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The Potential of Alginate and Corn Starch as Microencapsulant Materials to Protect The Viability of Probiotic *Pichia kudriavzevii* UNJCC Y-109

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Abstract: Microencapsulation improves the viability of probiotics in processed products and the digestive tract by protecting cells from harsh environments, including gastric acid, bile salts, and high temperatures. This study evaluated the effectiveness of alginate and cornstarch combinations for encapsulating *Pichia kudriavzevii* UNJCC Y-109 and *Saccharomyces cerevisiae* UNJCC Y-87 yeast cells as probiotics. Beads formed through encapsulation were characterized for color, shape, surface, and diameter, and their viability was tested under acidic pH and bile salt exposure. The beads exhibited irregular, oval-round shapes, smooth surfaces, and colors ranging from cold grey to white, with diameters of 1.56 ± 0.63 mm to 3.46 ± 0.06 mm. Encapsulation effectiveness was highest for *P. kudriavzevii* using 1% alginate and 3% cornstarch, yielding 89.82 ± 1.49% viability. For *S. cerevisiae*, 2% alginate and 3% cornstarch achieved optimal results with 92.74 ± 0.30% viability. Acidic pH tests showed survival rates exceeding 90% after 3 hours, while bile salt exposure at 2% concentration maintained over 80% viability. These findings demonstrate that alginate and cornstarch combinations effectively enhance the resilience of encapsulated probiotics, making them suitable for applications in functional foods and therapeutic products.

Keywords: alginate; cornstarch; microencapsulation; *Pichia kudriavzevii*; probiotic.

1. Introduction

The consumption of probiotics in optimal viability has a positive impact on the digestive system [1]. Microorganisms can be used as probiotic agents that can benefit humans and animals [2]. Probiotics are non-pathogenic live microorganisms that can provide health benefits to their hosts when consumed in optimal amounts [3]. Probiotic microorganisms must have the ability to survive in digestive tract conditions, including acidic conditions, bile salts, and digestive enzymes, have antibacterial properties, can colonize the intestinal epithelial tissue and function as probiotics [4]. There are several requirements for microbes as probiotic agents.

Requirements to be considered a probiotic agent include that microbes must be able to grow at pH and bile salt conditions in the stomach with a viability of 10^6-10^7 cfu/ml or 10^6-10^7 cfu/gram [5], have high viability when exposed to stomach acid [4]. Other requirements as a probiotic agent, besides being able to grow at low pH, were to produce anti-microbial compounds [6] such as organic acids, which affect the health of the digestive tract, the ability to survive through the gastrointestinal (GI) tract, and the prevention of diarrheal conditions and upper respiratory tract infections. Yeast is one of the microorganisms that have probiotic ability.

Yeasts are a class of eukaryotic, unicellular fungi that develop quickly [7]. Because they can grow in conditions with stomach and bile salts [8], some yeast species, including *Kluyveromyces*, *Debaryomyces*, *Issatchenkia*, *Yarrowia*, and *Pichia*, have potential as probiotic agents. Many scientists have pointed out the role of yeast species as probiotics. For example, *Saccharomyces boulardii*, a probiotic yeast, has been demonstrated by Cui *et al*. [9] to be useful in the treatment of a variety of digestive problems. The findings highlight the therapeutic potential of *S. boulardii* in regulating gastrointestinal health, and they are based on both preclinical and clinical trials. *Saccharomyces boulardii* can produce proteases that can neutralize bacterial toxins and improve the metabolic function of the intestinal mucosa [4].

According to Diosma *et al*. [10], *Kluyveromyces marxianus* has a high acceptability to bile salts and can survive in acidic stomach conditions with a survival rate of 70.6%. In addition, *Pichia kudriavzevii K. marxianus* exhibits higher vitality in gastric circumstances than *Saccharomyces* species, with a viability value of 7.30 log CFU/mL, exceeding the latter's value of 6.80 log CFU/mL. Encapsulation is an effective method for preserving the viability of probiotics in the digestive tract, which is essential for their therapeutic value [11].

Encapsulation is the process that involves placing a particular covering material on an inner substance [12]. Probiotic yeast cell biomass was used as the primary source of material for this investigation. According to Barajas-Álvarez *et al*. [13], this technique increases the capacity for the survival of probiotic cells in processed food as well as the functioning of the gut. Probiotic cells are efficiently shielded by encapsulation toward harsh processing, storage, and environmental factors such as bile salts, digestive enzymes, stomach acid, and high temperatures [14]. The choice of coating material and encapsulation process used has a major impact on the success of encapsulation [15].

Encapsulation materials that can be used are carrageenan, chitosan, whey protein, cellulose, alginate, gum arabic, and starch [16]. The material commonly used for encapsulation is alginate[17,18]. Alginate is widely used for encapsulation because it is cheap, easy to use, has non-toxic properties, good biocompatibility [19], and is easily destroyed under alkaline conditions so that it can release probiotic microorganisms into the small intestine [20]. Alginate is effective in protecting probiotic cells, ranging from 80-96% [21]. The alginate encapsulation

mechanism can occur due to the interaction between alginate polymers and divalent cations in the form of calcium ions. Calcium ions will be between the two alginate polymers or what is known as the “egg box” gel mechanism [22]. Still, there are some drawbacks to using alginate as an encapsulation material.

