The influence of prebiotic encapsulated materials on the stability of probiotic

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Abstract. The impact on probiotics from insufficient protection of microcapsules necessitates the development of more effective probiotic encapsulation. This is achieved by using encapsulating agents and adding a double coating layer to the microcapsules to enhance structural strength. This study aimed to investigate the survivability of the bacteria probiotic *Lactobacillus plantarum* TISTR 2075 encapsulated with alginate, gellan gum, creatine monohydrate and different prebiotics by using extrusion technique and coated with chitosan and hyaluronic acid by layer-by-layer technique. The encapsulation efficiency (%EE) was tested and the physical characteristics, morphology and mechanical strength of the gel encapsulations were also evaluated. Findings indicated that the encapsulation of probiotics with prebiotic added increased the encapsulation efficiency (70-94 %). Moreover, the addition of pullulan provided the microcapsules with high mechanical strength. These results confirmed that the enhancement of prebiotic pullulan to the encapsulating agents had significant effects on the survivability of probiotic cells.

1 Introduction

Probiotics are microorganisms promoting gastrointestinal health and enhancing immune responses when dispensed in adequate amounts. They offer several health benefits to the host, and their potent viability is worthy of protection [1]. Beneficial microorganisms must be assigned as bacteria cells live that can tolerate the gastrointestinal tract and locate the colon [2]. Moreover, probiotics have great potential for immunomodulatory activity, and even neurological health [3]. During processing and storage, probiotics are sensitive under the technological and gastrointestinal tract that could lead to the instability of the survivability and activity of probiotic bacteria cells [4]. A significant concern of probiotics is the viability during the process, the storage, and the delivery.

Prebiotics are fibers and carbohydrates that the human body cannot be digested but nurture as nourishment for gut bacteria such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), inulin, and resistant starch [5]. Their capability to preserve a healthy balance of intestine microorganism, raise the immune system, and enhance overall health [6, 7]. Encapsulation processes on the survival of *Lactobacillus plantarum* F1 and *Lactobacillus reuteri* 182 using the extrusion technique with 2% sodium alginate as encapsulation agent, combined with prebiotics, namely 4% trehalose and 2% inulin, and coated with 0.4% chitosan and subjected to freeze drier. The addition of trehalose and sodium alginate protected lactic acid bacteria (LAB) with the increasing of survival rate to more than 92.5%. The chitosan coating enhanced the spherical shape and improved mechanical strength. The prebiotic encapsulation resulted in better cell stability during one year storage, with values of 4.82 log CFU/g in alginate-trehalose beads and 5.64 log CFU/g in alginate-trehalose-inulin beads [8]. Kistaubayeva et al. [9] reported that the survival of *Lactobacillus rhamnosus* GG encapsulated with alginate and alginate plus pullulan were 74 and 94%, respectively. However, the viability of the probiotic was rapidly decreased in unencapsulated cells with low cell viability (43% survival rate). Apiwattanasiri et al. [10] suggested that the encapsulation of *Lactobacillus casei* using silk sericin-alginate-prebiotics and coated with silk sericin improved the survival rate of probiotic.

Microencapsulation is an encapsulation technique in which biocomponents are coated inside polymer microcapsules with nanometer to millimeter particles [11]. Microencapsulation technology is a well-confined method developed to conserve stability and protect active substances for long-term or within targeted delivery systems that help shield probiotics from adverse

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conditions in the stomach [4]. Traditionally, encapsulation with prebiotics ensures that the probiotic cells have a persistent supply of a food source, offers additional protection, improve their stability, and ensure that prebiotics assist the growth of the probiotics once they reach the gut [12].

The stability of microencapsulation is reduced during processing, storage, and during gastric and intestinal simulation juice. The microcapsules are coated with multiple layers of coating or layer-by-layer to effectively deliver probiotics [13]. Polysaccharide-protein conjugates offer a new dimension in encapsulation system design. This technique enhances the stability of colloids by using electrostatic technique to neutralize charges [14].

