed~~. Furthermore, ~~it also needs~~the capsules need to allow the probiotics to reach the lower gastrointestinal tract if they are to have a beneficial effect on humans. ~~Therefore~~Thus, the~~its~~ survival of the capsules during gastrointestinal digestion and their ~~its~~ ability to increase probiotic growth in the colon are~~is~~ important. Carbohydrates known to stimulate probiotic growth are ~~called~~ known as prebiotics. We previously optimized the probiotic encapsulation efficiency by varying the glucomannan concentration, and we also studied its role in protecting cells during pasteurization and cold storage (Aprilia et al., 2021). ~~However~~Yet, the~~its~~ role of the glucomannan concentration in protecting probiotic cells during digestion and ~~its~~ glucomannan's potential as a prebiotic remain unexplored.

~~This~~ The present study ~~aimed~~ sought to improve the probiotic encapsulation efficiency and properties of the hydrogel ~~formed by~~derived from glucomannan and chitosan by varying the cell concentration in an effort to increase the number of cells carried. It also ~~and examines~~ examined the effects of varying the cell concentration on ~~its~~ the hydrogel's physical properties, prebiotic activity score, and viability during simulated gastrointestinal exposure.

Materials and Methods

Materials

The primary material used in this study was glucomannan derived from porang tubers (*A. oncophyllus*), which was obtained from the Faculty of Agricultural Technology, Universitas Gadjah Mada (Yogyakarta, Indonesia). ~~Carboxymethylation~~ The carboxymethylation of the glucomannan ~~used~~ was performed using sodium chloroacetate, as previously described (Aprilia et al., 2017b). ~~The c~~The utilized chitosan, which had ~~with~~ a degree of deacetylation of 85%–89% ~~deacetylation~~, meaning that it me~~e~~ts established food quality criteria, was obtained from PT Biotech Surindo (Cirebon, West Java, Indonesia).

Preparation of the *Lactobacillus acidophilus* FNCC 0051 cells

The *L. acidophilus* FNCC 0051 cells used in this study were obtained from the Food and Nutrition Culture Collection (FNCC) of the~~,~~ Laboratory of Food Microbiology, Center for Food and Nutrition Studies, Universitas Gadjah Mada. ~~Cells~~ The cells, which were stored in a skim milk~~–~~glycerol suspension, were rejuvenated in~~in~~ de Man, Rogosa, and Sharpe (MRS) broth at 37°C overnight and then grown twice ~~successively~~. ~~The~~ Subsequently, the cell biomass was ~~then~~ harvested by means of centrifugation at 2400 g for 9 min at 4°C and then rinsed with saline solution.

Production of the hydrogel and determination of its encapsulation efficiency

The hydrogel was created by mixing porang glucomannan with chitosan using the complex coacervation method (Aprilia et al., 2021). ~~Encapsulation~~ The encapsulation of the probiotics in the hydrogel was ~~prepared~~ performed using~~with~~ three different cell numbers, namely ~~of~~ 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL. The cells were mixed with glucomannan ~~before~~ prior to the start of the coacervation process. The hydrogel's encapsulation efficiency was determined by releasing the cells ~~en~~trapped ~~cells in the hydrogel~~within it using a buffer solution at pH 8 and 37°C for 24 h (Aprilia et al., 2017b). The released cells were then ~~growth~~ grown in MRS agar to allow for the enumeration of the total viable cells. To calculate the encapsulation efficiency, the total viable cell number was~~s were~~ ~~then~~ divided by the number of initial cells ~~adding~~ added to the hydrogel mixture (Zeashan et al., 2020).

Determination of the hydrogel's properties

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Particle size, polydispersity index, and zeta potential

~~Particle~~ The particle size was estimated based on the hydrogel's diameter and simultaneously measured ~~simultaneously with~~on the basis of the polydispersity index using a particle size analyzer (SZ-100 series; Horiba ~~SZ-100 series;~~, Kyoto, Japan). The hydrogel's zeta potential was measured ~~with~~ using a Nano ZS Zetasizer (v.6.20; Malvern Instruments Ltd~~;~~ ., Malvern, UK).

Color

The hydrogel was freeze-dried and ground ~~before~~ prior to the color measurement. ~~Values of~~The redness (a*), yellowness (b*), and lightness (L*) values were determined ~~with~~ using a CR200 chromameter (Minolta~~,~~ ., Osaka, Japan). The whiteness index was calculated as previously described (Akgün, Ova Özcan, ~~and~~ & Övez, 2022).

Crystallinity percentage

The XRD of the hydrogels ~~was~~ determined ~~by~~ using a ~~Shimadzu~~ LabX XRD-6000 diffractometer (Shimadzu, Kyoto, Japan) equipped with a Cu Kα target at 40 kV and 30 mA, which had ~~with~~ a scanning rate of 4°/min. The pattern was collected in the 2θ range between 3.02 and 90°. ~~Crystallinity~~ The crystallinity percentage (%) was calculated by dividing the area under the peaks by the total area under the curve ~~area~~ (Yazdani et al., 2020).

Determination of the prebiotic activity score

The prebiotic activity score was calculated by subtracting the ratio of probiotic cell growth with prebiotics and glucose from the ratio of enteric cell growth with prebiotics and glucose, as previously described (Huebner, Wehling, ~~and~~ & Hutkins, 2007). The probiotic used was *L. acidophilus* FNCC 0051, ~~while~~ whereas the enteric cells used were *Escherichia coli* FNCC 0091. The test was performed by adding 1% (volume/volume [~~(~~v~~o~~l/v~~o~~l]) ~~of~~ probiotic cells into MRS broth containing 2% (weight/volume [w/v]) glucose or prebiotic and adding 1% (v/v) ~~of~~ enteric cells into M9 broth containing 2% (w/v) glucose or prebiotic. The cells were incubated at 37°C for 0 h, 24 h, and 48 h and then enumerated by means of the plate count method using MRS and nutrient agar. Each test was ~~replicated~~ performed three times.

Determination of *L. acidophilus* FNCC 0051 survival during exposure to simulated gastrointestinal conditions

~~Simulated~~ The utilized simulated gastric and intestinal juices were prepared ~~as~~ according to the method described by Xu et al. (2016). ~~Gastric~~ More specifically, the gastric juice was prepared by mixing 7 mL of pepsin in hydrochloric acid, 2 g of sodium chloride, and 1 M of sodium hydroxide. ~~Intestinal~~ The intestinal juice was prepared by mixing 1% pancreatic powder, 6.8 g of potassium dihydrogen phosphate, and 77 mL of 0.2 N sodium hydroxide. ~~Either~~ Next, 1 g of either ~~1 g of~~ free or encapsulated cells (in ~~the~~ hydrogel ~~of~~ derived from porang glucomannan-chitosan, konjac glucomannan-chitosan, and calcium alginate) was mixed with 9 mL of simulated gastrointestinal juices and incubated at 37°C for 120 min. The samples were withdrawn at intervals of 0 min, 30 min, 60 min, and 120 min to reflect~~for~~ gastric juice digestion and 0 min, 60 min, 90 min, and 120 min ~~for~~ to reflect intestinal juice digestion (Rather et al., 2017). The hydrogel was then rinsed twice with acetate buffer. The cells were enumerated using the pour plate technique on MRS agar after 48 h of incubation. The number of viable cells ~~after~~ following exposure was divided by the initial number of cells in order to determine the~~ir~~ cell survival rate during exposure to simulated gastrointestinal conditions (Zeashan et al., 2020). The hydrogel's appearance during exposure to simulated gastrointestinal conditions was observed using~~with~~ an optical BX51 microscope (Olympus Corp~~.~~ ., Tokyo, Japan) and an OptiLab pro digital camera (Miconos, Indonesia).

Results and Discussion

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Commented [.17]: Abbreviations should be spelled out upon first use, followed by the abbreviation itself in parentheses. Subsequently, only the abbreviation needs to be used in the text.

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Encapsulation ~~efficiency~~ efficiencies of hydrogels ~~in~~ with different numbers of cells

The encapsulation ~~efficiency~~ efficiencies of hydrogels ~~with~~in different numbers of initial cells ~~were shown~~are shown in **Table 1**. The data ~~showed~~ revealed that the encapsulation ~~efficiency~~ efficiencies of the hydrogels ~~was~~ ranged between 44.37%~~-~~ and 85.03%. The highest encapsulation efficiency was achieved when 10 log CFU/mL of cells was added to the mixture~~.~~, which~~This number~~ exceeded the Food and Agricultural Organization of the United Nations (FAO) criteria for probiotic products ~~(of~~ >6–7 log CFU/mL; ~~(~~Priya, Vijayalakshmi, ~~and~~ & Raichur, 2011). Previous studies using different encapsulants obtained different encapsulation efficiencies. For ~~example~~instance, the encapsulation of *L. acidophilus* in ~~the~~ hydrogel formed from sodium alginate and soy protein isolates achieved an encapsulation efficiency of 95%–98% ~~encapsulation efficiency~~, ~~while~~ whereas the encapsulation of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* in an emulsion achieved an encapsulation efficiency of 97%–99% ~~efficiency~~ (Mahmoodi Pour, Marhamatizadeh, & Fattahi, 2022; Zeashan et al., 2020~~; Mahmoodi Pour, Marhamatizadeh, and Fattahi, 2022~~). ~~Differences~~ The differences in the achieved encapsulation ~~efficiency~~ efficiencies might reflect the different encapsulant types and ~~the~~ encapsulation methods used (Zeashan et al., 2020). We previously showed that the same ratio of glucomannan and chitosan affected the encapsulation efficiency due to the chemical bonding of both polymers ~~and~~ as well as due to the difference in electrostatic values between the core and the polymer influencing the degree of cell entrapment (Aprilia et al., 2021).

Table 1. ~~The e~~Encapsulated cell numbers and hydrogel encapsulation ~~efficiency~~ efficiencies with different initial cell numbers.

Hydrogels with different cell concentrations (log CFU/mL)	Cell concentration before encapsulation (log CFU/mL)	Cell concentration after encapsulation (log CFU/g)	Encapsulation efficiency (%)
8	9.39 ± 0.00	4.47 ± 0.18	44.37±1.91[a]
9	9.56 ± 0.00	6.60 ± 0.13	65.83±1.37[b]
10	10.10 ± 0.00	7.94 ± 0.21	85.03±0.63[c]

Values represent the mean ± standard deviation (SD). Different superscript letters in the same column indicate significantly different results at the level of $p < 0.05$.

Properties of the hydrogels ~~in~~ with different cell concentrations ~~of cells~~

The appearance of the hydrogels generated from glucomannan and chitosan containing *L. acidophilus* was as shown in **Figure 1**. The polymer solution was clear before the encapsulation process, although it ~~and~~ became turbid after the encapsulation process. ~~It~~ This proved that there wasprovided evidence of the formation of particles that influenced the turbidity of the solution. After the drying process, the hydrogels exhibited a shape ~~looks like~~similar to that of ~~a~~ white cotton. The particle sizes and color values ~~of colors~~ of the hydrogels ~~were~~will be explained ~~in the next paragraph~~below.

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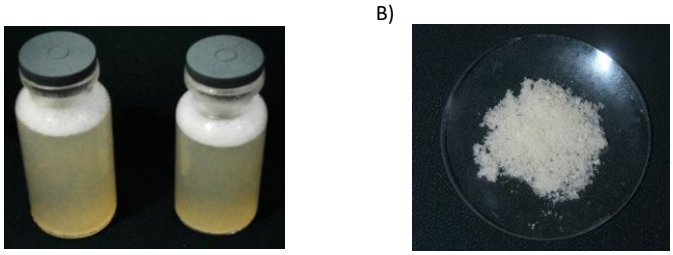

Figure 1. The appearance of hydrogels A) before drying and B) after the drying process.

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The sizes of the hydrogels encapsulating *L. acidophilus* was were measured found to be in the range of 0.7 µm to –9 µm, with most having a diameter of 2 µm to –3 µm (**Table 2**). Hydrogels Those hydrogels determined to be <100 µm in diameter are were classified as microgels. The cell concentration of cells significantly influenced the hydrogels' particle size ($p < 0.05$). The In fact, the more cells encapsulated within the a given hydrogel, the greater its diameter. It The particle size was also correlated with the encapsulation efficiency (**Table 1**), as since more cores can could be entrapped within larger hydrogel particles. The other factors influencing found to influence the particle size were the concentration and viscosity of the solution (Aprilia et al., 2021; Zeashan et al., 2020;). Aprilia *et al.*, 2021)

Table 2. Hydrogel pParticle sizes, polydispersity indexindexes, and zeta potentials of hydrogels with different initial cell concentrations.

Initial cell concentration (log CFU/mL)	Particle size (µm)	Polydispersity index	Zeta potential (mV)
8	2.23±0.11^{a}	1.23±0.17^{a}	24.40±0.75^{a}
9	2.79±0.19^{b}	1.39±0.04^{ab}	32.28±0.80^{b}
10	3.41±0.14^{c}	1.65±0.27^{b}	14.58±0.97^{c}

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

The polydispersity indexes of the hydrogel encapsulated cells were all >1 (**Table 2**), indicating a the broad particle distribution of particles of various sizes. The Overall, the index began to change when the initial cell concentration was 10 log CFU/mL. The Moreover, the greater the initial cell concentration, the higher the polydispersity index. This result result contrasts with the result of a previous study that found that the glucomannan concentration did to not influence the polydispersity index (Aprilia et al., 2021).

Hydrogel The hydrogels' zeta potentials became more electropositive as the cell concentration increased from 8 to 9 log CFU/mL but then decreased toas the cell concentration reached 10 log CFU/mL (**Table 2**). An increase in the number of cells number should cause result in a reduction in the hydrogel's charge due to the positive charge of empty hydrogels and the negative charge of cells (Aprilia et al., 2021), including *L. acidophilus* (Priya, Vijayalakshmi, & and Raichur, 2011). The observed pattern might be due tostem from the zeta potential being measured on the hydrogel's surface, which can bemeaning that it could have been affected by the pH of the surrounding environment (Barbosa et al., 2019).

The L*, b*, and whiteness values of the hydrogels increased after adding the addition of cells, while whereas the a* value decreased (**Table 3**). The utilized instrument determines determined these values based on the reflection by the cells of a direct light beam from a chromameter by the cells. Therefore, the more cells encapsulated within the hydrogel, the greater the reflection. Bacteria may also generate a distinct shades of colors like such as red. Based on the findings of a priorprevious study, *Lactobacillus pluvialis* could reflect an orange color from the pigment of canthaxanthin (Venil, Dufossé, & and Renuka Devi, 2020). This was finding is in agreement with this the present result, especially in terms of the increase of in the b* value after following the addition of *L. acidophilus.*

Table 3. Color values Hydrogel of hydrogels color values with different initial cell concentrations.

Initial cell concentration (log CFU/mL)	L*	a*	b*	Whiteness
control	65.06±0.12^{a}	7.02±0.09^{a}	12.50±0.08^{a}	62.24±0.15^{a}
8	76.97±0.32^{b}	5.42±0.01^{b}	14.24±0.11^{b}	72.38±0.21^{b}
9	79.48±0.33^{c}	5.61±0.07^{b}	15.14±0.01^{c}	73.89±0.25^{c}
10	77.39±0.23^{b}	4.22±0.23^{c}	13.24±0.13^{d}	73.46±0.30^{c}

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

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The XRD spectra represent the interaction between the diffraction intensity and the angle (**Figure 2**). ~~A~~ Moreover, a crystalline state ~~was~~ is indicated by the sharp diffraction peak, ~~while~~ whereas ~~the~~ an amorphous and solid state ~~was~~ is indicated by the declivous peak (Yanuriati et al., 2017). The X-ray diffractogram patterns of all the hydrogels showed a very broad band at 2θ between 5° and ~~–~~90°. In addition, all the hydrogels ~~had~~ exhibited~~almost the same~~ nearly identical highest peaks at around 2θ 7.06°–10.46°, 7.62°–11.00°, 7.48°–10.94°, and 7.16°–11.20° for those hydrogels without cells and with cells at concentrations of ~~log~~ 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively. These results differ from those concerning porang glucomannan, which exhibited~~had~~ its highest peaks at around 19°–20° and 35° (Yanuriati et al., 2017). However, there was a small peak in all the samples at around 2θ 10.5°, indicating the ~~existence~~ presence of chitosan (Yu, Lu, ~~&and~~ Xiao, 2007). This observation suggests that the mixture ~~between~~ of glucomannan hydrogel and cells strengthened the~~ir~~ associated chemical interaction, which is consistent with previous Fourier-transform infrared spectroscopy (FTIR) findings ~~(Aprilia et al., 2021), (Aprilia et al., 2021). It also suggests and~~ that some chitosan ~~had not interacted~~did not interact with the glucomannan. A ~~previous~~ prior study reported that the Schiff's crosslinking between glucomannan aldehyde groups and chitosan amino groups could suppress the chitosan's crystalline state, which is usually strengthened by ~~a~~ the hydrogen bond between the amino and hydroxyl groups (Yu, Lu, ~~and~~ & Xiao, 2007). We also found evidence of low crystallinity, with values of 26%, 25%, 17%, and 21% being determined for the hydrogels without cells and with cells at concentrations of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively. The addition of *L. acidophilus* appeared to have no effect on the diffraction peak, indicating that the entrapment of ~~-~~microbes within the hydrogel did not affect the interaction between the glucomannan and chitosan.

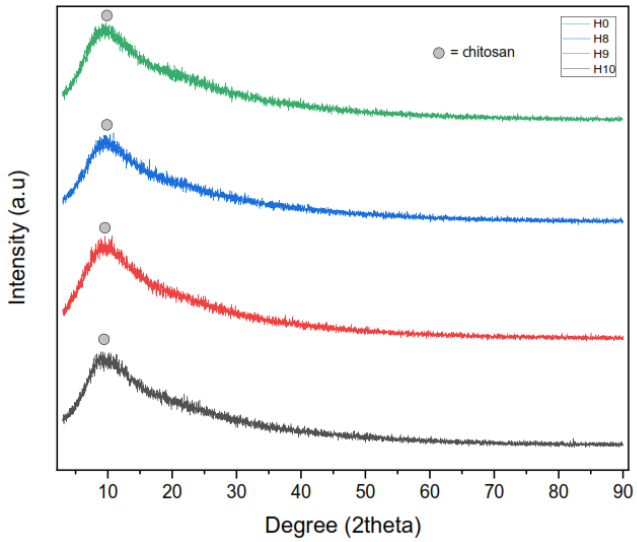

Figure 2. X-ray diffractogram for H0 (hydrogel without *L. acidophilus*), H8, H9, and H10 (hydrogels with *L. acidophilus* at concentrations of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively).

~~Hydrogel p~~Prebiotic activity of the hydrogels

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The *L. acidophilus* and *E. coli* cell density increased during 0 h, 24 h, and 48 hours of incubation in the presence of carbohydrates, glucose, inulin, and hydrogel (**Table 4**). Both bacteria showed no significant increase in almost all the carbohydrates, except for *L. acidophilus* with inulin and *E. coli* with glucose. These data suggest that only inulin can is able to specifically stimulate the growth of good bacteria and suppress the growth of enteric bacteria, which is consistent with its well-knownwidespread use as a commercial prebiotic worldwide.

Table 4. The dDensity of *L. acidophilus* FNCC 0051 cells in log 10 (CFU/mL) after 0 h, 24 h, and 48 hours of incubation with prebiotics, inulin, hydrogel, and glucose.