The application of alginate as an encapsulation material has several obstacles, namely susceptibility to stomach acid, loss of stability, and damage when passing through the digestive tract [20]. These obstacles can be overcome in various ways, such as mixing with other polymers, coating alginate beads with other compounds, and modifying alginate with supporting compounds [21]. Rashidinejad *et al*. [23] reported that combining alginate with Hi-Maize maize starch improves probiotic cell viability and minimizes damage during encapsulation. Hansen *et al*. [24] reported that starch helps probiotics survive by providing prebiotic nutrition, strengthening bead structures, and promoting the creation of polymer networks. Compared to non-encapsulated cells, *Lactobacillus casei* encapsulated with alginate and Hi-Maize exhibits greater survival rates [25]. Success is also affected by techniques [20]. By avoiding high temperatures, the extrusion process preserves probiotic viability while accommodating many cells [16]. Compared to emulsion techniques, it is easier to use, less expensive, and increases the survival of *L. reuteri* in acidic environments [26].

Jakarta State University Culture Collection (UNJCC) has a yeast that has the potential as a probiotic agent, namely *Pichia kudriavzevii* UNJCC Y-109. Several researchers reported that non-Saccharomyces yeast isolated from fermented food or beverages had tolerance to digestive tract conditions [27]. *P. kudriavzevii* has a fairly high ability to survive in stomach acid conditions at pH 1.2 and bile salts [28] and also a fairly good ability to auto-aggregate [29]. This study aimed to investigate the impact of encapsulating yeast using a combination of alginate and starch on its viability and effectiveness as a probiotic agent. The findings suggest that yeast cell encapsulation holds significant potential for the functional food industry, enhancing the viability of probiotics and paving the way for innovative probiotic products.

2. Materials and Methods

2.1. Materials.

The yeast isolates used in this study were obtained from the fermented drink Brem Lombok, collected by the Jakarta State University Culture Collection (UNJCC). The specific isolates used were *Pichia kudriavzevii* UNJCC Y-109 and *Saccharomyces cerevisiae* UNJCC Y-87, the latter serving as the control. The yeast cells were preserved using the freeze-drying method at -20°C.

2.2. The probiotic yeast cell suspension.

The preparation of the yeast cell suspension was conducted following the method described by Atia *et al*. [30]. Yeast cells were grown on Yeast Peptone Dextrose Agar (YPDA) medium, which consisted of 10 g yeast extract, 5 g peptone, 10 g glucose, and 20 g agar in 1000 mL of distilled water. This medium was sterilized in an autoclave before use. The yeast cells were incubated on a YPDA medium for 48 hours. After incubation, 5 mL of Phosphate Buffered Saline (PBS) was added to the yeast cultures. The PBS solution consisted of 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4 in 1000 mL of distilled water, and it was also sterilized by autoclaving. Yeast cell 10% (v/v) suspension was shaken at 200 rpm for 48 hours at 30°C, and the biomass of yeast cells was separated from the supernatant by centrifuging the

entire mixture at 3000 rpm for 10 minutes at 4°C. After the harvest, PBS solution was applied to wash the biomass pellets. A spectrophotometer was utilized to assess the density of the yeast cells, and they were adjusted to an optical density (OD) of 2 at 600 nm, or roughly 10^7 cfu/mL [31].

2.3. Preparation of coating material.

The coating material was prepared following the method described by Allan-Wojtas *et al*. [32]. A combination of alginate and cornstarch was used, with twelve different formulations: 1%:0%, 2%:0%, 3%:0%, 1%:1%, 1%:2%, 1%:3%, 2%:1%, 2%:2%, 2%:3%, 3%:1%, 3%:2%, and 3%:3% (w/v). Each mixture was homogenized and sterilized by autoclaving at 121°C for 20 minutes. The mixtures were allowed to cool to 38-40°C before use.

2.4. Encapsulation of probiotic yeast.

The probiotic yeast encapsulation procedure was based on the method by Suvarna *et al*. [19]. A yeast cell suspension was prepared at a concentration of 10^{-7} cfu/mL. This suspension was then mixed with the prepared coating material at a ratio of 1:10 (v/v) (cell suspension: coating material). The mixture was homogenized using a vortex mixer until uniform. The homogeneous mixture was then slowly dripped into a 1.5% $CaCl_2$ solution at a ratio of 1:9 (v/v) (mixture: $CaCl_2$ solution). The droplets were left for 30 minutes to form beads. The formed beads were separated using filter paper and transferred into sterile vials. The beads were stored in a refrigerator at 4°C for further testing [31].

2.5. Analysis of morphological beads.

The bead morphology study was performed using the references provided by Valero-Cases and Frutos [33]. Bead morphology involves examining the bead's diameter, shape, and surface. The diameter of a bead was measured with a digital caliper, and the form and surface were observed with a stereo microscope.

2.6. Analysis of encapsulation efficiency of yeast cell viability in beads.

The method for measuring the encapsulation efficiency and viability of yeast in the beads was based on the research by Mahmoud *et al*. [34]. This measurement aimed to determine the number of yeast cells contained within the beads. Initially, 1 gram of beads was added to 9 mL of a 2% sodium citrate solution and homogenized using a vortex mixer for 60 minutes until the beads fully disintegrated. Following this, 1 mL of the resulting suspension from the bead-breaking process was transferred into 9 mL of PBS and then serially diluted to levels 10^{-4}, 10^{-5}, and 10^{-6}. Subsequently, 0.1 mL of the suspension from each dilution was inoculated onto YPD agar using the spread plate method. The inoculated plates were incubated at 37°C for 48 hours. After the incubation period, the growing yeast colonies were observed and counted. Encapsulation efficiency was calculated using the following formula:

$$EE(\%) = \frac{Log\ CFU\ after\ encapsulation}{Log\ CFU\ before\ encapsulation} x\ 100 \qquad (1)$$