Amino acid (Creatin monohydrate) is used to supplement the structure of the microcapsules. Amino acids have smaller molecules than proteins, so they can be better attached with hydrogels to the structure of the microcapsules. Therefore, the objective of this investigation aimed to study the survival of the encapsulation probiotic bacteria *L. plantarum* TISTR 2075 with different encapsulated hydrogels and prebiotics. After that, chitosan and hyaluronic acid were applied to the samples as layer-by-layer coating materials. Utilization of the charge difference of positively charged chitosan followed by negatively charged hyaluronic acid was applied. The physical and morphological characteristics of microcapsules were analysed.

2 Materials and methods

2.1 Cell culture condition

The cell strain *Lactobacillus plantarum* TISTR 2075 acquired from Bangkok MIRCEN, Thailand. The cells were subcultured in MRS broth (Merck, Germany) medium. The cell cultures were incubated at 37°C for 24 h. Cell pellets were obtained by centrifugation at 4000 rpm for 15 min.

2.2 Probiotic encapsulation

Microencapsulated probiotics used the extrusion method. As discussed in section 2.1, 100 mL culture of the cell was prepared, and 3 mL of cell pellets were harvested by centrifugation. Cell suspension of 10^{10} CFU/mL was added with 20 mL of sterile encapsulants as in comprising 2% sodium alginate (ALDRICH, Norway), 0.2% gellan gum (MySkinRecipes, Thailand), 0.5% creatine monohydrate (Shixing, China) and a variety of 1% prebiotic such as fucoidan (MySkinRecipes, Thailand), pullulan (MySkinRecipes, Thailand) and yeast beta glucan (Angel Humam Health,China). The treatments of the experiment are indicated in Table 1. Then, encapsulant was mixed and extruded by using a sterile syringe connected to a sterile needle (diameter 0.6 mm) into 100 mL sterile of 0.2 M $CaCl_2$ (KemAus, Australia) for 30 min. Subsequently, the microcapsules were washed in 0.1% sterile peptone solution and removed to 100 mL of 0.4% (w/v) chitosan solution for 30 min. Next layer coatings were carried into 100 mL of 1% (w/v) hyaluronic acid for 30 min.

Table 1. Treatments of probiotic encapsulation.

Treatments	**Materials**	**Coating layers** 1% Chitosan + 1% Hyaluronic acid
AG-C (Control)	2% Sodium Alginate +1% Gellan gum	•
AGC-C	2% Sodium Alginate +1% Gellan gum +0.5% Creatine monohydrate	•
AGC-F-C	2% Sodium Alginate +1% Gellan gum +0.5% Creatine monohydrate +1% Fucoidan	•
AGC-Pu-C	2% Sodium Alginate +1% Gellan gum +0.5% Creatine monohydrate +1% Pullulan	•
AGC-Y-C	2% Sodium Alginate +1% Gellan gum +0.5% Creatine monohydrate +1% Yeast beta glucan	•

2.3 Efficiency of encapsulation

The efficiency of encapsulation assessed the viability of probiotics according to the encapsulation procedure using the following formula according to Apiwattanasiri et al. [10].

$$Encapsulation\ efficiency\ (EE,\%) = \frac{N}{N_0} \times 100 \qquad (1)$$

Here, N_0 and N indicate the number of viable cells remaining before and after encapsulation (log CFU/g).

2.4 Morphological and size

The morphology and the size of the particles were examined by using a stereo microscope (Motic, DM-143-FBGG, China).

2.5 Color

The samples were determined using a lab scan XE colorimeter (HunterLab, ColorFlex, USA). Then it was apprized as L*, a*, b*, C*, h* values.

2.6 Water activity (a_w)

The water activity was analyzed by a water activity meter (Aqualab, 3TE, USA). The microcapsules were set in the plastic cup to be analyzed at 25 ± 2°C. The microcapsules were measured and replicated 3 times.

2.7 Mechanical strength

The microcapsules were determined using a texture analyzer (TA.XT plus). A P/36R probe was applied with a pre-test speed of 1.00 mm/s, test speed of 2.00 mm/s and post-test speed of 10.00 mm/s. Briefly, a rupture force utilized to a microcapsule was determined as a measure of mechanical stability.

2.8 Statistical assessment

All treatments were represented in triplicate. The results are presented as mean ± standard deviation. The ANOVA was used to identify, using SPSS 28 Software (IBM SPSS, Armonk, USA). Significance was determined as p<0.05.