Prebiotic	*L. acidophilus*			*E. coli*		
	0 h	24 h	48 h	0 h	24 h	48 h
Glucose	6.94±1.32^{a}	8.35±0.81^{a}	9.17±0.01^{a}	6.65±0.92^{a}	8.54±0.09^{ab}	9.29±0.49^{b}
Inulin	6.59±0.19^{a}	7.33±0.49^{ab}	8.48±0.88^{b}	9.53±0.09^{a}	7.59±0.32^{a}	8.47±0.75^{a}
Hydrogel	9.37±0.10^{a}	9.58±0.46^{a}	10.15±0.21^{a}	8.80±1.13^{a}	8.17±0.86^{a}	9.02±2.18^{a}

Values represent the mean ± SD. Different superscript letters in the same row indicate significantly different results at the level of $p < 0.05$.

The prebiotic potential of the hydrogel was compared with that of inulin using on the basis of the prebiotic activity scores (**Figure 3**). The prebiotic activity score of the hydrogel was higher than that of inulin after 24 h of incubation, although it was reduced but became lower after 48 h, suggesting that the hydrogel was a the preferred energy source for the cells. This result is consistent with the XRD findings, which that confirmed the hydrogel to have an amorphous hydrogel state and, which has no long-range order, making it easier to digest,. Moreover,and the amount of carbohydrates will decrease with time. MeanwhileBy contrast, the known prebiotic inulin (Kamel et al., 2021) needed required a longer time to be available for the bacteria since it hasdue to its long polymeric carbon chains—, that is, chains of around 2–60 molecules (Samolińska and & Grela, 2017).

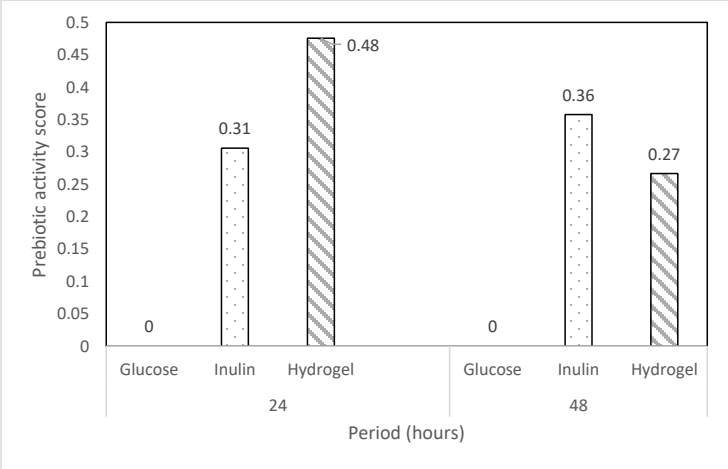

Figure 3. Prebiotic activity scores of *L. acidophilus* FNCC 0051 on glucose, inulin, and hydrogel.

Cell survival during exposure to simulated gastrointestinal conditions

Cell survival during exposure to gastric juice

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The *L. acidophilus* showed good viability during exposure to gastric juice at pH 2, ~~either~~ whether in its free form or when encapsulated in hydrogel (**Figure 4**). Generally, ~~T~~the growth of lactic acid bacteria is ~~generally~~ optimum at pH 6–7 (close to neutral pH). Some metabolic reactions change when the pH is <5 or <4.4. Indeed, some minerals will be lost at pH ≤2, ~~and~~ while prolonged storage at a low pH will increase the risk of cell death (Hayek dan Ibrahim, 2013). Our results in this regard are consistent with those of ~~a~~ previous ~~study~~ studies (Stasiak-Różańska et al., 2021; Zeashan et al., 2020)~~; Stasiak-Różańska *et al.*, 2021~~). Further study~~ies** are ~~needed~~ required to determine the effect of~~on~~ solid or solid-enriched macronutrient foods with a longer transit time (Müller, Canfora, ~~and~~ & Blaak, 2018). In addition, a shorter exposure time within the stomach enables cells to maintain homeostasis between the internal and external pH, which potentially ~~influencing~~ influenced the good viability ~~shown~~ found in this study.

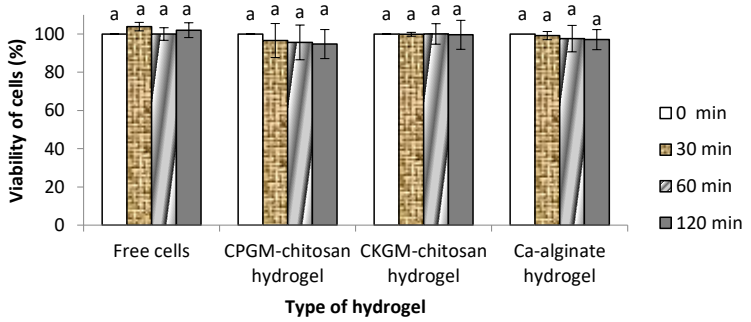

Figure 4. *L. acidophilus* FNCC 0051 viability during exposure to gastric juice for 120 min. Key: a, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

~~This~~ The present study also found that porang glucomannan-chitosan hydrogel might ~~have~~ exhibit a similar ability to protect cells ~~protecting~~ ~~ability~~ from the gastric environment as both konjac glucomannan–chitosan hydrogel and calcium-alginate hydrogel ($p > 0.05$). This finding accords with the ability of alginate ~~also~~ to protect~~ed~~ *L. plantarum* (Rather et al., 2017) and ~~–~~*Lactobacillus rhamnosus* from this harsh environment ~~for~~ over the course of 3 h of exposure (Oberoi et al., 2021).

The hydrogel was stable in the simulated gastric juice ~~for~~ throughout 120 min of exposure (**Figure 5**), which is consistent with the result of a previous swelling ratio study (Aprilia et al., 2021) that ~~found~~ ~~the~~determined the hydrogel ~~did~~ to not ~~deswell~~ deswell at ~~the~~ a pH <5. Deswelling causes ~~the~~ hydrogel to become smaller, which was previously thought to result in the release of cells from the hydrogel. However, the cells are still ~~en~~trapped in the hydrogel (**Figure 5**), which perhaps ~~reflecting~~ reflects the stronger electrostatic interaction between the glucomannan carbonyl group and ~~the~~ chitosan amine group in an acid environment (Aprilia et al., 2021). ~~Cells~~ The cells remain in the hydrogel because this interaction maintains the core. ~~Therefore~~Thus, deswelling could not be maximized, leading to only a small number of cells being released from the hydrogel. ~~There is a possibility~~It is possible that some empty hydrogels will shrink to the extent that they are no longer visible ~~at~~ after 60 min of exposure. These results are consistent with those of other studies ~~using~~ using hydrogels made from oxidized glucomannan and chitosan to ~~en~~trap diclofenac drugs, which ~~that~~ found <1% of cells to be~~was~~ released during exposure to simulated gastric fluid at pH 1.2 (Korkiatithaweechai et al., 2011). This ~~result~~ shows that the hydrogel cores were not released when ~~it~~ the hydrogel was exposed to low pH conditions.

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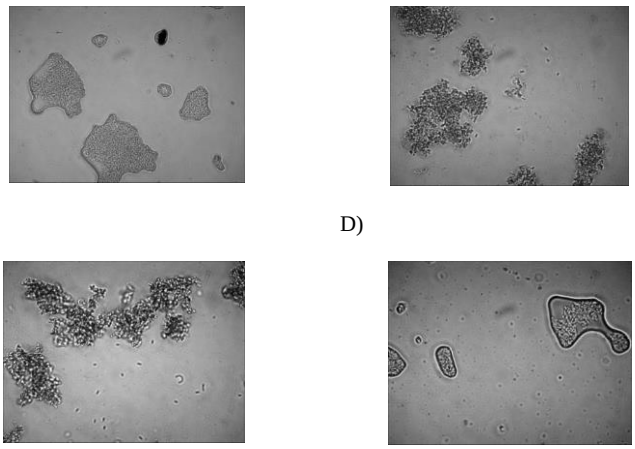

Figure 5. Microscopic appearance of hydrogel~~s~~ containing *L. acidophilus* FNCC 0051 (1300 × magnification) during exposure to gastric juice for (**A**) 0 min, (**B**) 30 min, (**C**) 60 min, and (**D**) 120 min.

Cell survival during exposure to intestinal juice

The viability of the free cells decreased significantly during exposure to intestinal juice for 60 min (**Figure 6**; $p < 0.05$). ~~Indeed~~Yet, the viability of the cells encapsulated in the hydrogel ~~could b~~wase maintained over 120 min of exposure, indicating that the encapsulation ~~increases~~ increased the viability of the *L. acidophilus* ~~viability~~. A decrease in the number of free cells may reflect cell death, which can be caused by factors other ~~factors other~~ than the pH of the medium. Priya et al. (2011) reported that while ~~,~~ bacteria showed good growth at pH 6.8, the presence of pancreatin~~,~~ (comprising amylase, trypsin, lipase, ribonuclease, and protease~~,~~) damaged the encapsulation wall, ~~causing~~ thereby resulting in cell death.

Figure 6 indicates that the porang glucomannan hydrogel ~~has~~ exhibited the same level of good protective effect as the konjac-chitosan glucomannan and ~~calcium~~ calcium-alginate hydrogels. In this study, the alginate-based hydrogel was used for the purpose of comparison ~~since~~ because it is widely used as an encapsulant due to its low price, good biocompatibility, and nontoxicity. A ~~previous~~ prior study ~~showed~~ found that the probiotic encapsulation of alginate increased ~~entrapped~~ the viability of the trapped cells when ~~viability~~ compared ~~to~~ with the free cells during exposure to a simulated gastrointestinal condition (Stasiak-Różańska et al., 2021). Therefore, the porang-chitosan glucomannan hydrogel ~~has~~ shows potential as a bacterial encapsulant.

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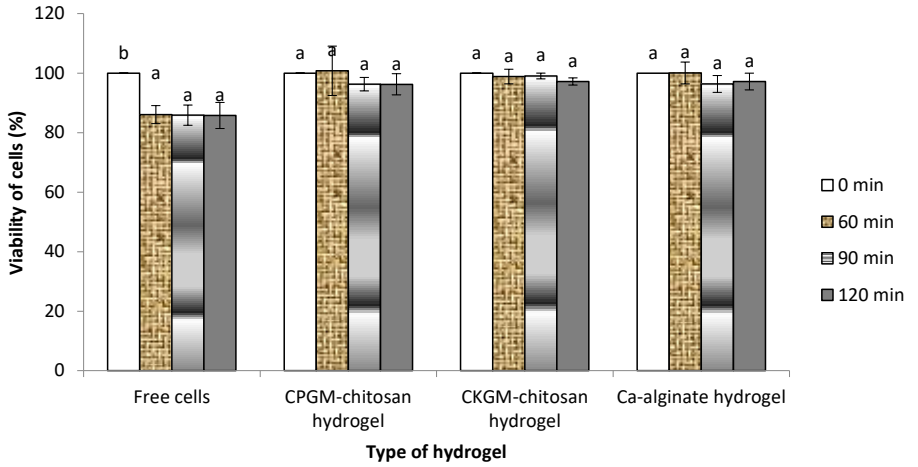

Figure 6. *L. acidophilus* FNCC 0051 cell viability during exposure to intestinal juice for 120 min. Key: a or b, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

The ~~hydrogel's~~ hydrogel's microscopic appearance was used to confirm the cell viability data. ~~Porang~~ Here, the porang glucomannan-chitosan hydrogel ~~was~~ remained stable for up to 2 h in the intestinal fluid. However, it was found to be larger after 60 min of exposure than after 0 min ~~exposure~~ (**Figure 7**), potentially reflecting its swelling behavior at pH 6.8. We ~~previously showed~~have previously shown that porang glucomannan-chitosan hydrogel begins to swell at pH >5 (Aprilia et al., 2021). The swelling of the hydrogel was evident until it reached 90 min of exposure. Moreover, many small hydrogels and cells were visible in the solution after 120 min of exposure. The swelling weakened the interaction of the hydrogels~~s~~, leading to some parts ~~of the hydrogel~~ being dissolved, ~~resulting~~ which resulted in both smaller hydrogels~~s~~ and the release of cells from the hydrogels. This result is consistent with that of another study that found konjac ~~glucomannan~~ glucomannan-carboxymethyl chitosan hydrogel with a bovine serum albumin core ~~showed~~ to show greater core release at pH 7.4 than at pH 5 due to the swelling enlarging its pores (Du et al., 2006). This core release also occurred when a chitosan-oxidized glucomannan hydrogel was exposed to simulated ~~intestine~~ intestinal fluid for 2–8 h (Korkiatithaweechai et al., 2011).

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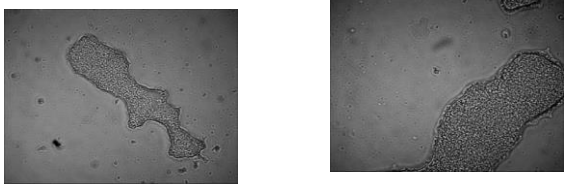

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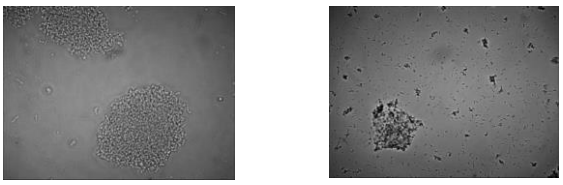

Figure 7. Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (1300 × magnification) during exposure to intestinal juice for (**A**) 0 min, (**B**) 30 min, (**C**) 60 min, and (**D**) 120 min.

Conclusions

The encapsulation of *L. acidophilus* in ~~a~~ hydrogel made from glucomannan and chitosan was improved by varying the concentration of the cells added. ~~Higher~~ In fact, higher concentrations ~~showed~~ were found to be associated with greater encapsulation efficiency, diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness. In addition, the hydrogel ~~showed~~ exhibited potential as a prebiotic, particularly after 24 h of incubation. ~~Moreover, t~~Moreover, the hydrogel protected the encapsulated cells, maintaining them during exposure to simulated gastrointestinal fluid. ~~Furthermore,~~Furthermore, the cell viability increased from 86% to 100% when ~~it~~ the hydrogel was exposed to intestinal juice, which was, comparable to the performance of the ~~to~~ alginate and konjac glucomannan hydrogels. Further animal studies are ~~needed~~ required to determine the cell viability in actual gastrointestinal conditions and assess the health effects of the hydrogel.

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10. Submit Revisi 2: 6 Oktober 2024

October 6, 2022

Dear Editor in Chief of The Scientific World Journal,

Please accept our revision entitled, **"Hydrogel from glucomannan-chitosan to improve survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid**" for consideration for publication in The Scientific World Journal.

We had revised all of the suggestion and correction from the reviewer. Please contact me if you have any question or concern regarding the manuscript. I look forward to receiving the results of the review.

Sincerely

Prof. Dr. Ir. Eni Harmayani, M.Sc

Responses the reviewer's comments

Manuscript ID:
Title: Hydrogel from glucomannan–chitosan to improve survival of *L. acidophilus* FNCC 0051 in simulated gastrointestinal fluid

Reviewer 2

No.	Reviewer's Comments	**Responses** (for author)
1.	It seems well-conducted and written and could be of interest to the readers of the Journal. I have no particular concerns to raise	There was no revision request.

Reviewer B

No.	Reviewer's Comments	**Responses** (for author)
1	The manuscript needs to be polished by an English native speaker to remove the grammatical errors throughout the manuscripts	We have proofread it by The Cambridge Proofreading LLC and Scribendi. The certificate is attached below.
2	It is absolutely essential you use the most recent papers in your manuscript. Unfortunately, you have employed older references and also the number of references is very low for a research article	We have added most recent papers and deleted some of old references. The added papers are shown in red fonts and the deleted papers are shown in "review version". Now 16 out of 25 references are recent papers (not more than 5 years)
3	The discussion section is not highlighted very well in your manuscript and it needs to review the previous research to reveal that what novelties are used in this study compared with others.	We have revised it in all of discussion section. The previous research are added in lines:151-162; 184-186; 195-197; 200-205; 108-213; 225-239; 265-268; 277-283; 290-292; 293-294; 297-299; 302-305; 318-320; 324-328; 336-337; 341-345.
4	The results also need to be revised and expanded. You need to use more tables and especially figures. Your current data is not sufficient to show the novelty of your work. It does not motivate the reviewers to read the manuscript when data has nothing to bring.	We have added the data presented in Table 3, Table 4, Figure 1, Figure 2, Figure 3.
5	The material and methods are written briefly and need to be expanded.	We have revised the material and method accordingly (line 78-143).
6	The name of the bacterial strains are not written fully when the authors have mentioned them for the first time. You should use the full name of the bacteria (Lactobacillus acidophilus) and then write L. acidophilus	Have been revised (line 2, 8)

7	In your opinion, what are the new aspects of this study compared with previous studies.? A huge amount of earlier research has investigated the role of hydrogels and nanoparticles on the viability of probiotics. The prior studies have investigated very well the effect of the hydrogels on the bacteria compared with your studies.	Our study investigated the hydrogel from porang glucomannan and chitosan that was applicated as bacterial encapsulant. The novelty of our findings are as follow: 1. We used porang glucomannan that has different character with konjac glucomannan (Line 20-22, 33-37, 42-49) 2. We applicated the hydrogel as probiotic encapsulant that has different character with the inanimate objects (line 48-49). We had to ensure that probiotic is still viable during processing and in gastrointestinal fluid.

Editorial Certificate

EDITORIAL

CERTIFICATE

Authors:	Document title:	Date Issued:
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~~Hydrogel~~ Use of hydrogel derived from glucomannan-chitosan to improve the survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid

Abstract

~~The~~ The probiotic encapsulating hydrogel ~~made~~ derived from porang (*Amorphophallus oncophyllus*) glucomannan~~,~~ and chitosan was investigated ~~for~~ with regard to its encapsulation efficiency, physical properties, prebiotic activity, and survival under simulated gastrointestinal conditions. ~~Encapsulation~~ The hydrogel's encapsulation efficiency was improved by varying the number ~~concentration~~ of the *Lactobacillus acidophilus* FNCC 0051, which also ~~increased~~ served to increase the diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness of the hydrogel. ~~The~~ Moreover, the hydrogel's prebiotic activity score was higher than that of inulin after 24 h of incubation, reflecting its role as a cell encapsulant, particularly when it comes to~~in~~ maintaining cells during exposure to simulated gastrointestinal fluid. ~~Cell~~ The cell viability increased from 86% to 100% when immersed in intestinal juice, which is comparable to the increase achieved using alginate and konjac glucomannan hydrogels. Future animal studies are ~~needed~~ required to determine the cell viability in actual gastrointestinal conditions ~~a~~and assess~~nd~~ the health effects of the hydrogel.

Keywords: hydrogel; viability; glucomannan; chitosan; gastrointestinal.

Introduction

Glucomannan is a functional polysaccharide that can be extracted from *Amorphophallus* tubers. While the glucomannan obtained from *Amorphophallus konjac* has a number of popular and commercial uses, several ~~studies~~ research groups are currently investigating the potential of glucomannan derived from other sources. *Amorphophallus oncophyllus*, which is commonly known as porang, is a local glucomannan source in Indonesia~~, commonly known as porang~~ (Harmayani, Aprilia, ~~and~~ & Marsono, 2014~~;~~)(Yanuriati et al., 2017). It has ~~specific~~ several characteristics that differ from those of konjac, including mannose/glucose ~~the~~ ~~mannose/glucose~~ molar ratio, degree of polymerization, and degree of acetylation, leading it to exhibit different solubility, viscosity, ~~water~~ ~~water~~water-holding capacity, and gelation properties (Harmayani, Aprilia, ~~and~~ & Marsono, 2014; Yanuriati et al., 2017). Therefore, the~~its~~ applications of porang may also differ depending on the function.