2.7. Analysis of survivability beads yeast probiotic encapsulant against gastric acid.

The method for analyzing the probiotic encapsulant survivability beads against stomach acid is based on Mokarram *et al*. [35]. Following incubation, the treated beads were transferred into 9 mL of 2% sodium citrate and homogenized using a vortex mixer for 60 minutes to disintegrate the beads and release the yeast cells. The viability of the encapsulated yeast cells was then determined using the total plate count (TPC) method. The survival percentage of yeast cells was calculated using the formula:

$$\text{Survival Percentage} = \frac{[(\text{Log CFU/mL})_{\text{After treatment}}}{\text{Log CFU/mL}_{\text{before treatment}}} \text{ x } 100 \qquad (2)$$

2.8. Analysis of survivability beads yeast probiotic encapsulant against bile salts.

The bile salt exposure test method refers to the study of Muthukumarasamy *et al*. [26] with modification of the bile salt concentration. The bile salt concentrations used were 0% (control), 0.5%, 1%, 1.5%, and 2%. A total of 1 gram of beads or 1 mL of yeast cell suspension without encapsulation at a concentration of 10^{-7} cfu/ml was put into 9 mL of PBS media containing bile salts (HIMEDIA) at a concentration of 0% (negative control); 0.5%; 1.0%; 1.5% and 2.0% (w/v). The beads were treated with bile salts into 9 mL of 2% sodium citrate and homogenized using a vortex for 60 minutes. A total of 1 mL of the suspension from the bead-breaking process was put into 9 mL of PBS and diluted with dilution levels of 10^{-3}, 10^{-4}, and 10^{-5}. Each treatment was repeated four times, and the formula calculated the survival percentage (%):

$$\text{Survival Percentage} = \frac{[(\text{LogCFU/mL})_{\text{After treatment}}}{(\text{LogCFU/mL})_{\text{before treatment}}} \text{x } 100 \qquad (3)$$

2.9. Statistical data analysis.

To evaluate the differences in the encapsulation efficiency of yeast probiotic beads and their survivability under gastric acid and bile salt conditions, an ANOVA (Analysis of Variance) was conducted. The analysis aimed to determine whether significant differences existed among the tested variables. The significance level was set at 95% (α = 5%).

3. Results and Discussion

3.1. Horse yeast morphological characteristics.

In this study, the yeast species *Pichia kudriavzevii* UNJCC Y-109 and *Saccharomyces cerevisiae* UNJCC Y-87 were analyzed for their morphological characteristics based on the methods described by Kurtzman *et al*. [36]. The morphological characterization revealed distinct differences between the two yeast types. *Pichia kudriavzevii* UNJCC Y-109 exhibited macroscopic features, including an ivory color, flat-brimmed appearance, convex elevation, and a mucoid texture (Figure 1). Microscopically, this yeast displayed oval cell shapes and a bipolar budding pattern. These distinctive traits highlight the unique morphological characteristics of *P. kudriavzevii* compared to *S. cerevisiae* UNJCC Y-87, underscoring the diversity among yeast species and their potential applications in various biotechnological processes.

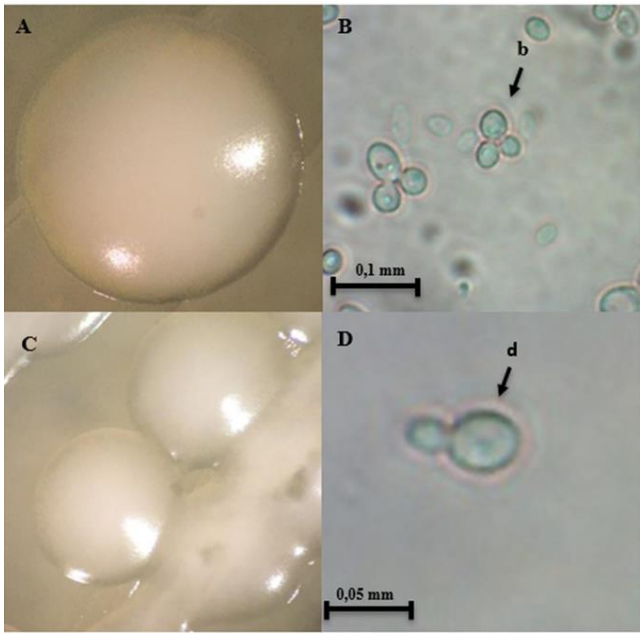

Figure 1. Macroscopic and microscopic characteristics of yeast on YPDA media after 48 hours at 30ºC, (**A**) *P. kudriavzevii* colonies UNJCC Y-109; (**B**) microscopic *P. kudriavzevii* UNJCC Y-109 (**b**) oval cell shape and bipolar budding; (**C**) *S. cerevisiae* colonies UNJCC Y-87; (**D**) microscopic *S. cerevisiae* UNJCC Y-87 (**d**) round, oval and budding cell shape monopolar acid (1000x magnification).

The morphological characteristics of *Pichia kudriavzevii* UNJCC Y-109 were consistent with those reported by Haile and Kang [37], who described this species as having white to ivory colonies, flat edges, convex elevation, and a mucoid or smooth texture. This yeast also exhibited oval cell shapes and bipolar budding, aligning with the findings of Helmy *et al*. [38]. However, while *P. kudriavzevii* is generally noted for a rough or butyrous texture [39].