~~Hydrogels~~ A hydrogel ~~are~~ is ~~one~~ a kind of technological glucomannan product that leverages its gelation properties. ~~They~~ Hydrogels ~~can form~~are formed through ~~-~~interactions between glucomannan and other polymers ~~to form~~that lead to the formation of a three-dimensional polymeric network (Stasiak-Różańska et al., 2021). This characteristic results in hydrogels exhibiting~~has~~ potential as ~~an~~ encapsulants. A previous study used a hydrogel created by crosslinking konjac, glucomannan, and chitosan, which ~~has~~ was found to have many advantages, including ~~being naturally formed~~natural formation without the need for a crosslinker, self-assembly, tolerance to different pH levels, and ~~its demonstrated~~demonstrable ability ~~in encapsulating~~to encapsulate drugs, proteins, and enzymes (Du et al., 2006; Korkiatithaweechai et al., 2011). A similar study ~~on~~ involving hydrogels formed by means of the interaction ~~of~~ between porang glucomannan and chitosan ~~considered~~ investigated the production of the primary carboxymethyl glucomannan material, the compatibility of the substitution degree of the carboxymethyl glucomannan involved in the hydrogel formation, the effect of the polymer concentration on the glucomannan properties, and ~~its~~ the application in relation to probiotic encapsulation (Aprilia et al., 2017a, 2017b, 2021). ~~Its~~ The key innovation of the study was the use of porang, which has ~~different~~ characteristics that differ from those of other glucomannan sources, such as the solubility, viscosity, ~~water~~ water-holding capacity, degree of polymerization, degree of acetylation, purity, and X-ray diffraction (XRD) pattern (Harmayani, Aprilia, ~~&~~ ~~and~~ Marsono, 2014; Yanuriati et al., 2017). ~~Other~~ The other differences include the type of modification used (carboxymethylation) and ~~its~~ the use of the hydrogel as a probiotic encapsulant. ~~In~~ By contrast, ~~the previous~~

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The name of the bacterial strains has been revised (written fully when the authors have mentioned them for the first time)

~~study~~prior studies ~~used~~ made use of the oxidation method (Korkiatithaweechai et al., 2011) and encapsulated drugs, proteins, and enzymes (Du et al., 2006; Korkiatithaweechai et al., 2011).

~~This~~ However, given that living cells have different characteristics to inanimate compounds, the role of this new hydrogel~~'s role~~ in encapsulating probiotics needs to be further studied ~~since the living cells have different characteristics to inanimate compounds~~. ~~The~~ Indeed, the new capsules should ensure the survival of the probiotics during food processing and storage, in addition to ensuring ~~and~~ sufficient delivery when consumed (>10^6–10^7 colony forming units [CFU]/mL) ~~when consumed~~. Furthermore, ~~it also needs~~the capsules need to allow the probiotics to reach the lower gastrointestinal tract if they are to have a beneficial effect on humans. ~~Therefore~~Thus, the~~its~~ survival of the capsules during gastrointestinal digestion and their ~~its~~ ability to increase probiotic growth in the colon are~~is~~ important. Carbohydrates known to stimulate probiotic growth are ~~called~~ known as prebiotics. We previously optimized the probiotic encapsulation efficiency by varying the glucomannan concentration, and we also studied its role in protecting cells during pasteurization and cold storage (Aprilia et al., 2021). ~~However~~Yet, the~~its~~ role of the glucomannan concentration in protecting probiotic cells during digestion and ~~its~~ glucomannan's potential as a prebiotic remain unexplored.

~~This~~ The present study ~~aimed~~ sought to improve the probiotic encapsulation efficiency and properties of the hydrogel ~~formed by~~derived from glucomannan and chitosan by varying the cell ~~concentration~~ number in an effort to increase the number of cells carried. It also ~~and examines~~ examined the effects of varying the cell ~~concentration~~ number on ~~its~~ the hydrogel's physical properties, prebiotic activity score, and viability during simulated gastrointestinal exposure.

Materials and Methods

Materials

The primary material used in this study was glucomannan derived from porang tubers (*A. oncophyllus*), which was obtained from the Faculty of Agricultural Technology, Universitas Gadjah Mada (Yogyakarta, Indonesia). ~~Carboxymethylation~~ The carboxymethylation of the glucomannan ~~used~~ was performed using sodium chloroacetate, as previously described (Aprilia et al., 2017b). ~~The c~~The utilized chitosan, which had ~~with~~ a degree of deacetylation of 85%–89% ~~deacetylation~~, meaning that it me~~et~~s established food quality criteria, was obtained from PT Biotech Surindo (Cirebon, West Java, Indonesia).

Preparation of the *Lactobacillus acidophilus* FNCC 0051 cells

The *L. acidophilus* FNCC 0051 cells used in this study were obtained from the Food and Nutrition Culture Collection (FNCC) of the, Laboratory of Food Microbiology, Center for Food and Nutrition Studies, Universitas Gadjah Mada. ~~Cells~~ The cells, which were stored in a skim milk–glycerol suspension, were rejuvenated in~~in~~ de Man, Rogosa, and Sharpe (MRS) broth at 37°C overnight and then grown twice ~~successively~~. ~~The~~ Subsequently, the cell biomass was ~~then~~ harvested by means of centrifugation at 2400 g for 9 min at 4°C and then rinsed with saline solution.

Production of the hydrogel and determination of its encapsulation efficiency

The hydrogel was created by mixing porang glucomannan with chitosan using the complex coacervation method (Aprilia et al., 2021). ~~Encapsulation~~ The encapsulation of the probiotics in the hydrogel was ~~prepared~~ performed using~~with~~ three different cell numbers, namely ~~of~~ 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL. The cells were mixed with glucomannan ~~before~~ prior to the start of the coacervation process. ~~.~~The hydrogel's encapsulation efficiency was determined by releasing the cells ~~en~~trapped ~~cells in the hydrogel~~within it using a buffer solution at pH 8 and 37°C for 24 h (Aprilia et al., 2017b). The released cells were then ~~growth~~ grown in MRS agar to allow for the enumeration of the total viable cells. To calculate the encapsulation efficiency, the ~~.~~ total viable cell number was~~s were then~~ divided by the number of initial cells ~~adding~~ added to the hydrogel mixture (Zeashan et al., 2020).

Commented [VA2]: The new use of hydrogel

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We have revised and expanded the material and methods (red fonts)

Determination of the hydrogel's properties

Particle size, polydispersity index, and zeta potential

Particle The particle size was estimated based on the hydrogel's diameter and simultaneously measured simultaneously withon the basis of the polydispersity index using a particle size analyzer (SZ-100 series; Horiba SZ-100 series;, Kyoto, Japan). The hydrogel's zeta potential was measured with using a Nano ZS Zetasizer (v.6.20; Malvern Instruments Ltd;., Malvern, UK).

Color

The hydrogel was freeze-dried and ground before prior to the color measurement. Values ofThe redness (a*), yellowness (b*), and lightness (L*) values were determined with using a CR200 chromameter (Minolta;., Osaka, Japan). The whiteness index was calculated as previously described (Akgün, Ova Özcan, and & Övez, 2022).

Crystallinity percentage

The XRD of the hydrogels was determined by using a Shimadzu LabX XRD-6000 diffractometer (Shimadzu, Kyoto, Japan) equipped with a Cu Kα target at 40 kV and 30 mA, which had with a scanning rate of 4°/min. The pattern was collected in the 2θ range between 3.02° and 90°. Crystallinity The crystallinity percentage (%) was calculated by dividing the area under the peaks by the total area under the curve area (Yazdani et al., 2020).

Determination of the prebiotic activity score

The prebiotic activity score was calculated by subtracting the ratio of probiotic cell growth with prebiotics and glucose from the ratio of enteric cell growth with prebiotics and glucose, as previously described (Huebner, Wehling, and & Hutkins, 2007). The probiotic used was *L. acidophilus* FNCC 0051, while whereas the enteric cells used were *Escherichia coli* FNCC 0091. The test was performed by adding 1% (volume/volume [(v/v)]) of probiotic cells into MRS broth containing 2% (weight/volume [w/v]) glucose or prebiotic and adding 1% (v/v) of enteric cells into M9 broth containing 2% (w/v) glucose or prebiotic. The cells were incubated at 37°C for 0 h, 24 h, and 48 h and then enumerated by means of the plate count method using MRS and nutrient agar. Each test was replicated performed three times.

Determination of *L. acidophilus* FNCC 0051 survival during exposure to simulated gastrointestinal conditions

Simulated The utilized simulated gastric and intestinal juices were prepared as according to the method described by Xu et al. (2016). Gastric More specifically, the gastric juice was prepared by mixing 7 mL of pepsin in hydrochloric acid, 2 g of sodium chloride, and 1 M of sodium hydroxide. Intestinal The intestinal juice was prepared by mixing 1% pancreatic powder, 6.8 g of potassium dihydrogen phosphate, and 77 mL of 0.2 N sodium hydroxide. Either Next, 1 g of either 1 g of free or encapsulated cells (in the hydrogel of derived from porang glucomannan-chitosan, konjac glucomannan-chitosan, and calcium alginate) was mixed with 9 mL of simulated gastrointestinal juices and incubated at 37°C for 120 min. The samples were withdrawn at intervals of 0 min, 30 min, 60 min, and 120 min to reflectfor gastric juice digestion and 0 min, 60 min, 90 min, and 120 min for to reflect intestinal juice digestion (Rather et al., 2017). The hydrogel was then rinsed twice with acetate buffer. The cells were enumerated using the pour plate technique on MRS agar after 48 h of incubation. The number of viable cells after following exposure was divided by the initial number of cells in order to determine their cell survival rate during exposure to simulated gastrointestinal conditions (Zeashan et al., 2020). The hydrogel's appearance during exposure to simulated gastrointestinal conditions was observed usingwith an optical BX51 microscope (Olympus Corp;., Tokyo, Japan) and an OptiLab pro digital camera (Miconos, Indonesia).

Results and Discussion

Encapsulation ~~efficiency~~ efficiencies of hydrogels ~~in~~ with different numbers of cells

The encapsulation ~~efficiency~~ efficiencies of hydrogels ~~within~~ different numbers of initial cells ~~were shown~~are shown in **Table 1**. The data ~~showed~~ revealed that the encapsulation ~~efficiency~~ efficiencies of the hydrogels ~~was~~ ranged between 44.37%~~-~~ and 85.03%. The highest encapsulation efficiency was achieved when 10 log CFU/mL of cells was added to the mixture~~.~~, which~~This number~~ exceed~~sed~~ the Food and Agricultural Organization of the United Nations (FAO) criteria for probiotic products (~~of~~ >6–7 log CFU/mL; ~~(~~Priya, Vijayalakshmi, ~~and~~ & Raichur, 2011). Previous studies using different encapsulants obtained different encapsulation efficiencies. For ~~example~~instance, the encapsulation of *L. acidophilus* in ~~the~~ hydrogel formed from sodium alginate and soy protein isolates achieved an encapsulation efficiency of 95%–98% ~~encapsulation efficiency~~, ~~while~~ whereas the encapsulation of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* in an emulsion achieved an encapsulation efficiency of 97%–99% ~~efficiency~~ (Mahmoodi Pour, Marhamatizadeh, & Fattahi, 2022; Zeashan et al., 2020~~; Mahmoodi Pour, Marhamatizadeh, and Fattahi, 2022~~). ~~Differences~~ The differences in the achieved encapsulation ~~efficiency~~ efficiencies might reflect the different encapsulant types and ~~the~~ encapsulation methods used (Zeashan et al., 2020). We previously showed that the same ratio of glucomannan and chitosan affected the encapsulation efficiency due to the chemical bonding of both polymers ~~and~~ as well as due to the difference in electrostatic values between the core and the polymer influencing the degree of cell entrapment (Aprilia et al., 2021).

Table 1. ~~The e~~Encapsulated cell numbers and hydrogel encapsulation ~~efficiency~~ efficiencies with different initial cell numbers.

Hydrogels with different cell ~~concentrations~~ numbers (log CFU/mL)	Cell ~~concentration~~ number before encapsulation (log CFU/mL)	Cell ~~concentration~~ number after encapsulation (log CFU/g)	Encapsulation efficiency (%)
8	9.39 ~~±~~ 0.00	4.47 ~~±~~ 0.18	44.37±1.91[a]
9	9.56 ~~±~~ 0.00	6.60 ~~±~~ 0.13	65.83±1.37[b]
10	10.10 ~~±~~ 0.00	7.94 ~~±~~ 0.21	85.03±0.63[c]

Values represent the mean ± standard deviation (SD). Different superscript letters in the same column indicate significantly different results at the level of $p < 0.05$.

Properties of the hydrogels ~~in~~ with different cell ~~concentrations~~ numbers ~~of cells~~

The appearance of the hydrogels generated from glucomannan and chitosan containing *L. acidophilus* was as shown in **Figure 1**. The polymer solution was clear before the encapsulation process, although it ~~and~~ became turbid after the encapsulation process. ~~It~~ This proved that there wasprovided evidence of the formation of particles that influenced the turbidity of the solution. After the drying process, the hydrogels exhibited a shape ~~looks like~~similar to that of ~~a~~ white cotton. The particle sizes and color values ~~of colors~~ of the hydrogels ~~were~~will be explained ~~in the next paragraph~~below.

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We have added the data and expanded the discussion. The data were presented in Table 3, Table 4, Figure 1, Figure 2, Figure 3.

Commented [VA5]: Response for Comments No. 3 of Reviewer B:

We added the properties of hydrogel, like the appearance, the colour measurements, and XRD spectra to characterize our product. Therefore, it can be the components that differs our research with other research.

A) B)

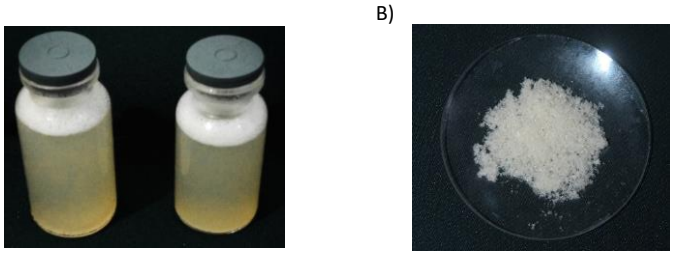

Figure 1. The appearance of hydrogels A) before drying and B) after the drying process.

Commented [VA6]: New data was added (yellow highlight)

The sizes of the hydrogels encapsulating *L. acidophilus* ~~was~~ were ~~measured~~ found to be in the range of 0.7 µm ~~to~~ –9 µm, with most having a diameter of 2 µm to –3 µm (**Table 2**). ~~Hydrogels~~ Those hydrogels determined to be <100 µm in diameter ~~are~~ were classified as microgels. The cell concentration ~~of cells~~ significantly influenced the hydrogels' particle size ($p < 0.05$). ~~The~~ In fact, the more cells encapsulated within ~~the~~ a given hydrogel, the greater its diameter. ~~It~~ The particle size was also correlated with the encapsulation efficiency (**Table 1**), ~~as since~~ more cores ~~can~~ could be ~~en~~trapped within larger hydrogel particles. The other factors ~~influencing~~ found to influence the particle size were the concentration and viscosity of the solution (Aprilia et al., 2021; Zeashan et al., 2020~~).~~). ~~Aprilia *et al.*, 2021)~~

Table 2. ~~Hydrogel p~~Particle sizes, polydispersity ~~index~~indexes, and zeta potentials of hydrogels with different initial cell concentrations.

Initial cell ~~concentration~~ number (log CFU/mL)	Particle size (µm)	Polydispersity index	Zeta potential (mV)
8	2.23±0.11[a]	1.23±0.17[a]	24.40±0.75[a]
9	2.79±0.19[b]	1.39±0.04[ab]	32.28±0.80[b]
10	3.41±0.14[c]	1.65±0.27[b]	14.58±0.97[c]

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

The polydispersity indexes of the hydrogel encapsulated cells were all >1 (**Table 2**), indicating ~~a~~ the broad ~~particle~~ distribution of particles of various sizes. ~~The~~ Overall, the index began to change when the initial cell number ~~concentration~~ was 10 log CFU/mL. ~~The~~ Moreover, the greater the initial cell ~~concentration~~number, the higher the polydispersity index. This ~~result~~ result contrasts with the result of a previous study that found ~~that~~ the glucomannan concentration ~~did~~ to not influence the polydispersity index (Aprilia et al., 2021).

~~Hydrogel~~ The hydrogels' zeta potentials became more electropositive as the cell ~~concentration~~ number increased from 8 to 9 log CFU/mL but then decreased ~~to~~as the cell ~~concentration~~ number reached 10 log CFU/mL (**Table 2**). An increase in the number of cells ~~number~~ should ~~cause~~ result in a reduction in the hydrogel's charge due to the positive charge of empty hydrogels and the negative charge of cells (Aprilia et al., 2021), including *L. acidophilus* (Priya, Vijayalakshmi, ~~&~~ and Raichur, 2011). The observed pattern might ~~be due to~~stem from the zeta potential being measured on the hydrogel's surface, ~~which can be~~meaning that it could have been affected by the pH of the surrounding environment (Barbosa et al., 2019).

The L*, b*, and whiteness values of the hydrogels increased after ~~adding~~ the addition of cells, ~~while~~ whereas the a* value decreased (**Table 3**). The utilized instrument ~~determines~~ determined these values based on the reflection by the cells of a direct light beam from a chromameter ~~by the cells~~. Therefore, the more cells encapsulated within the hydrogel, the greater the reflection. Bacteria~~l~~ may also generate ~~a~~

Commented [VA7]: New data was added (yellow highlight)

distinct shades of colors like such as red. Based on the findings of a priorprevious study, *Lactobacillus pluvialis* could reflect an orange color from the pigment of canthaxanthin (Venil, Dufossé, & and Renuka Devi, 2020). This was finding is in agreement with this the present result, especially in terms of the increase of in the b* value after following the addition of *L. acidophilus*.

Table 3. Color values Hydrogel of hydrogels color values with different initial cell numberconcentrations.

Initial cell number concentration (log CFU/mL)	L*	a*	b*	Whiteness
control	65.06±0.12[a]	7.02±0.09[a]	12.50±0.08[a]	62.24±0.15[a]
8	76.97±0.32[b]	5.42±0.01[b]	14.24±0.11[b]	72.38±0.21[b]
9	79.48±0.33[c]	5.61±0.07[b]	15.14±0.01[c]	73.89±0.25[c]
10	77.39±0.23[b]	4.22±0.23[c]	13.24±0.13[d]	73.46±0.30[c]

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

The XRD spectra represent the interaction between the diffraction intensity and the angle (**Figure 2**). A Moreover, a crystalline state was is indicated by the sharp diffraction peak, while whereas the an amorphous and solid state was is indicated by the declivous peak (Yanuriati et al., 2017). The X-ray diffractogram patterns of all the hydrogels showed a very broad band at 2θ between 5° and –90°. In addition, all the hydrogels had exhibitedalmost the same nearly identical highest peaks at around 2θ 7.06°–10.46°, 7.62°–11.00°, 7.48°–10.94°, and 7.16°–11.20° for those hydrogels without cells and with cells at numbersconcentrations of log 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively. These results differ from those concerning porang glucomannan, which exhibitedhad its highest peaks at around 19°–20° and 35° (Yanuriati et al., 2017). However, there was a small peak in all the samples at around 2θ 10.5°, indicating the existence presence of chitosan (Yu, Lu, &and Xiao, 2007). This observation suggests that the mixture between of glucomannan hydrogel and cells strengthened their associated chemical interaction, which is consistent with previous Fourier-transform infrared spectroscopy (FTIR) findings (Aprilia et al., 2021). (Aprilia et al., 2021). It also suggestsand that some chitosan had not interacteddid not interact with the glucomannan. A previous prior study reported that the Schiff's crosslinking between glucomannan aldehyde groups and chitosan amino groups could suppress the chitosan's crystalline state, which is usually strengthened by a the hydrogen bond between the amino and hydroxyl groups (Yu, Lu, and & Xiao, 2007). We also found evidence of low crystallinity, with values of 26%, 25%, 17%, and 21% being determined for the hydrogels without cells and with cells at numberconcentrations of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively. The addition of *L. acidophilus* appeared to have no effect on the diffraction peak, indicating that the entrapment of microbes within the hydrogel did not affect the interaction between the glucomannan and chitosan.