Differences in yeast morphology can be significantly influenced by dimorphism, a property observed in some yeast species that allows them to exhibit different morphological forms under varying conditions [40]. Dimorphism can be triggered by factors such as nutrient type and concentration, pH, and temperature. In this study, *Saccharomyces cerevisiae* UNJCC Y-87 displayed macroscopic characteristics of white coloration, flat edges, convex elevation, and a mucoid texture, consistent with observations by Haile and Kang [37]. Microscopically, S. cerevisiae UNJCC Y-87 showed a round cell shape and monopolar budding, which aligns with the findings of Helmy *et al*. [38]. These characteristics confirm that *S. cerevisiae* UNJCC Y-87 closely resembles the typical morphology of *S. cerevisiae*, underscoring the consistency of this yeast species' appearance under the conditions tested. Overall, the observed morphological traits of both *Pichia kudriavzevii* and *Saccharomyces cerevisiae* highlight the impact of internal and external factors, including dimorphism, on yeast morphology.

3.2. Alginate and cornstarch bead morphological characteristics.

To investigate the impacts of alginate and cornstarch concentration, the morphology of the beads—including their color, shape, surface, and diameter—was examined. 50% of the beads were cold grey I, 8% were cold grey II, and 42% were white. A concentration-dependent color shift was evident in the primarily white beads produced by higher alginate and cornstarch concentrations (3% each). The diameter of the alginate and cornstarch beads produced in this study varied greatly. Encapsulation of *P. kudriavzevii* UNJCC Y-109 with a combination of alginate and cornstarch produced beads ranging from 1.56 ± 0.63 mm – 3.46 ± 0.06 mm. These

results are consistent with a study by Miskiyah *et al*. [15], where the yellowish color of the bead is caused by the high concentration of yerba mate contained in the bead. Other studies reported a similar matter where combining alginate and starch would produce beads with a smooth or slippery surface [41]. Valero-Cases and Frutos [33] similarly reported 1.86–2.25 mm bead diameters when using a 21G syringe to encapsulate *L. plantarum* with 2% alginate and inulin [41]. Increasing the alginate concentration will increase the carboxylate anion units in the alginate matrix. It can bind to calcium ions in the $CaCl_2$ solution, so the alginate matrix formed will be larger, and the resulting diameter will be relatively larger. A study from Mokarram *et al*. [35] reported that the encapsulation of *L. rhamnosus* with a bead diameter of 23.7 ± 0.161µm decreased by 4 log CFU/mL after exposure to pH 1.5 for 2 hours.

3.3. Encapsulation efficiency of encapsulated yeast probiotic beads.

Çabuk *et al*. [42] reported that encapsulation efficiency (EE) is an important parameter in determining the effectiveness of the encapsulation process and the selection of the coating material used. The results of Encapsulation efficiency (EE) of *P. kudriavzevii* UNJCC Y-109 with a combination of alginate and cornstarch had percentages ranging from 84.01 ± 0.81% - 89.82 ± 1.49%, while the encapsulation efficiency of *S. cerevisiae* UNJCC Y-87 had percentages ranging from 86.90 ± 0.66% - 92.74 ± 0.30%. *P. kudriavzevii* UNJCC Y-109 encapsulation combined with 1% alginate and 3% cornstarch only decreased viability by 0.8 log CFU/mL. In contrast, *S. cerevisiae* UNJCC Y-87 with a combination of 2% alginate and 3% cornstarch only experienced a decrease in viability of 0.57 Log CFU/mL (Table 1).

Table 1. Encapsulation efficiency of *P. kudriavzevii* UNJCC Y-109 and *S. cerevisiae* UNJCC Y-87 with a combination of alginate and cornstarch.

Yeast strain	Concentration of Alginat:Cornstarch	Viability (Log CFU/mL) before treatment	Viability (Log CFU/mL) after treatment	Reduce viability (Log CFU/mL)	Encapsulation efficiency (%)
P.kudriavzevii UNJCC Y-109	1% : 0%	7.83	6.61 ± 0.03^{ab}	1.21	84.50 ± 0.49^{ab}
	2% : 0%		6.75 ± 0.02^{bcd}	1.07	86.28 ± 0.45^{bcd}
	3% : 0%		7.01 ± 0.02^{cd}	0.82	89.57 ± 0.38^{efg}
	1% : 1%		6.90 ± 0.02^{def}	0.92	88.21 ± 0.48^{def}
	1% : 2%		6.82 ± 0.08^{cd}	1.00	87.18 ± 1.28^{cd}
	1% : 3%		**7.03 ± 0.10^{efgh}**	**0.80**	**89.82 ± 1.49^{efgh}**
	2% : 1%		6.72 ± 0.04^{abc}	1.11	85.83 ±0.48^{abc}
	2% : 2%		6.87 ± 0.03^{cde}	0.96	87.69 ± 0.28^{cde}
	2% : 3%		6.57 ± 0.06^{a}	1.25	84.01 ± 0.81^{a}
	3% : 1%		6.73 ± 0.01^{abc}	1.10	85.94 ± 0.13^{abc}
	3% : 2%		6.61 ± 0.01^{ab}	1.21	84.58 ± 0.45^{ab}
	3% : 3%		6.74 ± 0.05$^{abcd0.58}$	1.08	86.15 ± 0.61^{abcd}
S.cerevisiae UNJCC Y-87	1% : 0%	7.79	7.21 ± 0.01^{i}	0.58	92.54 ± 0.40^{i}
	2% : 0%		7.16 ± 0.02^{hi}	0.63	91.88 ± 0.16^{hi}
	3% : 0%		6.77 ± 0.04^{cd}	1.02	86.90 ± 0.66^{cd}
	1% : 1%		7.16 ± 0.01^{hi}	0.63	91.96 ± 0.51^{hi}
	1% : 2%		7.15 ± 0.05^{ghi}	0.64	91.75 ± 0.47^{ghi}
	1% : 3%		7.11 ± 0.05^{ghi}	0.68	91.27 ± 1.01^{ghi}
	2% : 1%		7.10 ± 0.04^{ghi}	0.69	91.14 ± 0.83^{ghi}
	2% : 2%		6.98 ± 0.04^{efg}	0.81	89.60 ± 0.68^{efg}
	2% : 3%		**7.22 ± 0.01^{i}**	**0.57**	**92.74 ± 0.30^{i}**
	3% : 1%		7.07 ± 0.01^{ghi}	0.72	90.80 ± 0.33^{ghi}
	3% : 2%		7.11 ± 0.05^{ghi}	0.68	91.34 ± 1.02^{ghi}
	3% : 3%		7.00 ± 0.08^{cd}	0.78	89.93 ± 0.67^{fgh}