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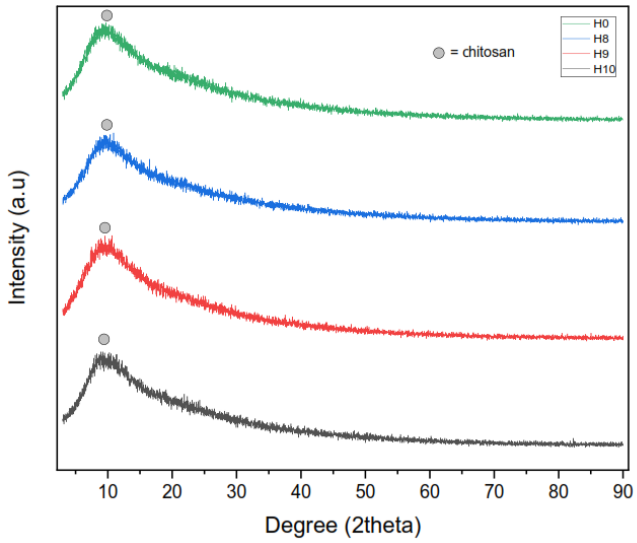

Figure 2. X-ray diffractogram for H0 (hydrogel without *L. acidophilus*), H8, H9, and H10 (hydrogels with *L. acidophilus* at number~~concentration~~s of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively).

~~Hydrogel p~~Prebiotic activity of the hydrogels

The *L. acidophilus* and *E. coli* cell density increased during 0 h, 24 h, and 48 h~~ours~~ of incubation in the presence of carbohydrates, glucose, inulin, and hydrogel (**Table 4**). Both bacteria showed no significant increase in almost all the carbohydrates, except for *L. acidophilus* with inulin and *E. coli* with glucose. These data suggest that only inulin ~~can~~ is able to specifically stimulate the growth of good bacteria and suppress the growth of enteric bacteria, which is consistent with its ~~well-known~~widespread use as a commercial prebiotic ~~worldwide~~.

Table 4. ~~The d~~Density of *L. acidophilus* FNCC 0051 cells in ~~log~~ 10 log (CFU/mL~~)~~ after 0 h, 24 h, and 48 h~~ours~~ of incubation with prebiotics, inulin, hydrogel, and glucose.

Prebiotic	*L. acidophilus*			*E. coli*		
	0 h	24 h	48 h	0 h	24 h	48 h
Glucose	6.94±1.32^{a}	8.35±0.81^{a}	9.17±0.01^{a}	6.65±0.92^{a}	8.54±0.09^{ab}	9.29±0.49^{b}
Inulin	6.59±0.19^{a}	7.33±0.49^{ab}	8.48±0.88^{b}	9.53±0.09^{a}	7.59±0.32^{a}	8.47±0.75^{a}
Hydrogel	9.37±0.10^{a}	9.58±0.46^{a}	10.15±0.21^{a}	8.80±1.13^{a}	8.17±0.86^{a}	9.02±2.18^{a}

Values represent the mean ± SD. Different superscript letters in the same row indicate significantly different results at the level of $p < 0.05$.

The prebiotic potential of the hydrogel was compared with that of inulin ~~using~~ on the basis of the prebiotic activity scores (**Figure 3**). The prebiotic activity score of the hydrogel was higher than that of inulin after 24 h of incubation, although it was reduced ~~but became lower~~ after 48 h, suggesting that the hydrogel was ~~a~~ the preferred energy source for the cells. This result is consistent with the XRD findings, which ~~that~~ confirmed the hydrogel to have an amorphous ~~hydrogel~~ state and, ~~which has~~ no long-range order, making it easier to digest~~.~~. Moreover, ~~and~~ the amount of carbohydrates will decrease with time. ~~Meanwhile~~By

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'hydrogel prebiotic activity could improve our functional product that also be the innovation of this research. The score prebiotic activity was also calculated by using the data of inulin activities that had been proved as commercial prebiotic.

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contrast, the known prebiotic inulin (Kamel et al., 2021) ~~needed~~ required a longer time to be available for the bacteria ~~since it has~~due to its long polymeric carbon chains~~—~~, ~~that is~~, chains of around 2–60 molecules (Samolińska ~~and~~ & Grela, 2017).

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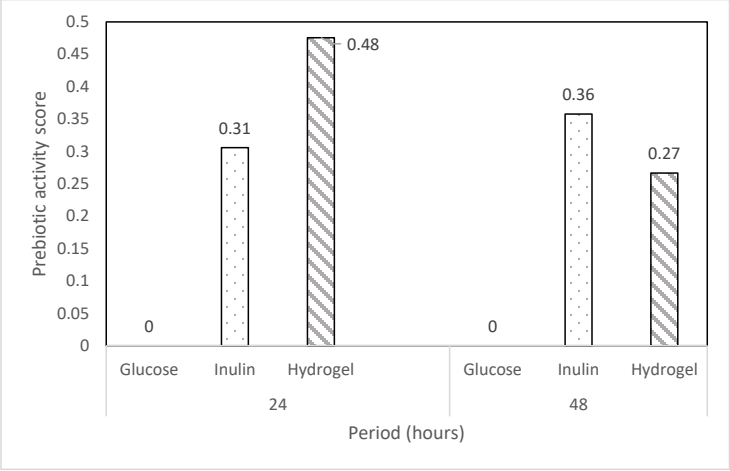


Figure 3. Prebiotic activity score~~s~~ of *L. acidophilus* FNCC 0051 on glucose, inulin, and hydrogel.

Cell survival during exposure to simulated gastrointestinal conditions

Cell survival during exposure to gastric juice

The *L. acidophilus* showed good viability during exposure to gastric juice at pH 2, ~~either~~ whether in its free form or when encapsulated in hydrogel (**Figure 4**). Generally, ~~T~~the growth of lactic acid bacteria is ~~generally~~ optimum at pH 6–7 (close to neutral pH). Some metabolic reactions change when the pH is <5 or <4.4. Indeed, some minerals will be lost at pH ≤2, ~~and~~ while prolonged storage at a low pH will increase the risk of cell death (Hayek dan Ibrahim, 2013). Our results in this regard are consistent with those of ~~a~~ previous ~~study~~ studies (Stasiak-Różańska et al., 2021; Zeashan et al., 2020)~~; Stasiak-Różańska *et al.*, 2021)~~. Further study~~ies are ~~needed~~ required to determine the effect of~~on~~ solid or solid-enriched macronutrient foods with a longer transit time (Müller, Canfora, ~~and~~ & Blaak, 2018). In addition, a shorter exposure time within the stomach enables cells to maintain homeostasis between the internal and external pH, which potentially ~~influencing~~ influenced the good viability ~~shown~~ found in this study.

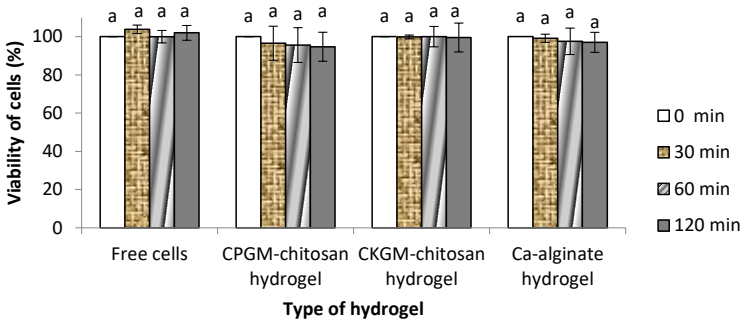
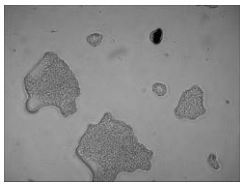


Figure 4. *L. acidophilus* FNCC 0051 viability during exposure to gastric juice for 120 min. Key: a, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

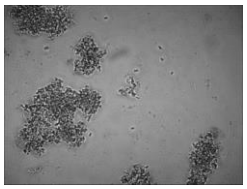
~~This~~ The present study also found that porang glucomannan-chitosan hydrogel might ~~have~~ exhibit a similar ability to protect cells ~~protecting ability~~ from the gastric environment as both konjac glucomannan–chitosan hydrogel and calcium-alginate hydrogel ($p > 0.05$). This finding accords with the ability of alginate ~~also~~ to protect~~ed~~ *L. plantarum* (Rather et al., 2017) and *Lactobacillus rhamnosus* from this harsh environment ~~for~~ over the course of 3 h of exposure (Oberoi et al., 2021).

The hydrogel was stable in the simulated gastric juice ~~for~~ throughout 120 min of exposure (**Figure 5**), which is consistent with the result of a previous swelling ratio study (Aprilia et al., 2021) that ~~found the~~determined the hydrogel ~~did~~ to not ~~deswell~~ deswell at ~~the~~ a pH <5. Deswelling causes ~~the~~ hydrogel to become smaller, which was previously thought to result in the release of cells from the hydrogel. However, the cells are still ~~en~~trapped in the hydrogel (**Figure 5**), which perhaps ~~reflecting~~ reflects the stronger electrostatic interaction between the glucomannan carbonyl group and ~~the~~ chitosan amine group in an acid environment (Aprilia et al., 2021). ~~Cells~~ The cells remain in the hydrogel because this interaction maintains the core. ~~Therefore~~Thus, deswelling could not be maximized, leading to only a small number of cells being released from the hydrogel. ~~There is a possibility~~It is possible that some empty hydrogels will shrink to the extent that they are no longer visible ~~at~~ after 60 min of exposure. These results are consistent with those of other studies ~~using~~ using hydrogels made from oxidized glucomannan and chitosan to ~~en~~trap diclofenac drugs, which ~~that~~ found <1% of cells to be~~was~~ released during exposure to simulated gastric fluid at pH 1.2 (Korkiatithaweechai et al., 2011). This ~~result~~ shows that the hydrogel cores were not released when ~~it~~ the hydrogel was exposed to low pH conditions.

A)



B)



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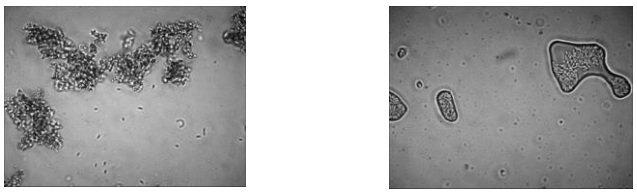

Figure 5. Microscopic appearance of hydrogel~~s~~ containing *L. acidophilus* FNCC 0051 (1300 × magnification) during exposure to gastric juice for (**A**) 0 min, (**B**) 30 min, (**C**) 60 min, and (**D**) 120 min.

Cell survival during exposure to intestinal juice

The viability of the free cells decreased significantly during exposure to intestinal juice for 60 min (**Figure 6**; $p < 0.05$). ~~Indeed~~Yet, the viability of the cells encapsulated in the hydrogel ~~could b~~was~~e~~ maintained over 120 min of exposure, indicating that the encapsulation ~~increases~~ increased the viability of the *L. acidophilus* ~~viability~~. A decrease in the number of free cells may reflect cell death, which can be caused by factors other ~~factors other~~ than the pH of the medium. Priya et al. (2011) reported that while ~~,~~ bacteria showed good growth at pH 6.8, the presence of pancreatin~~,~~ (comprising amylase, trypsin, lipase, ribonuclease, and protease~~,~~) damaged the encapsulation wall, ~~causing~~ thereby resulting in cell death.

Figure 6 indicates that the porang glucomannan hydrogel ~~has~~ exhibited the same level of good protective effect as the konjac-chitosan glucomannan and ~~calcium~~ calcium-alginate hydrogels. In this study, the alginate-based hydrogel was used for the purpose of comparison ~~since~~ because it is widely used as an encapsulant due to its low price, good biocompatibility, and nontoxicity. A ~~previous~~ prior study ~~showed~~ found that the probiotic encapsulation of alginate increased ~~entrapped~~ the viability of the trapped cells when ~~viability~~ compared ~~to~~ with the free cells during exposure to a simulated gastrointestinal condition (Stasiak-Różańska et al., 2021). Therefore, the porang-chitosan glucomannan hydrogel ~~has~~ shows potential as a bacterial encapsulant.

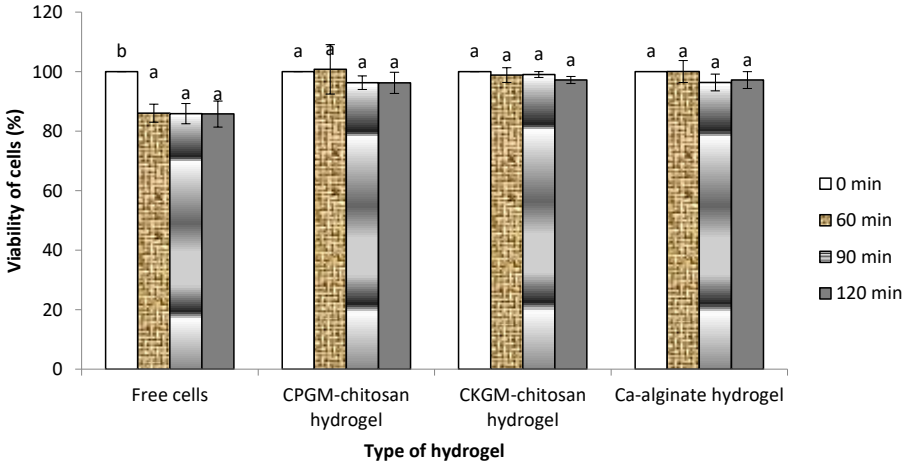

Figure 6. *L. acidophilus* FNCC 0051 cell viability during exposure to intestinal juice for 120 min. Key: a or b, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

The ~~hydrogel's~~ hydrogel's microscopic appearance was used to confirm the cell viability data. ~~Porang~~ Here, the porang glucomannan-chitosan hydrogel ~~was~~ remained stable for up to 2 h in the intestinal fluid. However, it was found to be larger after 60 min of exposure than after 0 min ~~exposure~~ (**Figure 7**), potentially reflecting its swelling behavior at pH 6.8. We ~~previously showed~~have previously shown that porang glucomannan-chitosan hydrogel begins to swell at pH >5 (Aprilia et al., 2021). The swelling of the hydrogel was evident until it reached 90 min of exposure. Moreover, many small hydrogels and cells were visible in the solution after 120 min of exposure. The swelling weakened the interaction of the hydrogels~~s~~, leading to some parts ~~of the hydrogel~~ being dissolved, ~~resulting~~ which resulted in both smaller hydrogels~~s~~ and the release of cells from the hydrogels. This result is consistent with that of another study that found konjac ~~glucomannan~~ glucomannan-carboxymethyl chitosan hydrogel with a bovine serum albumin core ~~showed~~ to show greater core release at pH 7.4 than at pH 5 due to the swelling enlarging its pores (Du et al., 2006). This core release also occurred when a chitosan-oxidized glucomannan hydrogel was exposed to simulated ~~intestine~~ intestinal fluid for 2–8 h (Korkiatithaweechai et al., 2011).

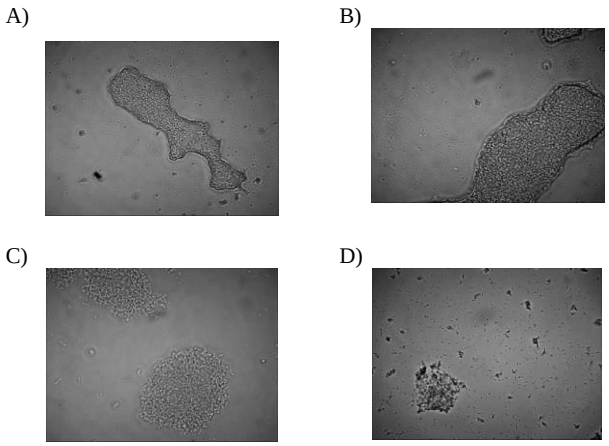

Figure 7. Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (1300 × magnification) during exposure to intestinal juice for (**A**) 0 min, (**B**) 30 min, (**C**) 60 min, and (**D**) 120 min.

Conclusions

The encapsulation of *L. acidophilus* in ~~a~~ hydrogel made from glucomannan and chitosan was improved by varying the number ~~concentration~~ of the cells added. ~~Higher~~ In fact, higher ~~concentrations~~ numbers ~~showed~~ were found to be associated with greater encapsulation efficiency, diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness. In addition, the hydrogel ~~showed~~ exhibited potential as a prebiotic, particularly after 24 h of incubation. ~~Moreover, t~~Moreover, the hydrogel protected the encapsulated cells, maintaining them during exposure to simulated gastrointestinal fluid. ~~Furthermore,~~Furthermore, the cell viability increased from 86% to 100% when ~~it~~ the hydrogel was exposed

to intestinal juice, which was, comparable to the performance of the to alginate and konjac glucomannan hydrogels. Further animal studies are needed required to determine the cell viability in actual gastrointestinal conditions and assess the health effects of the hydrogel.

Data Aavailability

The data used to support the findings of this study are included within the article.

Conflict of Iinterest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was supported by the Riset Inovatif Produktif RISPRO pProject of Lembaga Pengelola Dana Pendidikan (LPDP) (Indonesia Endowment Fund for Education) for 2016–2017 and the Research Directorate and Reputation Team towards World Class University – Quality Assurance Office of Universitas Gadjah Mada (according to Aassignment Letter letter Numbernumber: 6144/UN1.P.III/DIT-LIT/PT/2021 dated September 27, 2021).

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Commented [VA11]: The addition references added were coloured with red fonts.
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We have revised it and now 21 of 25 papers are included in research paper, while the new references (not more than 5 years) are 16 of 25 papers (>50%).

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11. Reviu 2 (10 November 2022)

Message for Author

A: Please add new references in the introduction section. Some of recent references (in 2022 and 2023) in the field are missing. B: Please enhance this sentence. You may extend it. "Yet, the role of the glucomannan concentration in protecting probiotic cells during digestion and glucomannan's potential as a prebiotic remain unexplored." C: The figures and tables must be separately attached in the supplemental files. Make sure the figures are editable (the text in the figures). PDF format could be suitable for publication. You can find some examples in the published works. D. Please go through the entire manuscript to double check accuracy and ensure errors-free.

12. Revisi 3: 11 November 2022

November 11, 2022

Dear Editor in Chief of The Scientific World Journal,

Please accept our revision entitled, **"Hydrogel from glucomannan-chitosan to improve survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid**" for consideration for publication in The Scientific World Journal.

We had revised all of the suggestion and correction from editor. Please contact me if you have any question or concern regarding the manuscript. I look forward to receiving the results of the review.

Sincerely

Prof. Dr. Ir. Eni Harmayani, M.Sc

Responses the reviewer's comments

Manuscript ID:
Title: Hydrogel from glucomannan–chitosan to improve survival of *L. acidophilus* FNCC 0051 in simulated gastrointestinal fluid

Editor

No.	Reviewer's Comments	**Responses** (for author)
1.	Please add new references in the introduction section. Some of recent references (in 2022 and 2023) in the field are missing.	We have added new recent references (yellow highlight).
2.	Please enhance this sentence. You may extend it. "Yet, the role of the glucomannan concentration in protecting probiotic cells during digestion and glucomannan's potential as a prebiotic remain unexplored	We have corrected and extended the sentences as follows: "Yet, the impact of probiotic cells concentration as the core on the encapsulation efficiency and the properties of the hydrogel remain unexplored."
3	The figures and tables must be separately attached in the supplemental files. Make sure the figures are editable (the text in the figures). PDF format could be suitable for publication. You can find some examples in the published works.	We have separated the figures and tables.
4	Please go through the entire manuscript to double check accuracy and ensure errors-free.	We have done it

Hydrogel derived from glucomannan-chitosan to improve the survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid

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Abstract

The probiotic encapsulating hydrogel derived from porang (*Amorphophallus oncophyllus*) glucomannan and chitosan was investigated with regard to its encapsulation efficiency, physical properties, prebiotic activity, and survival under simulated gastrointestinal conditions. The hydrogel's encapsulation efficiency was improved by varying the number of the *Lactobacillus acidophilus* FNCC 0051, which also served to increase the diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness of the hydrogel. Moreover, the hydrogel's prebiotic activity score was higher than that of inulin after 24 h of incubation, reflecting its role as a cell encapsulant, particularly when it comes to maintaining cells during exposure to simulated gastrointestinal fluid. The cell viability increased from 86% to 100% when immersed in intestinal juice, which is comparable to the increase achieved using alginate and konjac glucomannan hydrogels. Future animal studies are required to determine the cell viability in actual gastrointestinal conditions and assess the health effects of the hydrogel.