Note: Numbers followed by the same letters are not significantly different at α=0.05 of the Duncan Multiple Range Test (DMRT).

The Encapsulation efficiency (EE) of *S. cerevisiae* UNJCC Y-87 at a concentration of 2% alginate and 3% cornstarch was also significantly different from that of 2% alginate and

2% cornstarch with successive values of 92.74 ± 0.30% and 89.60 ± 0.68% (Table 1). Based on this, 1% alginate and 3% cornstarch are effective concentrations for encapsulating the cells of *P. kudriavzevii* UNJCC Y-109. In comparison, 2% alginate and 3% cornstarch effectively encapsulate *S. cerevisiae* UNJCC Y-87 but are not significantly different from a concentration of 1% alginate and 0% cornstarch.

Encapsulation with the addition of cornstarch is proven to increase the percentage of Encapsulation Efficiency. Adding cornstarch can reduce the porosity of the bead so that the bead structure formed will be stronger and more compact [43]. These results align with the study of Wu *et al*. [44]. The effectiveness of bacterial encapsulation of the Rs-2 strain with a combination of alginate and starch (1%: 2%) reached 90.4%. Other studies report that adding starch can increase Encapsulation Efficiency compared to alginate encapsulation alone [19], and the presence of Ca^{2+} ions can cause cytoplasmic membrane instability during the bead formation process. Donthidi *et al*. [45] and Straccia *et al*. [46] reported variations in the effectiveness of encapsulation in several bacteria of the genus Lactobacillus that were encapsulated using a combination of alginate and starch. Straccia *et al*. [46] reported that the more Ca^{2+} ion binding sites, the more stable and thicker the resulting bead. This statement is supported by Besiri *et al*. [47], where the amount of Ca^{2+} ions available in the $CaCl_2$ solution strongly influences the stability of the bead. This is related to the degree of syneresis. The degree of syneresis can indicate the ability of the gel to return to its original shape [48].

3.4. Survivability beads yeast probiotic encapsulant against gastric acid.

Variations in incubation time are used to determine whether the bead can survive exposure to stomach acid and have the potential to be applied to protect probiotic cells. An incubation time of 3 hours in acidic gastric conditions is the threshold time for a probiotic to be said to be tolerant to acidic conditions [49]. The beads tested were beads with a concentration of 1% alginate: 3% cornstarch for the yeast *P. kudriavzevii* UNJCC Y-109, while for the yeast *S. cerevisiae* UNJCC Y-87, namely beads with a concentration of 2% alginate: 3% cornstarch.

The decrease in the viability of *P. kudriavzevii* UNJCC Y-109, which was encapsulated with 1% alginate and 3% cornstarch, occurred after exposure to pH 2 for 3 hours with a value of 6.95 ± 0.05 log CFU/mL (Table 2). *P. kudriavzevii* UNJCC Y-109 without encapsulation experienced the greatest decrease in the 3rd hour of exposure to pH 2 with a value of 7.56 ± 0.04 log CFU/mL. These results are still included in the optimal category, where the optimal number of probiotics consumed is around 6-7 log CFU/mL [50]. The longer the exposure to pH 2, the viability of *P. kudriavzevii* UNJCC Y-109 will decrease, both encapsulated and unencapsulated. The highest viability was produced at a concentration of 0% alginate: 0% cornstarch in the first hour of exposure to pH 2 with a value of 7.65 ± 0.07 log CFU/mL. In comparison, the lowest viability was produced at a concentration of 1% alginate and 3% cornstarch with an incubation time of 3 hours. Based on exposure to pH 2 for 0 hours, 1 hour, 2 hours, and 3 hours, the survival percentage of *P. kudriavzevii* UNJCC Y-109 encapsulated with a combination of alginate and cornstarch after 3 hours of exposure to pH 2 resulted in a higher survival percentage than without encapsulation with values of 98.43 ± 0.77% and 96.53 ± 0.55% (Table 2). Encapsulation of *P. kudriavzevii* UNJCC Y-109 with 1% alginate: 3% cornstarch had no significant effect in protecting the survival percentage of yeast from exposure to stomach acid. Similar results were found in the study of Pankasemsuk *et al*. [25] that encapsulation of *L. casei* 01 with a combination of alginate and 2% corn starch after 2 hours

of exposure to pH 2 had no significant effect on protecting cell viability from exposure to stomach acid. Mokarram *et al*. [35] also reported that encapsulation of *L. acidophilus* and *L. rhamnosus* with alginate had no significant effect after exposure to stomach acid. There is still little information about the encapsulation of *P. kudriavzevii*, so the results of this study provide new information about the encapsulation of *P. kudriavzevii*.