Keywords: hydrogel; viability; glucomannan; chitosan; gastrointestinal.

Introduction

Glucomannan is a functional polysaccharide that can be extracted from *Amorphophallus* tubers. While the glucomannan obtained from *Amorphophallus konjac* has a number of popular and commercial uses, several research groups are currently investigating the potential of glucomannan derived from other sources. *Amorphophallus oncophyllus*, which is commonly known as porang, is a local glucomannan source in Indonesia (Harmayani, Aprilia, & Marsono, 2014; Yanuriati et al., 2017). It has several characteristics that differ from those of konjac, including mannose/glucose molar ratio, degree of polymerization, and degree of acetylation, leading it to exhibit different solubility, viscosity, water-holding capacity, and gelation properties (Harmayani, Aprilia, & Marsono, 2014; Yanuriati et al., 2017). Therefore, the applications of porang may also differ depending on the function.

A hydrogel is a kind of technological glucomannan product that leverages its gelation properties. Hydrogels are formed through interactions between glucomannan and other polymers that lead to the formation of a three-dimensional polymeric network (Stasiak-Różańska et al., 2021). This characteristic results in hydrogels exhibiting potential as encapsulants. A previous study used a hydrogel created by crosslinking konjac, glucomannan, and chitosan, which was found to have many advantages, including natural formation without the need for a crosslinker, self-assembly, tolerance to different pH levels, and demonstrable ability to encapsulate drugs, proteins, and enzymes (Du et al., 2006; Korkiatithaweechai et al., 2011). A similar study involving hydrogels formed by means of the interaction between porang glucomannan and chitosan investigated the production of the primary carboxymethyl glucomannan material, the compatibility of the substitution degree of the carboxymethyl glucomannan involved in the hydrogel formation, the effect of the polymer concentration on the glucomannan properties, and the application in relation to probiotic encapsulation (Aprilia et al., 2017a, 2017b, 2021). The key innovation of the study was the use of porang, which has characteristics that differ from those of other glucomannan

sources, such as the solubility, viscosity, water-holding capacity, degree of polymerization, degree of acetylation, purity, and X-ray diffraction (XRD) pattern (Harmayani, Aprilia, & Marsono, 2014; Yanuriati et al., 2017). The other differences include the type of modification used (carboxymethylation) and the use of the hydrogel as a probiotic encapsulant. By contrast, prior studies made use of the oxidation method (Korkiatithaweechai et al., 2011) and encapsulated drugs, proteins, and enzymes (Du et al., 2006; Korkiatithaweechai et al., 2011). The use of carboxymethyl konjac glucomannan-chitosan as probiotic encapsulant recently studied, but it was combined with calcium-alginate hydrogel bead system (Dinga *et al.*, 2022). They were also found to be used as secondary emulsion to carry curcumin (Wang *et al.*, 2023).

However, given that living cells have different characteristics to inanimate compounds, the role of this new hydrogel in encapsulating probiotics needs to be further studied. Indeed, the new capsules should ensure the survival of the probiotics during food processing and storage, in addition to ensuring sufficient delivery when consumed (>10^6–10^7 colony forming units [CFU]/mL). Furthermore, the capsules need to allow the probiotics to reach the lower gastrointestinal tract if they are to have a beneficial effect on humans. Thus, the survival of the capsules during gastrointestinal digestion and their ability to increase probiotic growth in the colon are important. Carbohydrates known to stimulate probiotic growth are known as prebiotics. We previously optimized the probiotic encapsulation efficiency by varying the glucomannan concentration, and we also studied its role in protecting cells during pasteurization and cold storage (Aprilia et al., 2021). Yet, the impact of probiotic cells concentration as the core on the encapsulation efficiency and the properties of the hydrogel remain unexplored.

The present study sought to improve the probiotic encapsulation efficiency and properties of the hydrogel derived from glucomannan and chitosan by varying the cell number in an effort to increase the number of cells carried. It also examined the effects of varying the cell number on the hydrogel's physical properties, prebiotic activity score, and viability during simulated gastrointestinal exposure.

Materials and Methods

Materials

The primary material used in this study was glucomannan derived from porang tubers (*A. oncophyllus*), which was obtained from the Faculty of Agricultural Technology, Universitas Gadjah Mada (Yogyakarta, Indonesia). The carboxymethylation of the glucomannan was performed using sodium chloroacetate, as previously described (Aprilia et al., 2017b). The utilized chitosan, which had a degree of deacetylation of 85%–89%, meaning that it met established food quality criteria, was obtained from PT Biotech Surindo (Cirebon, West Java, Indonesia).

Preparation of the *Lactobacillus acidophilus* FNCC 0051 cells

The *L. acidophilus* FNCC 0051 cells used in this study were obtained from the Food and Nutrition Culture Collection (FNCC) of the Laboratory of Food Microbiology, Center for Food and Nutrition Studies, Universitas Gadjah Mada. The cells, which were stored in a skim milk-glycerol suspension, were rejuvenated in de Man, Rogosa, and Sharpe (MRS) broth at 37°C overnight and then grown twice. Subsequently, the cell biomass was harvested by means of centrifugation at 2400 g for 9 min at 4°C and then rinsed with saline solution.

Production of the hydrogel and determination of its encapsulation efficiency

The hydrogel was created by mixing porang glucomannan with chitosan using the complex coacervation method (Aprilia et al., 2021). The encapsulation of the probiotics in the hydrogel was performed using three different cell numbers, namely 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL. The cells were mixed with glucomannan prior to the start of the coacervation process. The hydrogel's encapsulation efficiency was determined by releasing the cells trapped within it using a buffer solution at pH 8 and 37°C for 24 h (Aprilia et al., 2017b). The released cells were then grown in MRS agar to allow for the enumeration of the

total viable cells. To calculate the encapsulation efficiency, the total viable cell number was divided by the number of initial cells added to the hydrogel mixture (Zeashan et al., 2020).

Determination of the hydrogel's properties

Particle size, polydispersity index, and zeta potential

The particle size was estimated based on the hydrogel's diameter and simultaneously measured on the basis of the polydispersity index using a particle size analyzer (SZ-100 series; Horiba, Kyoto, Japan). The hydrogel's zeta potential was measured using a Nano ZS Zetasizer (v.6.20; Malvern Instruments Ltd., Malvern, UK).

Color

The hydrogel was freeze-dried and ground prior to the color measurement. The redness (a*), yellowness (b*), and lightness (L*) values were determined using a CR200 chromameter (Minolta, Osaka, Japan). The whiteness index was calculated as previously described (Akgün, Ova Özcan, & Övez, 2022).

Crystallinity percentage

The XRD of the hydrogel was determined using a LabX XRD-6000 diffractometer (Shimadzu, Kyoto, Japan) equipped with a Cu Kα target at 40 kV and 30 mA, which had a scanning rate of 4°/min. The pattern was collected in the 2θ range between 3.02° and 90°. The crystallinity percentage (%) was calculated by dividing the area under the peaks by the total area under the curve (Yazdani et al., 2020).

Determination of the prebiotic activity score

The prebiotic activity score was calculated by subtracting the ratio of probiotic cell growth with prebiotics and glucose from the ratio of enteric cell growth with prebiotics and glucose, as previously described (Huebner, Wehling, & Hutkins, 2007). The probiotic used was *L. acidophilus* FNCC 0051, whereas the enteric cells used were *Escherichia coli* FNCC 0091. The test was performed by adding 1% (volume/volume [v/v]) probiotic cells into MRS broth containing 2% (weight/volume [w/v]) glucose or prebiotic and adding 1% (v/v) enteric cells into M9 broth containing 2% (w/v) glucose or prebiotic. The cells were incubated at 37°C for 0 h, 24 h, and 48 h and then enumerated by means of the plate count method using MRS and nutrient agar. Each test was performed three times.

Determination of *L. acidophilus* FNCC 0051 survival during exposure to simulated gastrointestinal conditions

The utilized simulated gastric and intestinal juices were prepared according to the method described by Xu et al. (2016). More specifically, the gastric juice was prepared by mixing 7 mL of pepsin in hydrochloric acid, 2 g of sodium chloride, and 1 M of sodium hydroxide. The intestinal juice was prepared by mixing 1% pancreatic powder, 6.8 g of potassium dihydrogen phosphate, and 77 mL of 0.2 N sodium hydroxide. Next, 1 g of either free or encapsulated cells (in hydrogel derived from porang glucomannan-chitosan, konjac glucomannan-chitosan, and calcium alginate) was mixed with 9 mL of simulated gastrointestinal juices and incubated at 37°C for 120 min. The samples were withdrawn at intervals of 0 min, 30 min, 60 min, and 120 min to reflect gastric juice digestion and 0 min, 60 min, 90 min, and 120 min to reflect intestinal juice digestion (Rather et al., 2017). The hydrogel was then rinsed twice with acetate buffer. The cells were enumerated using the pour plate technique on MRS agar after 48 h of incubation. The number of viable cells following exposure was divided by the initial number of cells in order to determine the cell survival rate during exposure to simulated gastrointestinal conditions (Zeashan et al., 2020). The hydrogel's appearance during exposure to simulated gastrointestinal conditions was observed using an optical BX51 microscope (Olympus Corp., Tokyo, Japan) and an OptiLab pro digital camera (Miconos, Indonesia).

Results and Discussion

Encapsulation efficiencies of hydrogels with different numbers of cells

The encapsulation efficiencies of hydrogels with different numbers of initial cells are shown in **Table 1**. The data revealed that the encapsulation efficiencies of the hydrogels ranged between 44.37% and 85.03%. The highest encapsulation efficiency was achieved when 10 log CFU/mL of cells was added to the mixture, which exceeded the Food and Agricultural Organization of the United Nations (FAO) criteria for probiotic products (>6–7 log CFU/mL; Priya, Vijayalakshmi, & Raichur, 2011). Previous studies using different encapsulants obtained different encapsulation efficiencies. For instance, the encapsulation of *L. acidophilus* in hydrogel formed from sodium alginate and soy protein isolates achieved an encapsulation efficiency of 95%–98%, whereas the encapsulation of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* in an emulsion achieved an encapsulation efficiency of 97%–99% (Mahmoodi Pour, Marhamatizadeh, & Fattahi, 2022; Zeashan et al., 2020). The differences in the achieved encapsulation efficiencies might reflect the different encapsulant types and encapsulation methods used (Zeashan et al., 2020). We previously showed that the same ratio of glucomannan and chitosan affected the encapsulation efficiency due to the chemical bonding of both polymers as well as due to the difference in electrostatic values between the core and the polymer influencing the degree of cell entrapment (Aprilia et al., 2021).

Properties of the hydrogels with different cell numbers

The appearance of the hydrogels generated from glucomannan and chitosan containing *L. acidophilus* was as shown in **Figure 1**. The polymer solution was clear before the encapsulation process, although it became turbid after the encapsulation process. This provided evidence of the formation of particles that influenced the turbidity of the solution. After the drying process, the hydrogels exhibited a shape similar to that of white cotton. The particle sizes and color values of the hydrogels will be explained below.

The sizes of the hydrogels encapsulating *L. acidophilus* were found to be in the range of 0.7 µm to 9 µm, with most having a diameter of 2 µm to 3 µm (**Table 2**). Those hydrogels determined to be <100 µm in diameter were classified as microgels. The cell concentration significantly influenced the hydrogels' particle size ($p < 0.05$). In fact, the more cells encapsulated within a given hydrogel, the greater its diameter. The particle size was also correlated with the encapsulation efficiency (**Table 1**), as more cores could be trapped within larger hydrogel particles. The other factors found to influence the particle size were the concentration and viscosity of the solution (Aprilia et al., 2021; Zeashan et al., 2020).

The polydispersity indexes of the hydrogel encapsulated cells were all >1 (**Table 2**), indicating the broad distribution of particles of various sizes. Overall, the index began to change when the initial cell number was 10 log CFU/mL. Moreover, the greater the initial cell number, the higher the polydispersity index. This result contrasts with the result of a previous study that found the glucomannan concentration to not influence the polydispersity index (Aprilia et al., 2021).

The hydrogels' zeta potentials became more electropositive as the cell number increased from 8 to 9 log CFU/mL but then decreased as the cell number reached 10 log CFU/mL (**Table 2**). An increase in the number of cells should result in a reduction in the hydrogel's charge due to the positive charge of empty hydrogels and the negative charge of cells (Aprilia et al., 2021), including *L. acidophilus* (Priya, Vijayalakshmi, & Raichur, 2011). The observed pattern might stem from the zeta potential being measured on the hydrogel's surface, meaning that it could have been affected by the pH of the surrounding environment (Barbosa et al., 2019).

The L*, b*, and whiteness values of the hydrogels increased after the addition of cells, whereas the a* value decreased (**Table 3**). The utilized instrument determined these values based on the reflection by the cells of a direct light beam from a chromameter. Therefore, the more cells encapsulated within the hydrogel, the greater the reflection. Bacteria may also generate distinct shades of colors such as red. Based on the findings of a prior study, *Lactobacillus pluvialis* could reflect an orange color from the pigment of canthaxanthin (Venil, Dufossé, & Renuka Devi, 2020). This finding is in agreement with the present result, especially in terms of the increase in the b* value following the addition of *L. acidophilus.*

The XRD spectra represent the interaction between the diffraction intensity and the angle (**Figure 2**). Moreover, a crystalline state is indicated by the sharp diffraction peak, whereas an amorphous and solid state is indicated by the declivous peak (Yanuriati et al., 2017). The X-ray diffractogram patterns of all the hydrogels showed a very broad band at 2θ between 5° and 90°. In addition, all the hydrogels exhibited nearly identical highest peaks at around 2θ 7.06°–10.46°, 7.62°–11.00°, 7.48°–10.94°, and 7.16°–11.20° for those hydrogels without cells and with cells at numbers of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively. These results differ from those concerning porang glucomannan, which exhibited its highest peaks at around 19°–20° and 35° (Yanuriati et al., 2017). However, there was a small peak in all the samples at around 2θ 10.5°, indicating the presence of chitosan (Yu, Lu, & Xiao, 2007). This observation suggests that the mixture of glucomannan hydrogel and cells strengthened the associated chemical interaction, which is consistent with previous Fourier-transform infrared spectroscopy (FTIR) findings (Aprilia et al., 2021). It also suggests that some chitosan did not interact with the glucomannan. A prior study reported that the Schiff's crosslinking between glucomannan aldehyde groups and chitosan amino groups could suppress the chitosan's crystalline state, which is usually strengthened by the hydrogen bond between the amino and hydroxyl groups (Yu, Lu, & Xiao, 2007). We also found evidence of low crystallinity, with values of 26%, 25%, 17%, and 21% being determined for the hydrogels without cells and with cells at numbers of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively. The addition of *L. acidophilus* appeared to have no effect on the diffraction peak, indicating that the entrapment of microbes within the hydrogel did not affect the interaction between the glucomannan and chitosan.

Prebiotic activity of the hydrogels

The *L. acidophilus* and *E. coli* cell density increased during 0 h, 24 h, and 48 h of incubation in the presence of carbohydrates, glucose, inulin, and hydrogel (**Table 4**). Both bacteria showed no significant increase in almost all the carbohydrates, except for *L. acidophilus* with inulin and *E. coli* with glucose. These data suggest that only inulin is able to specifically stimulate the growth of good bacteria and suppress the growth of enteric bacteria, which is consistent with its widespread use as a commercial prebiotic.

The prebiotic potential of the hydrogel was compared with that of inulin on the basis of the prebiotic activity scores (**Figure 3**). The prebiotic activity score of the hydrogel was higher than that of inulin after 24 h of incubation, although it was reduced after 48 h, suggesting that the hydrogel was the preferred energy source for the cells. This result is consistent with the XRD findings, which confirmed the hydrogel to have an amorphous state and no long-range order, making it easier to digest. Moreover, the amount of carbohydrates will decrease with time. By contrast, the known prebiotic inulin (Kamel et al., 2021) required a longer time to be available for the bacteria due to its long polymeric carbon chains—that is, chains of around 2–60 molecules (Samolińska & Grela, 2017).

Cell survival during exposure to simulated gastrointestinal conditions

Cell survival during exposure to gastric juice

The *L. acidophilus* showed good viability during exposure to gastric juice at pH 2, whether in its free form or when encapsulated in hydrogel (**Figure 4**). Generally, the growth of lactic acid bacteria is optimum at pH 6–7 (close to neutral pH). Some metabolic reactions change when the pH is <5 or <4.4. Indeed, some minerals will be lost at pH ≤2, while prolonged storage at a low pH will increase the risk of cell death (Hayek dan Ibrahim, 2013). Our results in this regard are consistent with those of previous studies (Stasiak-Różańska et al., 2021; Zeashan et al., 2020). Further studies are required to determine the effect of solid or solid-enriched macronutrient foods with a longer transit time (Müller, Canfora, & Blaak, 2018). In addition, a shorter exposure time within the stomach enables cells to maintain homeostasis between the internal and external pH, which potentially influenced the good viability found in this study.

The present study also found that porang glucomannan-chitosan hydrogel might exhibit a similar ability to protect cells from the gastric environment as both konjac glucomannan-chitosan hydrogel and calcium-alginate hydrogel ($p > 0.05$). This finding accords with the ability of alginate to protect *L. plantarum* (Rather

et al., 2017) and *Lactobacillus rhamnosus* from this harsh environment over the course of 3 h of exposure (Oberoi et al., 2021).

The hydrogel was stable in the simulated gastric juice throughout 120 min of exposure (**Figure 5**), which is consistent with the result of a previous swelling ratio study (Aprilia et al., 2021) that determined the hydrogel to not deswell at a pH <5. Deswelling causes hydrogel to become smaller, which was previously thought to result in the release of cells from the hydrogel. However, the cells are still trapped in the hydrogel (**Figure 5**), which perhaps reflects the stronger electrostatic interaction between the glucomannan carbonyl group and chitosan amine group in an acid environment (Aprilia et al., 2021). The cells remain in the hydrogel because this interaction maintains the core. Thus, deswelling could not be maximized, leading to only a small number of cells being released from the hydrogel. It is possible that some empty hydrogels will shrink to the extent that they are no longer visible after 60 min of exposure. These results are consistent with those of other studies using hydrogels made from oxidized glucomannan and chitosan to trap diclofenac drugs, which found <1% of cells to be released during exposure to simulated gastric fluid at pH 1.2 (Korkiatithaweechai et al., 2011). This shows that the hydrogel cores were not released when the hydrogel was exposed to low pH conditions.

Cell survival during exposure to intestinal juice

The viability of the free cells decreased significantly during exposure to intestinal juice for 60 min (**Figure 6**; $p < 0.05$). Yet, the viability of the cells encapsulated in the hydrogel was maintained over 120 min of exposure, indicating that the encapsulation increased the viability of the *L. acidophilus*. A decrease in the number of free cells may reflect cell death, which can be caused by factors other than the pH of the medium. Priya et al. (2011) reported that while bacteria showed good growth at pH 6.8, the presence of pancreatin (comprising amylase, trypsin, lipase, ribonuclease, and protease) damaged the encapsulation wall, thereby resulting in cell death.

Figure 6 indicates that the porang glucomannan hydrogel exhibited the same level of good protective effect as the konjac-chitosan glucomannan and calcium-alginate hydrogels. In this study, the alginate-based hydrogel was used for the purpose of comparison because it is widely used as an encapsulant due to its low price, good biocompatibility, and nontoxicity. A prior study found that the probiotic encapsulation of alginate increased the viability of the trapped cells when compared with the free cells during exposure to a simulated gastrointestinal condition (Stasiak-Różańska et al., 2021). Therefore, the porang-chitosan glucomannan hydrogel shows potential as a bacterial encapsulant.

The hydrogel's microscopic appearance was used to confirm the cell viability data. Here, the porang glucomannan-chitosan hydrogel remained stable for up to 2 h in the intestinal fluid. However, it was found to be larger after 60 min of exposure than after 0 min (**Figure 7**), potentially reflecting its swelling behavior at pH 6.8. We have previously shown that porang glucomannan-chitosan hydrogel begins to swell at pH >5 (Aprilia et al., 2021). The swelling of the hydrogel was evident until it reached 90 min of exposure. Moreover, many small hydrogels and cells were visible in the solution after 120 min of exposure. The swelling weakened the interaction of the hydrogels, leading to some parts being dissolved, which resulted in both smaller hydrogels and the release of cells from the hydrogels. This result is consistent with that of another study that found konjac glucomannan-carboxymethyl chitosan hydrogel with a bovine serum albumin core to show greater core release at pH 7.4 than at pH 5 due to the swelling enlarging its pores (Du et al., 2006). This core release also occurred when a chitosan-oxidized glucomannan hydrogel was exposed to simulated intestinal fluid for 2–8 h (Korkiatithaweechai et al., 2011).

Conclusions

The encapsulation of *L. acidophilus* in hydrogel made from glucomannan and chitosan was improved by varying the number of the cells added. In fact, higher numbers were found to be associated with greater encapsulation efficiency, diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness. In addition, the hydrogel exhibited potential as a prebiotic, particularly after 24 h of incubation. Moreover, the hydrogel protected the encapsulated cells, maintaining them during exposure

to simulated gastrointestinal fluid. Furthermore, the cell viability increased from 86% to 100% when the hydrogel was exposed to intestinal juice, which was comparable to the performance of the alginate and konjac glucomannan hydrogels. Further animal studies are required to determine the cell viability in actual gastrointestinal conditions and assess the health effects of the hydrogel.

Data Availability

The data used to support the findings of this study are included in the article.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgments

This research was supported by the Riset Inovatif Produktif project Lembaga Pengelola Dana Pendidikan (Indonesia Endowment Fund for Education) for 2016–2017 and the Research Directorate and Reputation Team towards World Class University-Quality Assurance Office of Universitas Gadjah Mada (assignment letter number 6144/UN1.P.III/DIT-LIT/PT/2021 dated September 27, 2021).

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TABLE 1: Encapsulated cell numbers and hydrogel encapsulation efficiencies with different initial cell numbers.

Hydrogels with different cell numbers (log CFU/mL)	Cell number before encapsulation (log CFU/mL)	Cell number after encapsulation (log CFU/g)	Encapsulation efficiency (%)
8	9.39±0.00	4.47±0.18	44.37±1.91^{a}
9	9.56±0.00	6.60±0.13	65.83±1.37^{b}
10	10.10±0.00	7.94±0.21	85.03±0.63^{c}

Values represent the mean ± standard deviation (SD). Different superscript letters in the same column indicate significantly different results at the level of $p < 0.05$.

TABLE 2: Particle sizes, polydispersity indexes, and zeta potentials of hydrogels with different initial cell concentrations.

Initial cell number (log CFU/mL)	Particle size (µm)	Polydispersity index	Zeta potential (mV)
8	2.23±0.11^{a}	1.23±0.17^{a}	24.40±0.75^{b}
9	2.79±0.19^{b}	1.39±0.04^{ab}	32.28±0.80^{c}
10	3.41±0.14^{c}	1.65±0.27^{b}	14.58±0.97^{a}

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

TABLE 3: Color values of hydrogels with different initial cell numbers.

Initial cell number (log CFU/mL)	L*	a*	b*	Whiteness
control	65.06±0.12^{a}	7.02±0.09^{c}	12.50±0.08^{a}	62.24±0.15^{a}
8	76.97±0.32^{b}	5.42±0.01^{b}	14.24±0.11^{c}	72.38±0.21^{b}
9	79.48±0.33^{c}	5.61±0.07^{b}	15.14±0.01^{d}	73.89±0.25^{c}
10	77.39±0.23^{b}	4.22±0.23^{a}	13.24±0.13^{b}	73.46±0.30^{c}

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

TABLE 4: Density of *Lactobacillus acidophilus* FNCC 0051 and *Escherichia coli* cells in 10 log CFU/mL after 0 h, 24 h, and 48 h of incubation with prebiotics, inulin, hydrogel, and glucose.

Prebiotic	*Lactobacillus acidophilus*			*Escherichia coli*		
	0 h	24 h	48 h	0 h	24 h	48 h
Glucose	6.94±1.32^{a}	8.35±0.81^{a}	9.17±0.01^{b}	6.65±0.92^{a}	8.54±0.09^{ab}	9.29±0.49^{b}
Inulin	6.59±0.19^{a}	7.33±0.49^{ab}	8.48±0.88^{a}	9.53±0.09^{a}	7.59±0.32^{a}	8.47±0.75^{a}
Hydrogel	9.37±0.10^{a}	9.58±0.46^{a}	10.15±0.21^{b}	8.80±1.13^{a}	8.17±0.86^{a}	9.02±2.18^{a}

Values represent the mean ± SD. Different superscript letters in the same row indicate significantly different results at the level of $p < 0.05$.

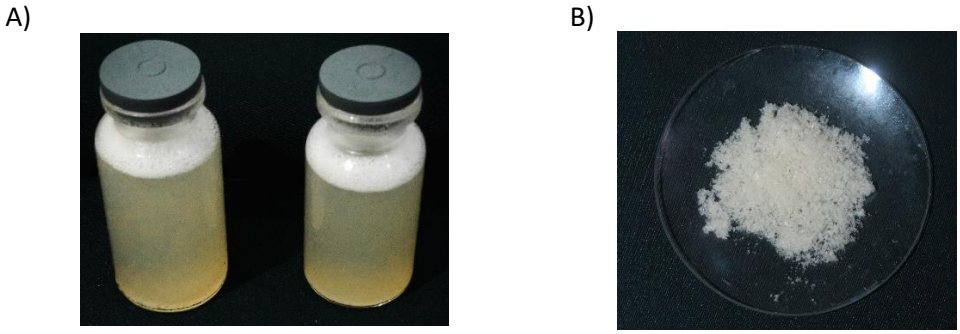

FIGURE 1: The appearance of hydrogels A) before drying and B) after the drying process.

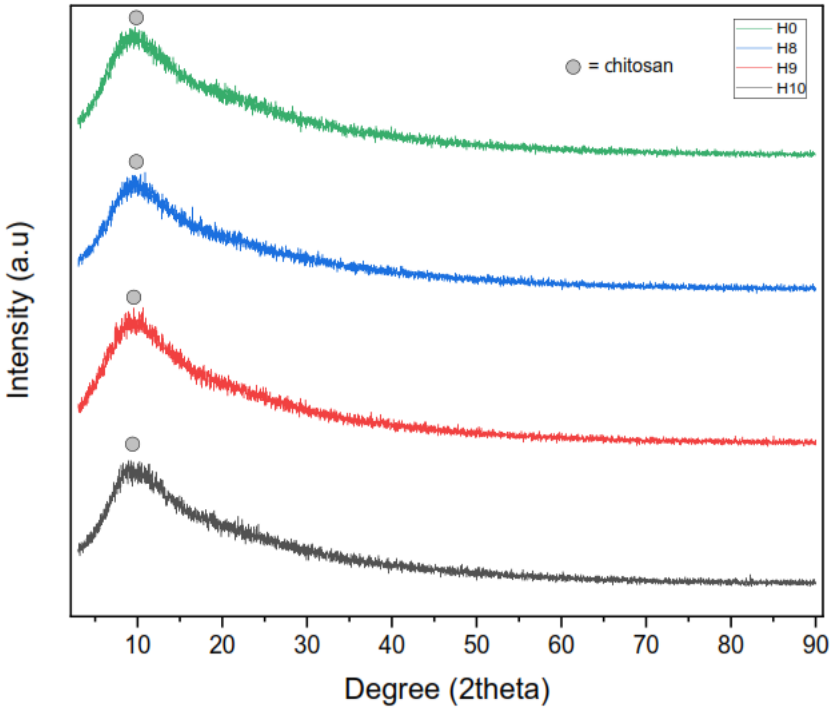

FIGURE 2: X-ray diffractogram for H0 (hydrogel without *L. acidophilus*), H8, H9, and H10 (hydrogels with *L. acidophilus* at numbers of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively).

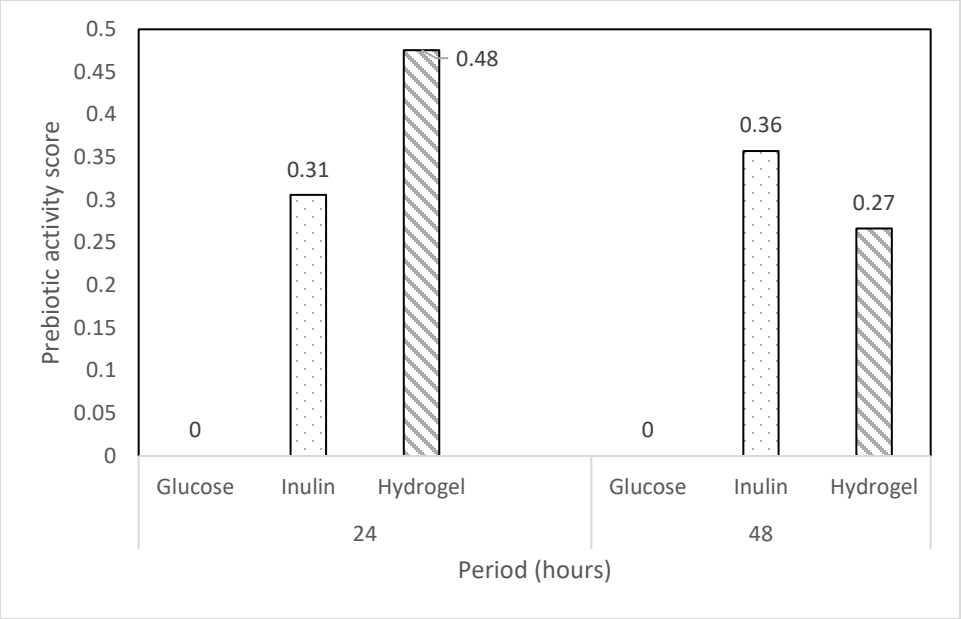

FIGURE 3: Prebiotic activity score of *L. acidophilus* FNCC 0051 on glucose, inulin, and hydrogel.

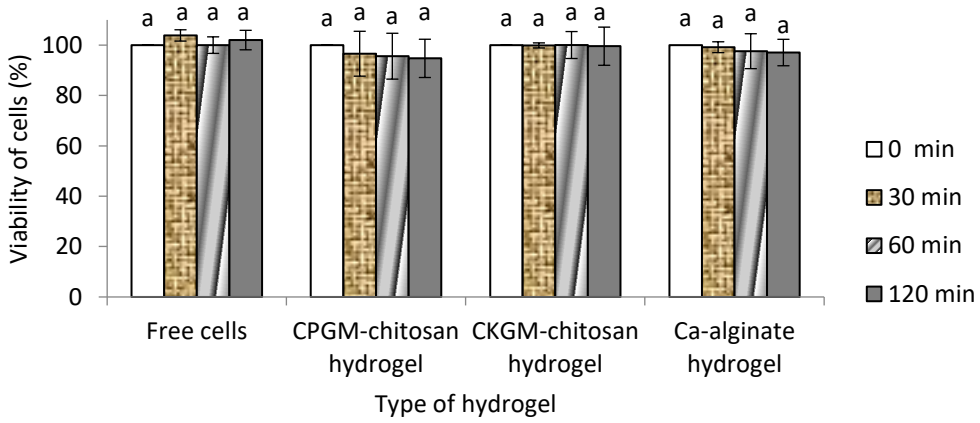

FIGURE 4**:** *L. acidophilus* FNCC 0051 viability during exposure to gastric juice for 120 min. Key: a, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

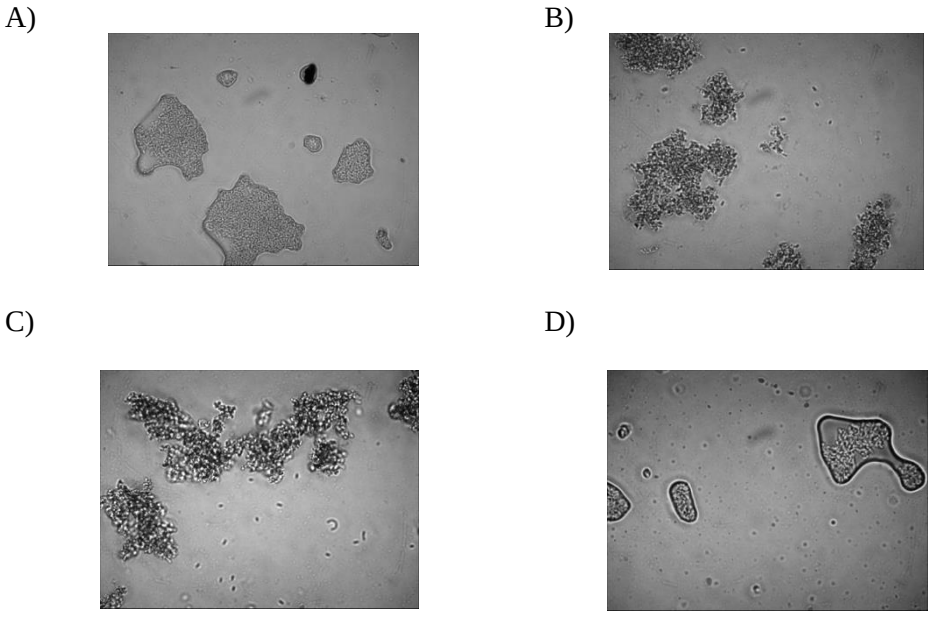

FIGURE 5: Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (1300 × magnification) during exposure to gastric juice for (A) 0 min, (B) 30 min, (C) 60 min, and (D) 120 min.

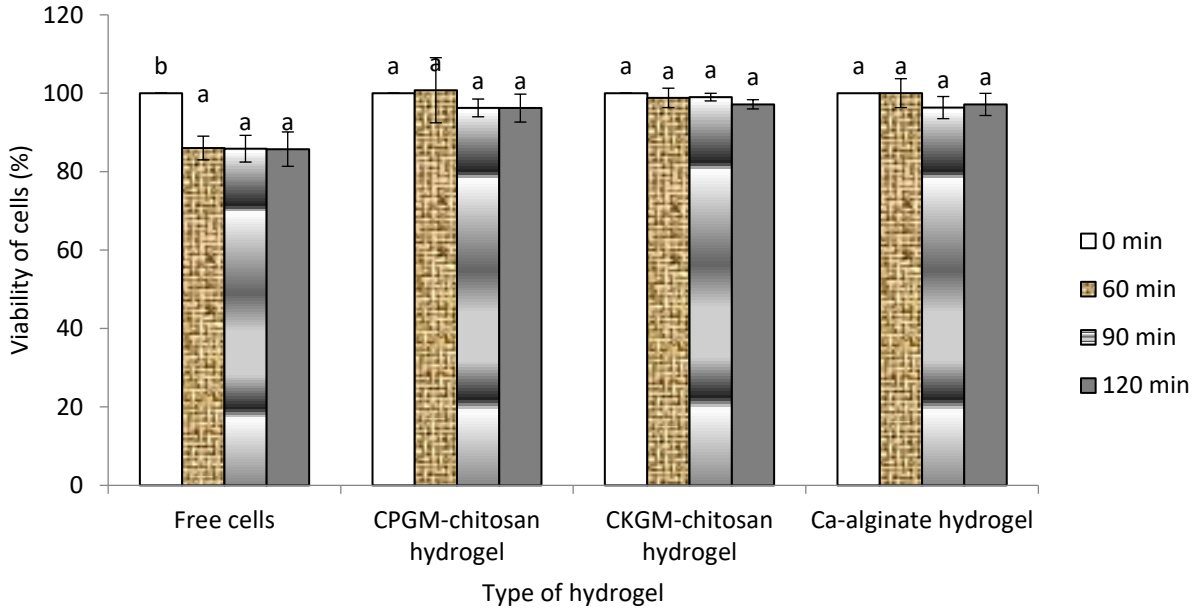

FIGURE 6: *L. acidophilus* FNCC 0051 cell viability during exposure to intestinal juice for 120 min. Key: a or b, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

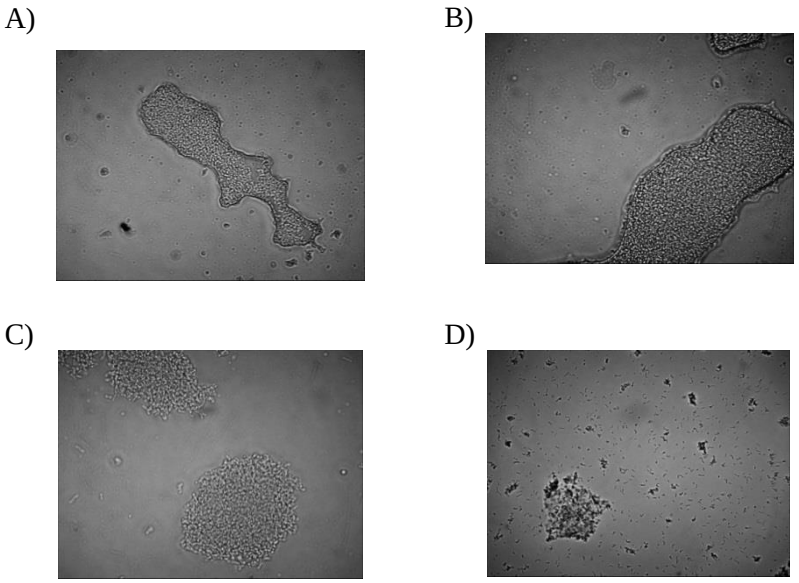

FIGURE 7: Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (1300 × magnification) during exposure to intestinal juice for (A) 0 min, (B) 60 min, (C) 90 min, and (D) 120 min.

13. Submit Revisi 4: 21 November 2022

November 21, 2022

Dear Editor in Chief of The Scientific World Journal,

Please accept our revision entitled, **"Hydrogel from glucomannan-chitosan to improve survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid**" for consideration for publication in The Scientific World Journal.

We had revised all of the suggestion and correction from editor. Please contact me if you have any question or concern regarding the manuscript. I look forward to receiving the results of the review.