Table 2. Viability and survival rate (log CFU/mL) of *P. kudriavzevii* UNJCC Y-109 at pH 2 with incubation times of 0 hours, 1 hour, 2 hours, and 3 hours.

Concentration of Alginat: Cornstarch	Incubation time (hour)			
	0	**1**	**2**	**3**
0% : 0%	7.83 ± 0.04d	7.65 ± 0.07d	7.61 ± 0.05c	7.56 ± 0.04c
1% : 3%	7.06 ± 0.05b	7.02 ± 0.03ab	7.02 ± 0.04ab	6.95 ± 0.05a

Percentage of survival of *P.kudriavzevii* UNJCC Y-109 at pH 2 with incubation times of 0 hours, 1 hour, 2 hours and 3 hours

Concentration of Alginat: Cornstarch	Incubation time (hours)	Concentration of Alginat: Cornstarch	Incubation time (hours)	Concentration of Alginat: Cornstarch
	0	**1**	**2**	**3**
0%: 0%	100 ± 0.00	97.87 ± 0.71	97.34 ± 0.57	96.53 ± 0.55
1%: 3%	100 ± 0.00	99.52 ± 0.37	99.49 ± 0.38	98.43 ± 0.77

Note: Numbers followed by the same letters are not significantly different at α=0.05 of the Duncan Multiple Range Test (DMRT)

Further test results showed a significant difference in the viability of *S. cerevisiae* UNJCC Y-87 at concentrations of 0% alginate and 0% cornstarch after an incubation time of pH 2 for 1 hour and 3 hours and not significantly different at 2 hours of incubation time (Table 3). At concentrations of 2% alginate and 3% cornstarch, there was no significant difference after the incubation time of pH 2 for 1, 2, and 3 hours. The survival percentage of *S. cerevisiae* UNJCC Y-87 after exposure to pH 2 showed that all treatments experienced a decrease in the survival percentage, both free cells and with encapsulation. Concentrations of 2% alginate and 3% cornstarch also produced the same thing, where real differences occurred at only 1 and 3 hours of incubation. When the concentration of 0% alginate: 0% cornstarch and 2% alginate: 3% cornstarch is compared, it produces significantly different survival percentage values after exposure to pH 2 for 1, 2, and 3 hours. The survival percentage of *S. cerevisiae* UNJCC Y-87 without encapsulation was higher than with the encapsulation treatment. Similar to the results of the study by Bevilacqua *et al*. [51], where the viability of *S. cerevisiae* without encapsulation was higher than that given the encapsulation treatment using 2% alginate with successive values of 7.18 ± 0.61 log CFU/mL and 6.74 ± 0.18 log CFU/mL after exposure to pH 2. Other studies also reported the same thing, where the survival percentage of S. succinic without encapsulation was higher than that of being treated with encapsulation with 2% alginate and 2% chicory [52]. The good adaptability of *S. cerevisiae* can cause this. *S. cerevisiae* has adaptations to acidic conditions by increasing the activity of acid transporter proteins such as ATPase, Pma1, and Pdr12 proteins [53]. In addition, *S. cerevisiae* also has a Vacuolar Proton-translocating ATPase (V-ATPase) protein, which removes intercellular protons outside the cell, resulting in osmotic stability [53]. The greatest decrease in cell viability in encapsulating *S. cerevisiae* UNJCC Y-87 with a combination of 2% alginate:3% cornstarch was greatest in the first hour of exposure to pH 2 of 0.31 log CFU/mL. This indicates a change in the bead structure, which can lead to a decrease in cell viability.

Table 3. Viability and survival rate (log CFU/mL) of *S. cerevisiae* UNJCC Y-87 at pH 2 with incubation times of 0 hours, 1 hour, 2 hours, and 3 hours

Concentration of Alginat: Cornstarch	Incubation time (log CFU/mL)			
	0 hour	**1 hour**	**2 hour**	**3 hour**
0% : 0%	7.79 ± 0.03e	7.76 ± 0.3d	7.65 ± 0.03cd	7.59 ± 0.07c
2% : 3%	7.23 ± 0.05b	6.92 ± 0.11ab	6.84 ± 0.03ab	6.73 ± 0.02a

Percentage of survival of *S. cerevisiae* UNJCC Y-87 at pH 2 with incubation times of 0 hours, 1 hour, 2 hours and 3 hours.

Concentration of Alginat: Cornstarch	Incubation time (hour)	Concentration of Alginat: Cornstarch	Incubation time (hour)	Concentration of Alginat : Cornstarch
	0 hour	**1 hour**	**2 hour**	**3 hour**
0% : 0%	100 ± 0.00d	99.64 ± 0.71d	98.25 ± 0.62cd	97.48 ± 0.84c
2% : 3%	100 ± 0.00d	95.74 ± 0.97b	94.64 ± 0.18ab	93.14 ± 0.21a

Note: Numbers followed by the same letters are not significantly different at α=0.05 of the Duncan Multiple Range Test (DMRT).