Sincerely

Prof. Dr. Ir. Eni Harmayani, M.Sc

Responses the reviewer's comments

Manuscript ID:
Title: Hydrogel from glucomannan–chitosan to improve survival of *L. acidophilus* FNCC 0051 in simulated gastrointestinal fluid

Editor

No.	Reviewer's Comments	**Responses** (for author)
1	Delete some outdated references and update some more recent references in the field to enhance the link between the current literature (i.e., 2021-2022) and your research; some of recent references (in 2021 and 2022) in the field are missing.	We have updated some references in blue highlighted font, but we can not updated several references due to the only previous and as the main source of our works (in green highlighted font)
2	The location of tables and figures must be presented in the work.	We have revised it.
3	Figures must be editable	We attached the original figure.
4	Structure of this work must be presented in introduction section. The rest of this work is as follows.	We have explained the structure and the rest of the work in introduction (red fonts)
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Hydrogel derived from glucomannan-chitosan to improve the survival of *Lactobacillus acidophilus* FNCC 0051 in simulated gastrointestinal fluid

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Abstract

The probiotic encapsulating hydrogel derived from porang (*Amorphophallus oncophyllus*) glucomannan and chitosan was investigated with regard to its encapsulation efficiency, physical properties, prebiotic activity, and survival under simulated gastrointestinal conditions. The hydrogel's encapsulation efficiency was improved by varying the number of the *Lactobacillus acidophilus* FNCC 0051, which also served to increase the diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness of the hydrogel. Moreover, the hydrogel's prebiotic activity score was higher than that of inulin after 24 h of incubation, reflecting its role as a cell encapsulant, particularly when it comes to maintaining cells during exposure to simulated gastrointestinal fluid. The cell viability increased from 86% to 100% when immersed in intestinal juice, which is comparable to the increase achieved using alginate and konjac glucomannan hydrogels. Future animal studies are required to determine the cell viability in actual gastrointestinal conditions and assess the health effects of the hydrogel.

Keywords: hydrogel; viability; glucomannan; chitosan; gastrointestinal.

Introduction

Glucomannan is a functional polysaccharide that can be extracted from *Amorphophallus* tubers. While the glucomannan obtained from *Amorphophallus konjac* has a number of popular and commercial uses, several research groups are currently investigating the potential of glucomannan derived from other sources. *Amorphophallus oncophyllus*, which is commonly known as porang, is a local glucomannan source in Indonesia (Harmayani, Aprilia, & Marsono, 2014; Yanuriati et al., 2017). It has several characteristics that differ from those of konjac, including mannose/glucose molar ratio, degree of polymerization, and degree of acetylation, leading it to exhibit different solubility, viscosity, water-holding capacity, and gelation properties (Harmayani, Aprilia, & Marsono, 2014; Yanuriati et al., 2017). Therefore, the applications of porang may also differ depending on the function.

A hydrogel is a kind of technological glucomannan product that leverages its gelation properties. Hydrogels are formed through interactions between glucomannan and other polymers that lead to the formation of a three-dimensional polymeric network (Stasiak-Różańska et al., 2021). This characteristic results in hydrogels exhibiting potential as encapsulants. A previous study used a hydrogel created by crosslinking konjac, glucomannan, and chitosan, which was found to have many advantages, including natural formation without the need for a crosslinker, self-assembly, tolerance to different pH levels, and demonstrable ability to encapsulate drugs, proteins, and enzymes (Du et al., 2006; Korkiatithaweechai et al., 2011). A similar study involving hydrogels formed by means of the interaction between porang glucomannan and chitosan investigated the production of the primary carboxymethyl glucomannan material, the compatibility of the substitution degree of the carboxymethyl glucomannan involved in the hydrogel formation, the effect of the polymer concentration on the glucomannan properties, and the application in relation to probiotic encapsulation (Aprilia et al., 2017a, 2017b, 2021). The key innovation of the study was the use of porang, which has characteristics that differ from those of other glucomannan

sources, such as the solubility, viscosity, water-holding capacity, degree of polymerization, degree of acetylation, purity, and X-ray diffraction (XRD) pattern (Harmayani, Aprilia, & Marsono, 2014; Yanuriati et al., 2017). The other differences include the type of modification used (carboxymethylation) and the use of the hydrogel as a probiotic encapsulant. By contrast, prior studies made use of the oxidation method (Korkiatithaweechai et al., 2011) and encapsulated drugs, proteins, and enzymes (Du et al., 2006; Korkiatithaweechai et al., 2011). The use of carboxymethyl konjac glucomannan-chitosan as probiotic encapsulant recently studied, but it was combined with calcium-alginate hydrogel bead system (Dinga *et al.*, 2022). They were also found to be used as secondary emulsion to carry curcumin (Wang *et al.*, 2023).

However, given that living cells have different characteristics to inanimate compounds, the role of this new hydrogel in encapsulating probiotics needs to be further studied. Indeed, the new capsules should ensure the survival of the probiotics during food processing and storage, in addition to ensuring sufficient delivery when consumed (>10^6–10^7 colony forming units [CFU]/mL). Furthermore, the capsules need to allow the probiotics to reach the lower gastrointestinal tract if they are to have a beneficial effect on humans. Thus, the survival of the capsules during gastrointestinal digestion and their ability to increase probiotic growth in the colon are important.

We previously studied the properties of the hydrogel produced in the difference glucomannan concentration and evaluated its probiotic encapsulation efficiency, also its role in protecting cells during pasteurization and cold storage (Aprilia et al., 2021). Encapsulation efficiency could not only improved by varying the concentration of added polymer, but also added core (Li *et al.*, 2022). The impact of probiotic cells number as the core on the encapsulation efficiency and the properties of the hydrogel in this works remain unexplored. The present study sought to improve the probiotic encapsulation efficiency by varying the number of cells and to evaluate the hydrogel physical properties. It was also examined the ability of hydrogel to maintain probiotic during simulated gastrointestinal exposure and its potency as prebiotic.

Materials and Methods

Materials

The primary material used in this study was glucomannan derived from porang tubers (*A. oncophyllus*), which was obtained from the Faculty of Agricultural Technology, Universitas Gadjah Mada (Yogyakarta, Indonesia). The carboxymethylation of the glucomannan was performed using sodium chloroacetate, as previously described (Aprilia et al., 2017b). The utilized chitosan, which had a degree of deacetylation of 85%–89%, meaning that it met established food quality criteria, was obtained from PT Biotech Surindo (Cirebon, West Java, Indonesia).

Preparation of the *Lactobacillus acidophilus* FNCC 0051 cells

The *L. acidophilus* FNCC 0051 cells used in this study were obtained from the Food and Nutrition Culture Collection (FNCC) of the Laboratory of Food Microbiology, Center for Food and Nutrition Studies, Universitas Gadjah Mada. The cells, which were stored in a skim milk-glycerol suspension, were rejuvenated in de Man, Rogosa, and Sharpe (MRS) broth at 37°C overnight and then grown twice. Subsequently, the cell biomass was harvested by means of centrifugation at 2400 g for 9 min at 4°C and then rinsed with saline solution.

Production of the hydrogel and determination of its encapsulation efficiency

The hydrogel was created by mixing porang glucomannan with chitosan using the complex coacervation method (Aprilia et al., 2021). The encapsulation of the probiotics in the hydrogel was performed using three different cell numbers, namely 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL. The cells were mixed with glucomannan prior to the start of the coacervation process. The hydrogel's encapsulation efficiency was determined by releasing the cells trapped within it using a buffer solution at pH 8 and 37°C for 24 h (Aprilia et al., 2017b). The released cells were then grown in MRS agar to allow for the enumeration of the

total viable cells. To calculate the encapsulation efficiency, the total viable cell number was divided by the number of initial cells added to the hydrogel mixture (Zeashan et al., 2020).

Determination of the hydrogel's properties

Particle size, polydispersity index, and zeta potential

The particle size was estimated based on the hydrogel's diameter and simultaneously measured on the basis of the polydispersity index using a particle size analyzer (SZ-100 series; Horiba, Kyoto, Japan). The hydrogel's zeta potential was measured using a Nano ZS Zetasizer (v.6.20; Malvern Instruments Ltd., Malvern, UK).

Color

The hydrogel was freeze-dried and ground prior to the color measurement. The redness (a*), yellowness (b*), and lightness (L*) values were determined using a CR200 chromameter (Minolta, Osaka, Japan). The whiteness index was calculated as previously described (Akgün, Ova Özcan, & Övez, 2022).

Crystallinity percentage

The XRD of the hydrogel was determined using a LabX XRD-6000 diffractometer (Shimadzu, Kyoto, Japan) equipped with a Cu Kα target at 40 kV and 30 mA, which had a scanning rate of 4°/min. The pattern was collected in the 2θ range between 3.02° and 90°. The crystallinity percentage (%) was calculated by dividing the area under the peaks by the total area under the curve (Yazdani et al., 2020).

Determination of the prebiotic activity score

The prebiotic activity score was calculated by subtracting the ratio of probiotic cell growth with prebiotics and glucose from the ratio of enteric cell growth with prebiotics and glucose, as previously described (Zakaria *et al.*, 2018). The probiotic used was *L. acidophilus* FNCC 0051, whereas the enteric cells used were *Escherichia coli* FNCC 0091. The test was performed by adding 1% (volume/volume [v/v]) probiotic cells into MRS broth containing 2% (weight/volume [w/v]) glucose or prebiotic and adding 1% (v/v) enteric cells into M9 broth containing 2% (w/v) glucose or prebiotic. The cells were incubated at 37°C for 0 h, 24 h, and 48 h and then enumerated by means of the plate count method using MRS and nutrient agar. Each test was performed three times.

Determination of *L. acidophilus* FNCC 0051 survival during exposure to simulated gastrointestinal conditions

The utilized simulated gastric and intestinal juices were prepared according to the method described by Xu et al. (2016). More specifically, the gastric juice was prepared by mixing 7 mL of pepsin in hydrochloric acid, 2 g of sodium chloride, and 1 M of sodium hydroxide. The intestinal juice was prepared by mixing 1% pancreatic powder, 6.8 g of potassium dihydrogen phosphate, and 77 mL of 0.2 N sodium hydroxide. Next, 1 g of either free or encapsulated cells (in hydrogel derived from porang glucomannan-chitosan, konjac glucomannan-chitosan, and calcium alginate) was mixed with 9 mL of simulated gastrointestinal juices and incubated at 37°C for 120 min. The samples were withdrawn at intervals of 0 min, 30 min, 60 min, and 120 min to reflect gastric juice digestion and 0 min, 60 min, 90 min, and 120 min to reflect intestinal juice digestion (Rather et al., 2017). The hydrogel was then rinsed twice with acetate buffer. The cells were enumerated using the pour plate technique on MRS agar after 48 h of incubation. The number of viable cells following exposure was divided by the initial number of cells in order to determine the cell survival rate during exposure to simulated gastrointestinal conditions (Zeashan et al., 2020). The hydrogel's appearance during exposure to simulated gastrointestinal conditions was observed using an optical BX51 microscope (Olympus Corp., Tokyo, Japan) and an OptiLab pro digital camera (PT Miconos, Indonesia).

Results and Discussion

Encapsulation efficiencies of hydrogels with different numbers of cells

The encapsulation efficiencies of hydrogels with different numbers of initial cells are shown in **Table 1**. The data revealed that the encapsulation efficiencies of the hydrogels ranged between 44.37% and 85.03%. The highest encapsulation efficiency was achieved when 10 log CFU/mL of cells was added to the mixture, which exceeded the Food and Agricultural Organization of the United Nations (FAO) criteria for probiotic products (> 6–7 log CFU/mL; Isa and Razavi, 2021). Previous studies using different encapsulants obtained different encapsulation efficiencies. For instance, the encapsulation of *L. acidophilus* in hydrogel formed from sodium alginate and soy protein isolates achieved an encapsulation efficiency of 95%–98%, whereas the encapsulation of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* in an emulsion achieved an encapsulation efficiency of 97%–99% (Mahmoodi Pour, Marhamatizadeh, & Fattahi, 2022; Zeashan et al., 2020). The differences in the achieved encapsulation efficiencies might reflect the different encapsulant types and encapsulation methods used (Zeashan et al., 2020). We previously showed that the same ratio of glucomannan and chitosan affected the encapsulation efficiency due to the chemical bonding of both polymers as well as due to the difference in electrostatic values between the core and the polymer influencing the degree of cell entrapment (Aprilia et al., 2021).

TABLE 1: Encapsulated cell numbers and hydrogel encapsulation efficiencies with different initial cell numbers.

Hydrogels with different cell numbers (log CFU/mL)	Cell number before encapsulation (log CFU/mL)	Cell number after encapsulation (log CFU/g)	Encapsulation efficiency (%)
8	9.39 ± 0.00	4.47 ± 0.18	44.37 ± 1.91[a]
9	9.56 ± 0.00	6.60 ± 0.13	65.83 ± 1.37[b]
10	10.10 ± 0.00	7.94 ± 0.21	85.03 ± 0.63[c]

Values represent the mean ± standard deviation (SD). Different superscript letters in the same column indicate significantly different results at the level of $p < 0.05$.

Properties of the hydrogels with different cell numbers

The appearance of the hydrogels generated from glucomannan and chitosan containing *L. acidophilus* was as shown in **Figure 1**. The polymer solution was clear before the encapsulation process, although it became turbid after the encapsulation process. This provided evidence of the formation of particles that influenced the turbidity of the solution. After the drying process, the hydrogels exhibited a shape similar to that of white cotton. The particle sizes and color values of the hydrogels will be explained below.

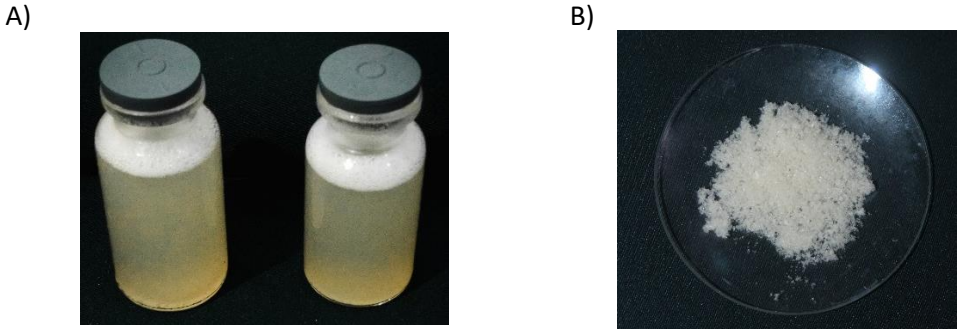

FIGURE 1: The appearance of hydrogels A) before drying and B) after the drying process.

The sizes of the hydrogels encapsulating *L. acidophilus* were found to be in the range of 0.7 µm to 9 µm, with most having a diameter of 2 µm to 3 µm (**Table 2**). Those hydrogels determined to be <100 µm in diameter were classified as microgels. The cell concentration significantly influenced the hydrogels' particle size ($p < 0.05$). In fact, the more cells encapsulated within a given hydrogel, the greater its diameter. The particle size was also correlated with the encapsulation efficiency (**Table 1**), as more cores could be trapped within larger hydrogel particles. The other factors found to influence the particle size were the concentration and viscosity of the solution (Aprilia et al., 2021; Zeashan et al., 2020).

TABLE 2: Particle sizes, polydispersity indexes, and zeta potentials of hydrogels with different initial cell concentrations.

Initial cell number (log CFU/mL)	Particle size (µm)	Polydispersity index	Zeta potential (mV)
8	2.23 ± 0.11^{a}	1.23 ± 0.17^{a}	24.40 ± 0.75^{b}
9	2.79 ± 0.19^{b}	1.39 ± 0.04^{ab}	32.28 ± 0.80^{c}
10	3.41 ± 0.14^{c}	1.65 ± 0.27^{b}	14.58 ± 0.97^{a}

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

The polydispersity indexes of the hydrogel encapsulated cells were all >1 (**Table 2**), indicating the broad distribution of particles of various sizes. Overall, the index began to change when the initial cell number was 10 log CFU/mL. Moreover, the greater the initial cell number, the higher the polydispersity index. This result contrasts with the result of a previous study that found the glucomannan concentration to not influence the polydispersity index (Aprilia et al., 2021).

The hydrogels' zeta potentials became more electropositive as the cell number increased from 8 to 9 log CFU/mL but then decreased as the cell number reached 10 log CFU/mL (**Table 2**). An increase in the number of cells should result in a reduction in the hydrogel's charge due to the positive charge of empty hydrogels and the negative charge of cells (Aprilia et al., 2021), including *L. acidophilus* (Priya, Vijayalakshmi, & Raichur, 2011). The observed pattern might stem from the zeta potential being measured on the hydrogel's surface, meaning that it could have been affected by the pH of the surrounding environment (Barbosa et al., 2019).

The L*, b*, and whiteness values of the hydrogels increased after the addition of cells, whereas the a* value decreased (**Table 3**). The utilized instrument determined these values based on the reflection by the cells of a direct light beam from a chromameter. Therefore, the more cells encapsulated within the hydrogel, the greater the reflection. Bacteria may also generate distinct shades of colors such as red. Based on the findings of a prior study, *Lactobacillus pluvialis* could reflect an orange color from the pigment of canthaxanthin (Venil, Dufossé, & Renuka Devi, 2020). This finding is in agreement with the present result, especially in terms of the increase in the b* value following the addition of *L. acidophilus.*

TABLE 3: Color values of hydrogels with different initial cell numbers.

Initial cell number (log CFU/mL)	L*	a*	b*	Whiteness
control	65.06 ± 0.12^{a}	7.02 ± 0.09^{c}	12.50 ± 0.08^{a}	62.24 ± 0.15^{a}
8	76.97 ± 0.32^{b}	5.42 ± 0.01^{b}	14.24 ± 0.11^{c}	72.38 ± 0.21^{b}
9	79.48 ± 0.33^{c}	5.61 ± 0.07^{b}	15.14 ± 0.01^{d}	73.89 ± 0.25^{c}
10	77.39 ± 0.23^{b}	4.22 ± 0.23^{a}	13.24 ± 0.13^{b}	73.46 ± 0.30^{c}

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

The XRD spectra represent the interaction between the diffraction intensity and the angle (**Figure 2**). Moreover, a crystalline state is indicated by the sharp diffraction peak, whereas an amorphous and solid state is indicated by the declivous peak (Yanuriati et al., 2017). The X-ray diffractogram patterns of all the

hydrogels showed a very broad band at 2θ between 5° and 90°. In addition, all the hydrogels exhibited nearly identical highest peaks at around 2θ 7.06°–10.46°, 7.62°–11.00°, 7.48°–10.94°, and 7.16°–11.20° for those hydrogels without cells and with cells at numbers of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively. These results differ from those concerning porang glucomannan, which exhibited its highest peaks at around 19°–20° and 35° (Yanuriati et al., 2017). However, there was a small peak in all the samples at around 2θ 10.5°, indicating the presence of chitosan (Yu, Lu, & Xiao, 2007). This observation suggests that the mixture of glucomannan hydrogel and cells strengthened the associated chemical interaction, which is consistent with previous Fourier-transform infrared spectroscopy (FTIR) findings (Aprilia et al., 2021). It also suggests that some chitosan did not interact with the glucomannan. A prior study reported that the Schiff's crosslinking between glucomannan aldehyde groups and chitosan amino groups could suppress the chitosan's crystalline state, which is usually strengthened by the hydrogen bond between the amino and hydroxyl groups (Yu, Lu, & Xiao, 2007). We also found evidence of low crystallinity, with values of 26%, 25%, 17%, and 21% being determined for the hydrogels without cells and with cells at numbers of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively. The addition of *L. acidophilus* appeared to have no effect on the diffraction peak, indicating that the entrapment of microbes within the hydrogel did not affect the interaction between the glucomannan and chitosan.