According to Feltre *et al*. [54], alginate encapsulation with gelatinized starch will undergo structural changes after one hour of exposure to gastric acid. Based on the results of gastric acid exposure testing at 1 hour, 2 hours, and 3 hours of incubation time, all good treatments on *P. kudriavzevii* UNJCC Y-109 and *S. cerevisiae* UNJCC Y-87 had a survival percentage of >90%. The provision that microbes can become probiotic agents is that they can survive with a percentage of more than 70% [55].

Exposure to acidic pH, such as in the stomach, can cause changes in the structure of alginate and cornstarch beads. When conditions are acidic (pH 2), ion exchange occurs between Ca^{2+} and H^{+}. The Ca^{2+} metal ion that binds to the carboxyl group (COO^{-}) can be replaced by monovalent H^{+} ions to become COOH. This was caused by the pKa value of sodium alginate, which was higher than the pH of the solution, which was 3.2. The pKa value describes a compound's ability to accept or release protons [56]. This structural change can cause a decrease in yeast cell viability. Under acidic conditions, when pH < 4, the carboxylic group present in the α-L-Guluronic (G) residue will experience protonation. Protonation is the addition of a hydrogen ion (proton) to a molecule. This causes a decrease in electrostatic repulsion, resulting in a smaller bead size when compared to divalent ions (Ca^{2+}). If the pH of the solution is <2, alginate will precipitate in the form of very small and inelastic beads [57].

3.5. Survivability beads yeast probiotic encapsulant against bile salt.

Further of the test was survivability beads yeast probiotic encapsulant exposure to bile salt. Variations in incubation time were used to determine whether the bead could survive exposure to bile salt and have the potential to be applied to protect probiotic cells. The beads tested were beads with a concentration of 1% alginate: 3% cornstarch for the yeast *P. kudriavzevii* UNJCC Y-109, while for the yeast *S. cerevisiae* UNJCC Y-87, namely beads with a concentration of 2% alginate: 3% cornstarch.

Encapsulation of *P. kudriavzevii* UNJCC Y-109 with a concentration of 1% alginate and 3% cornstarch resulted in a fluctuating survival percentage (Table 4). The results showed a significant difference between the concentration of 0% alginate: 0% cornstarch in exposure to bile salts with concentrations of 1% and 1.5%, but exposure to bile salts of 0%, 0.5%, and 1% resulted in viability which was not significantly different. Concentrations of 1% alginate and 3% cornstarch on exposure to bile salts of 1.5% and 2% resulted in significantly different viability with values of 6.22 ± 0.13 and 6.59 ± 0.02 Log CFU/mL but not significantly different at concentrations of 0.5% and 1%. The decrease in survival occurred after exposure to bile salt concentrations of 0.5% with a percentage of 82.99 ± 0.64%. The increase in the survival

percentage of *P. kudriavzevii* UNJCC Y-109 occurred in the encapsulation treatment after exposure to bile salts. This could be due to the hydrolysis of corn starch carried out by *P. kudriavzevii* UNJCC Y-109. In the encapsulation process, starch plays a role in supporting matrix formation and acts as a prebiotic [19]. *P. kudriavzevii* can produce extracellular enzymes, namely amylase [58], beta-glucanase, and glucoamylase [59]. The amylase enzyme can hydrolyze amylose and amylopectin found in corn starch [60]. The degradation of amylose and amylopectin will produce glucose, maltose, and maltodextrin, which can be used as a carbon source, so the number of yeasts in the bead can increase [61].

The survival percentage of *P. kudriavzevii* UNJCC Y-109 without the encapsulation treatment was higher than the encapsulation treatment. *P. kudriavzevii* UNJCC Y-109 survived after exposure to bile salts with a concentration of 2% for 3 hours with a percentage of 95.69%. Several similar studies reported that *P. kudriavzevii* survived exposure to bile salts. Research by Helmy *et al*. [38] reported that *P. kudriavzevii* survived exposure to 0.5% to 2% of bile salts. Research by Ogunremi *et al*. [62] reported that *P. kudriavzevii* survived in 2% bile salts with a percentage of 66.66%. Research by Chelliah *et al*. [63] reported that *P. kudriavzevii* survived in conditions of 0.5% bile salts for 72 hours with a percentage of 70%.

Table 4. Viability and Survival (log CFU/mL) of *P. kudriavzevii* UNJCC Y-109 at bile salt concentrations of 0%, 0.5%, 1%, 1.5% and 2%.

Concentration of Alginate: Cornstarch	**Bile salt concentration**				
	0%	**0.5%**	**1%**	**1.5%**	**2%**
0% : 0%	7.83 ± 0.03f	7.78 ± 0.02f	7.72 ± 0.00f	7.51 ± 0.08e	7.49 ± 0.03e
1% : 3%	7.06 ± 0.03d	5.86 ± 0.00a	5.87 ± 0.03a	6.22 ± 0.13b	6.59 ± 0.02c

Percentage of survival of *P. kudriavzevii* UNJCC Y-109 at bile salt concentrations of 0%, 0.5%, 1%, 1.5% and 2%.

Concentration of Alginat: Cornstarch	**Bile salt concentration (%)**	**Concentration of Alginat: Cornstarch**	**Bile salt concentration (%)**	**Concentration of Alginat: Cornstarch**	**Bile salt concentration (%)**
	0%	**0.5%**	**1%**	**1.5%**	**2%**
0% : 0%	100 ± 0.00e	99.43 ± 0.22e	98.62 ± 0.65e	96.00 ± 1.76d	95.69 ± 1.15d
1% : 3%	100 ± 0.00e	82.99 ± 0.64a	83.14 ± 1.07a	90.85 ± 1.32b	93.32 ± 0.71c

Note: Numbers followed by the same letters are not significantly different at α=0.05 of the Duncan Multiple Range Test (DMRT)

The viability of *S. cerevisiae* UNJCC Y-87 with encapsulation of 2% alginate: 3% cornstarch showed that there was a significant difference in the interaction of the concentrations of 2% alginate: 3% cornstarch at concentrations of 1.5% and 2% of lead salt, but not significantly different at concentrations of 0.5%, 1%, and 1.5%. The results showed a significant decrease of 1.33 log CFU/mL after exposure to 2% bile salts for 3 hours (Table 5). This decrease was greater when compared to the concentration of 0% alginate: 0% cornstarch, which only decreased by 0.35 log CFU/mL under the same conditions, so it can be seen that the encapsulation of 2% alginate and 3% cornstarch is not optimal enough to protect the viability of S. cerevisiae UNJCC Y-87 on exposure to 2% bile salt. Similar to the results of a study by Bevilacqua *et al*. [51] reported that encapsulation of *S. cerevisiae* with 2% alginate decreased viability more than cells without encapsulation, with successive values of 5.76 ± 0.22 log CFU/mL and 6.98 ± 0.44 log CFU/mL after exposure to 0.3% bile salt. The two-way ANOVA statistical test results were also carried out on the survival percentage of *S. cerevisiae* UNJCC Y-87.

Table 5. Viability and Survival Rate (log CFU/mL) of *S. cerevisiae* UNJCC Y-87 at bile salt concentrations of 0%, 0.5%, 1.5%, and 2%.

Concentration of Alginat: Cornstarch	Bile salt concentration (%)				
	0	**0.5**	**1**	**1.5**	**2**
0% : 0%	7.78 ± 0.03d	7.67 ± 0.03d	7.66 ± 0.01d	7.70 ± 0.01d	7.43 ± 0.04d
2% : 3%	7.06 ± 0.05c	6.91 ± 0.19bc	6.64 ± 0.14b	6.22 ± 0.20b	5.73 ± 0.19a
Percentage of survival of *S. cerevisiae* UNJCC Y-87 at bile salt concentrations of 0%, 0.5%, 1%. 1.5% and 2%					
Concentration of Alginate: Cornstarch	**Bile salt concentration (%)**				
	0	**0.5**	**1**	**1.5**	**2**
0% : 0%	100 ± 0.00d	98.50 ± 0.37bcd	98.45 ± 0.31bcd	98.99 ± 0.40cd	95.43 ± 0.66bcd
2% : 3%	100 ± 0.00d	97.89 ± 2.00bcd	94.03 ± 1.33bc	93.77 ± 3.15b	81.15 ± 4.66a

Note: Numbers followed by the same letters are not significantly different at α=0.05 of the Duncan Multiple Range Test (DMRT)

P. kudriavzevii UNJCC Y-109 and *S. cerevisiae* UNJCC Y-87 without encapsulation had a higher viability and survival rate when compared to the encapsulation treatment using a combination of alginate and cornstarch. Both test yeasts had a high percentage of survival, namely > 80% after exposure to various concentrations of bile salts. This is due to the ability of yeast to produce bile salt hydrolase (BSH) enzymes. The BSH enzyme will change the structure of primary bile salts into secondary bile salts through deconjugation by releasing glycine and taurine compounds [64].

Encapsulation of *P. kudriavzevii* UNJCC Y-109 and *S. cerevisiae* UNJCC Y-87 with alginate-cornstarch showed >80% survival after 3 hours in 0.5–2% bile salts. Similarly, Mandal *et al*. [65] reported 88.4% survival for *L. casei* encapsulated with 4% alginate and 3% resistant starch. After three hours in 2% bile salts, *L. casei* encapsulated with alginate and 2% corn starch had a 78% survival rate, according to Pankasemsuk *et al*. [25]. Research by Suvarna *et al*. [19] reported that the encapsulation of *Pichia barkeri* with alginate and gelatinized starch resulted in the viability of 6 log CFU/mL after passing gastrointestinal exposure. Alginate-corn starch (Hi-Maize) encapsulation preserved *L. acidophilus* viability up to 95.69% in 1% bile salts for 6 hours, according to Iyer [66] study. Alginate structural changes brought on by Ca^{2+}-Na^{+} ion exchange destabilize the alginate chain and increase water penetration, which results in a loss in survival [56].

4. Conclusions

Pichia kudriavzevii UNJCC Y-109 (EE 89.82%) responded best to 1% alginate with 3% cornstarch, whereas *Saccharomyces cerevisiae* UNJCC Y-87 (EE 92.74%) responded best to 2% alginate with 3% cornstarch. Both cultures demonstrated strong encapsulation for probiotic usage, surviving >80% in 0.5–2% bile salts and >90% in pH 2 stomach acid.

Author Contributions

Conceptualization, D.S. and A.S.; methodology, A.S.; software, S.R.; validation, L.K.T., S.R., and R.H.B..; formal analysis, L.A.; investigation, A.S.; resources, M.A.S.; writing—original draft preparation, R.H.B., D.S.; writing—review and editing, R.H.B.S.; D.S; R.Y.; visualization, T.K.; supervision, L.H.H.; project administration, H.E.E..; funding acquisition, L.A. All authors have read and agreed to the published version of the manuscript.

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Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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