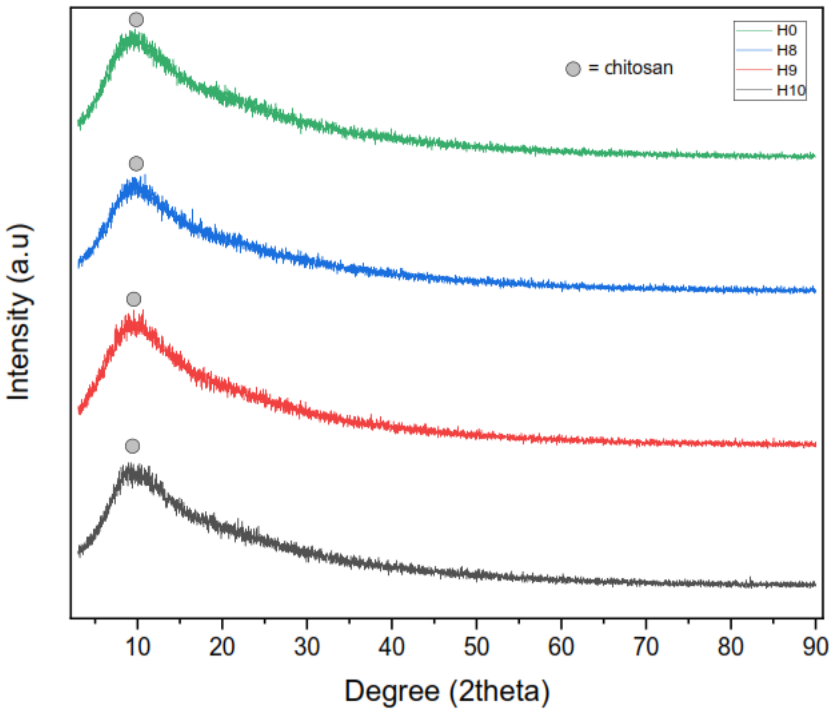

FIGURE 2: X-ray diffractogram for H0 (hydrogel without *L. acidophilus*), H8, H9, and H10 (hydrogels with *L. acidophilus* at numbers of 8 log CFU/mL, 9 log CFU/mL, and 10 log CFU/mL, respectively).

Prebiotic activity of the hydrogels

The *L. acidophilus* and *E. coli* cell density increased during 0 h, 24 h, and 48 h of incubation in the presence of carbohydrates, glucose, inulin, and hydrogel (**Table 4**). Both bacteria showed no significant increase in almost all the carbohydrates, except for *L. acidophilus* with inulin and *E. coli* with glucose. These data suggest that only inulin is able to specifically stimulate the growth of good bacteria and suppress the growth of enteric bacteria, which is consistent with its widespread use as a commercial prebiotic.

TABLE 4: Density of *Lactobacillus acidophilus* FNCC 0051 and *Escherichia coli* cells in 10 log CFU/mL after 0 h, 24 h, and 48 h of incubation with prebiotics, inulin, hydrogel, and glucose.

Prebiotic	*Lactobacillus acidophilus*			*Escherichia coli*		
	0 h	24 h	48 h	0 h	24 h	48 h
Glucose	6.94 ± 1.32^{a}	8.35 ± 0.81^{a}	9.17 ± 0.01^{b}	6.65 ± 0.92^{a}	8.54 ± 0.09^{ab}	9.29 ± 0.49^{b}
Inulin	6.59 ± 0.19^{a}	7.33 ± 0.49^{ab}	8.48 ± 0.88^{a}	9.53 ± 0.09^{a}	7.59 ± 0.32^{a}	8.47 ± 0.75^{a}
Hydrogel	9.37 ± 0.10^{a}	9.58 ± 0.46^{a}	10.15 ± 0.21^{b}	8.80 ± 1.13^{a}	8.17 ± 0.86^{a}	9.02 ± 2.18^{a}

Values represent the mean ± SD. Different superscript letters in the same row indicate significantly different results at the level of $p < 0.05$.

The prebiotic potential of the hydrogel was compared with that of inulin on the basis of the prebiotic activity scores (**Figure 3**). The prebiotic activity score of the hydrogel was higher than that of inulin after 24 h of incubation, although it was reduced after 48 h, suggesting that the hydrogel was the preferred energy source for the cells. This result is consistent with the XRD findings, which confirmed the hydrogel to have an amorphous state and no long-range order, making it easier to digest. Moreover, the amount of carbohydrates will decrease with time. By contrast, the known prebiotic inulin (Kamel et al., 2021) required a longer time to be available for the bacteria due to its long polymeric carbon chains—that is, chains of around 2–60 molecules (Samolińska & Grela, 2017).

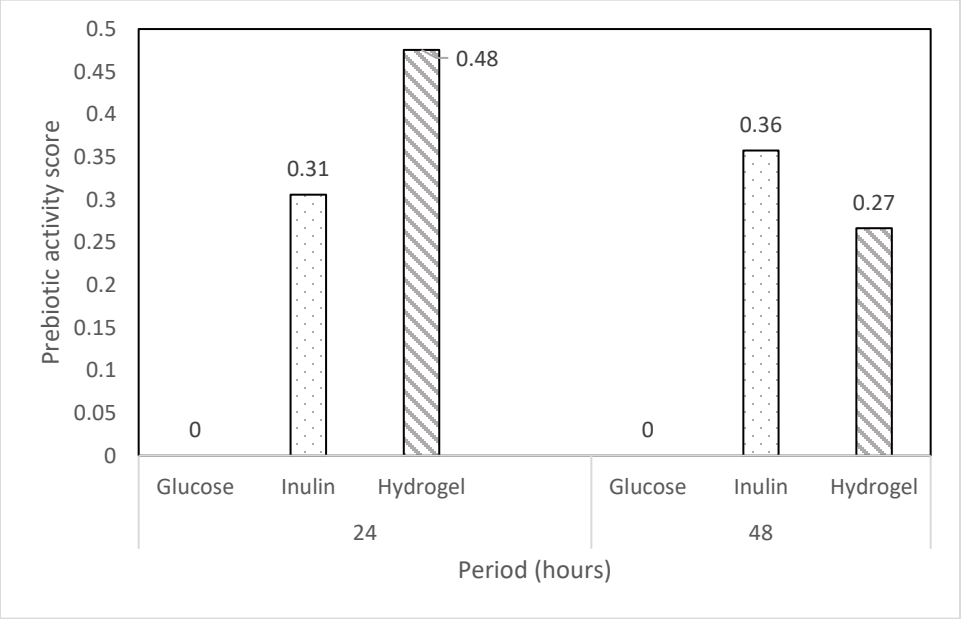

FIGURE 3: Prebiotic activity score of *L. acidophilus* FNCC 0051 on glucose, inulin, and hydrogel.

Cell survival during exposure to simulated gastrointestinal conditions

Cell survival during exposure to gastric juice

The *L. acidophilus* showed good viability during exposure to gastric juice at pH 2, whether in its free form or when encapsulated in hydrogel (**Figure 4**). Generally, the growth of lactic acid bacteria is optimum at pH 6–7 (close to neutral pH). Some metabolic reactions change when the pH is <5 or <4.4. Indeed, some minerals will be lost at pH ≤2, while prolonged storage at a low pH will increase the risk of cell death (Hayek dan Ibrahim, 2013). Our results in this regard are consistent with those of previous studies (Stasiak-Różańska et al., 2021; Zeashan et al., 2020). Further studies are required to determine the effect of solid or solid-enriched macronutrient foods with a longer transit time (Müller, Canfora, & Blaak, 2018). In addition, a shorter exposure time within the stomach enables cells to maintain homeostasis between the internal and external pH, which potentially influenced the good viability found in this study.

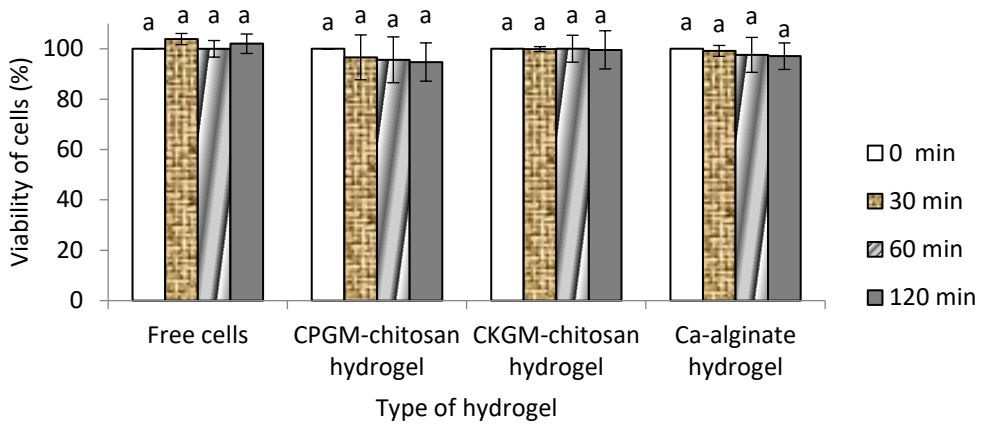

FIGURE 4**:** *L. acidophilus* FNCC 0051 viability during exposure to gastric juice for 120 min. Key: a, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

The present study also found that porang glucomannan-chitosan hydrogel might exhibit a similar ability to protect cells from the gastric environment as both konjac glucomannan-chitosan hydrogel and calcium-alginate hydrogel ($p > 0.05$). This finding accords with the ability of alginate to protect *L. plantarum* (Rather et al., 2017) and *Lactobacillus rhamnosus* from this harsh environment over the course of 3 h of exposure (Oberoi et al., 2021).

The hydrogel was stable in the simulated gastric juice throughout 120 min of exposure (**Figure 5**), which is consistent with the result of a previous swelling ratio study (Aprilia et al., 2021) that determined the hydrogel to not de-swell at a pH < 5. Deswelling causes hydrogel to become smaller, which was previously thought to result in the release of cells from the hydrogel. However, the cells are still trapped in the hydrogel (**Figure 5**), which perhaps reflects the stronger electrostatic interaction between the glucomannan carbonyl group and chitosan amine group in an acid environment (Aprilia et al., 2021). The cells remain in the hydrogel because this interaction maintains the core. Thus, deswelling could not be maximized, leading to only a small number of cells being released from the hydrogel. It is possible that some empty hydrogels will shrink to the extent that they are no longer visible after 60 min of exposure. These results are consistent with those of other studies using hydrogels made from oxidized glucomannan and chitosan to trap diclofenac drugs, which found < 1% of cells to be released during exposure to simulated gastric fluid at pH 1.2 (Korkiatithaweechai et al., 2011). This shows that the hydrogel cores were not released when the hydrogel was exposed to low pH conditions.

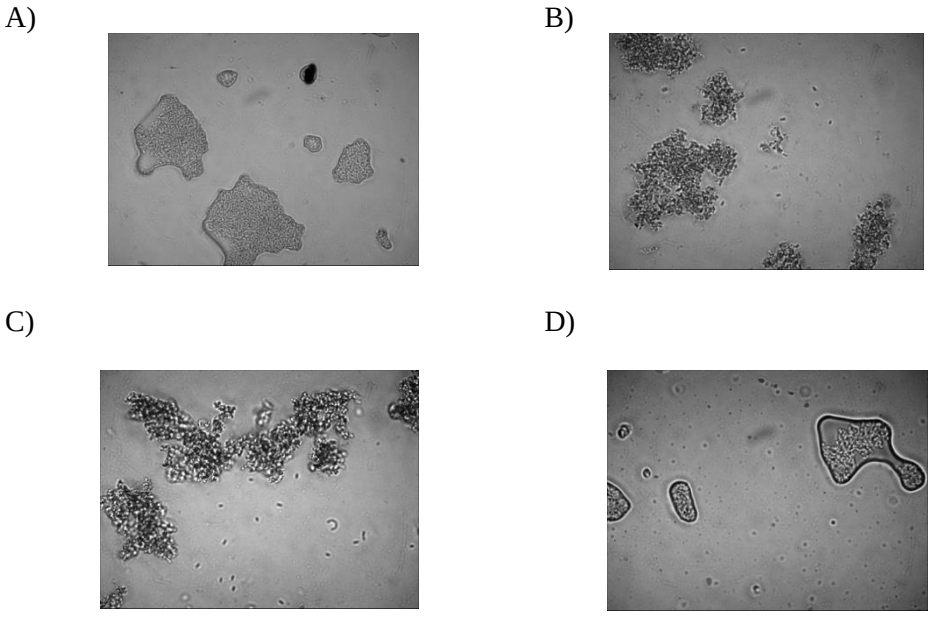

FIGURE 5: Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (1300 × magnification) during exposure to gastric juice for (A) 0 min, (B) 30 min, (C) 60 min, and (D) 120 min.

Cell survival during exposure to intestinal juice

The viability of the free cells decreased significantly during exposure to intestinal juice for 60 min (**Figure 6**; $p < 0.05$). Yet, the viability of the cells encapsulated in the hydrogel was maintained over 120 min of exposure, indicating that the encapsulation increased the viability of the *L. acidophilus*. A decrease in the number of free cells may reflect cell death, which can be caused by factors other than the pH of the medium. Priya et al. (2011) reported that while bacteria showed good growth at pH 6.8, the presence of pancreatin (comprising amylase, trypsin, lipase, ribonuclease, and protease) damaged the encapsulation wall, thereby resulting in cell death.

Figure 6 indicates that the porang glucomannan hydrogel exhibited the same level of good protective effect as the konjac-chitosan glucomannan and calcium-alginate hydrogels. In this study, the alginate-based hydrogel was used for the purpose of comparison because it is widely used as an encapsulant due to its low price, good biocompatibility, and nontoxicity. A prior study found that the probiotic encapsulation of alginate increased the viability of the trapped cells when compared with the free cells during exposure to a simulated gastrointestinal condition (Stasiak-Różańska et al., 2021). Therefore, the porang-chitosan glucomannan hydrogel shows potential as a bacterial encapsulant.

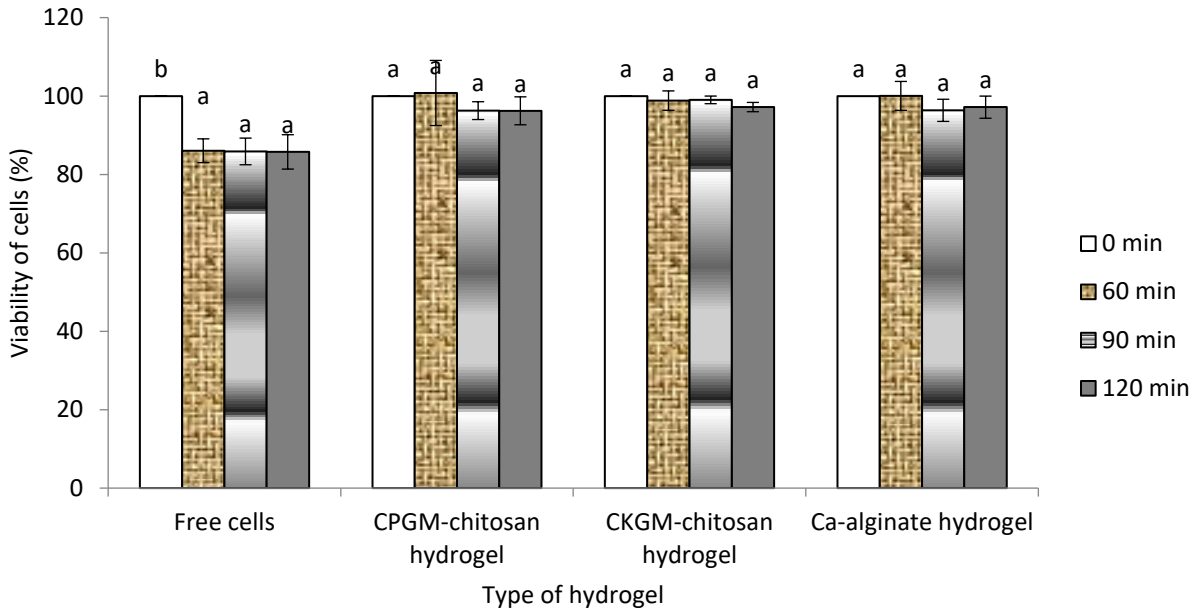

FIGURE 6: *L. acidophilus* FNCC 0051 cell viability during exposure to intestinal juice for 120 min. Key: a or b, $p < 0.05$; CPGM, carboxymethyl porang glucomannan; CKGM, carboxymethyl konjac glucomannan.

The hydrogel's microscopic appearance was used to confirm the cell viability data. Here, the porang glucomannan-chitosan hydrogel remained stable for up to 2 h in the intestinal fluid. However, it was found to be larger after 60 min of exposure than after 0 min (**Figure 7**), potentially reflecting its swelling behavior at pH 6.8. We have previously shown that porang glucomannan-chitosan hydrogel begins to swell at pH >5 (Aprilia et al., 2021). The swelling of the hydrogel was evident until it reached 90 min of exposure. Moreover, many small hydrogels and cells were visible in the solution after 120 min of exposure. The swelling weakened the interaction of the hydrogels, leading to some parts being dissolved, which resulted in both smaller hydrogels and the release of cells from the hydrogels. This result is consistent with that of another study that found konjac glucomannan-carboxymethyl chitosan hydrogel with a bovine serum albumin core to show greater core release at pH 7.4 than at pH 5 due to the swelling enlarging its pores (Du et al., 2006). This core release also occurred when a chitosan-oxidized glucomannan hydrogel was exposed to simulated intestinal fluid for 2–8 h (Korkiatithaweechai et al., 2011).

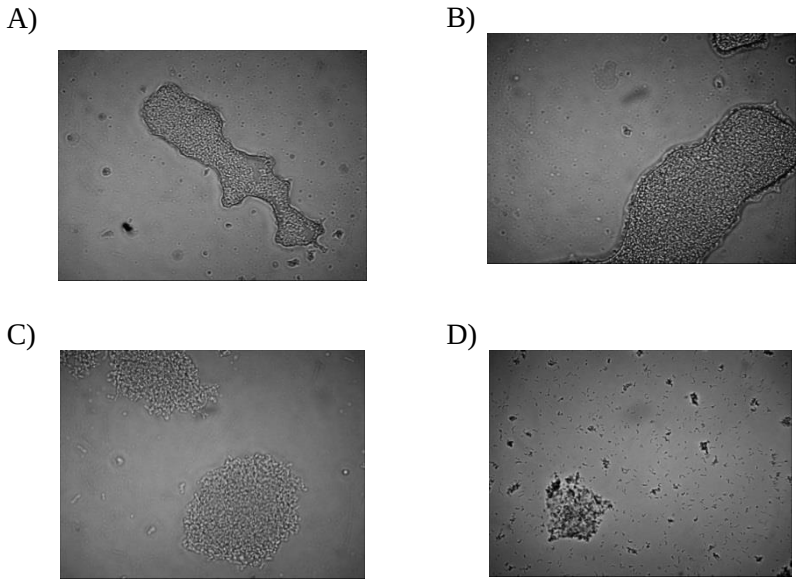

FIGURE 7: Microscopic appearance of hydrogel containing *L. acidophilus* FNCC 0051 (1300 × magnification) during exposure to intestinal juice for (A) 0 min, (B) 60 min, (C) 90 min, and (D) 120 min.

Conclusions

The encapsulation of *L. acidophilus* in hydrogel made from glucomannan and chitosan was improved by varying the number of the cells added. In fact, higher numbers were found to be associated with greater encapsulation efficiency, diameter (2–3 mm), polydispersity index (1.23–1.65), positive zeta potential, whiteness, and brightness. In addition, the hydrogel exhibited potential as a prebiotic, particularly after 24 h of incubation. Moreover, the hydrogel protected the encapsulated cells, maintaining them during exposure to simulated gastrointestinal fluid. Furthermore, the cell viability increased from 86% to 100% when the hydrogel was exposed to intestinal juice, which was comparable to the performance of the alginate and konjac glucomannan hydrogels. Further animal studies are required to determine the cell viability in actual gastrointestinal conditions and assess the health effects of the hydrogel.

Data Availability

The data used to support the findings of this study are included in the article.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgments

This research was supported by the Riset Inovatif Produktif project Lembaga Pengelola Dana Pendidikan (Indonesia Endowment Fund for Education) for 2016–2017 and the Research Directorate and Reputation Team towards World Class University-Quality Assurance Office of Universitas Gadjah Mada (assignment letter number 6144/UN1.P.III/DIT-LIT/PT/2021 dated September 27, 2021).

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