3 Results and discussion

3.1 The encapsulation efficiency of microcapsules

As shown in Figure 1, the viability after encapsulation of all treatments tended to decrease after encapsulation. The encapsulation efficiency of probiotic *L. plantarum* TISTR 2075 by extrusion encapsulation technique in this study was found to be in the range of 71-94%. AGC-Y-C had the lowest encapsulation efficiency (71.83%). However, AG-C, AGC-C, AGC-F-C and AGC-Pu-C showed high encapsulation efficiency of higher than 90%. Pullulan is a polysaccharide that, when combined with proteins, increases the viscosity and structural strength of the microcapsules. The pullulan structure is a complex interlacing of fibers that increases the area available for probiotics to bind to the microcapsule structure [15]. Fucoidan is a polysaccharide with encapsulation properties. When used in combination with proteins and hydrogels, the encapsulation efficiency of fucoidan encapsulated probiotics is greatly improved. This is due to the difference in charge between the negative charge of the sulfate fucoidan and the positive charge of the protein [16]. Surprisingly, the AG-C sample without prebiotics and creatine monohydrate had an encapsulation efficiency comparable to that of the microcapsules with prebiotics and creatine monohydrate added. According to many researches, microcapsules without prebiotics may have a high encapsulation efficiency, but their survival rate following storage will be low. Jeznienė et al. [8] showed that encapsulation with prebiotic addition (inulin) provided a higher survival rate during storage than encapsulation without prebiotic addition. Thinkohkaew et al. [17] reported that the *Lactobacillus casei* microcapsules with added prebiotics (inulin) had a high probiotic survival rate of approximately 7.6 log CFU/g, but the microcapsules without added inulin had a survival rate of approximately 6.8 log CFU/g after 28 days at 4°C storage. The AGC-Y-C microcapsules decreased up to 2.99 log CFU/g, while the microcapsules encapsulated with other encapsulant reduced 0.10-0.66 log CFU/g. Consistent with the findings of Thinkohkaew et al. [18], the encapsulation efficiency of the encapsulated probiotic using alginate combined with gellan gum and coated with chitosan improved the survival rate from 80.35% to 97.82%. Gellan gum would improve the encapsulation efficiency
further through the ionic interactions with alginate. The use of beta-glucan-alginate encapsulation of *L. acidophilus* LA-5 via spray drying has an encapsulation efficiency between 78.20%. Yeast beta-glucan is an outstanding polysaccharide with a honeycomb-like structure. This structure, crosslinked with alginate, effectively locks in the probiotics and protects them [19]. Ahmadi et al. [19] showed that the encapsulation of probiotics supplemented with higher ratio of yeast beta glucan strengthened the structure of microcapsules and improved the efficiency of probiotic encapsulation. The microcapsules encapsulated by prebiotic supplementation and adding a layer to coat the microcapsules in two layers. The microcapsules had higher encapsulation efficiency and stability from adding a coating layer to the microcapsules. The single-layer coating of the microcapsules helped protect the probiotics from the unsuitable or adverse external conditions affecting probiotics. However, increasing the number of coating layers would diminish the effect on the probiotics encapsulated within the microcapsule structure [10].

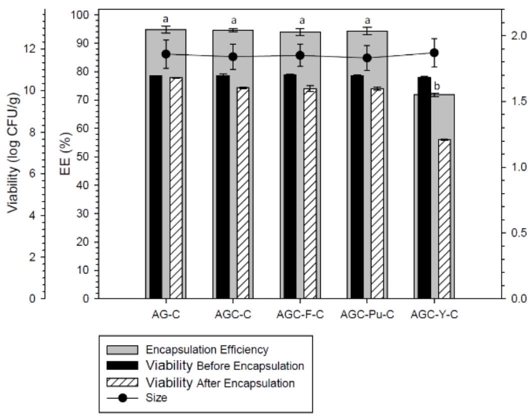

Fig. 1. The efficiency of encapsulation, viability before and after encapsulation, and particle size of microcapsules. Different letters on the top of the bar indicated a significant difference (p<0.05) between groups.

3.2 Morphological and particle size

Probiotics encapsulated with different types of encapsulants were examined under a stereo microscope. It can be seen that the microcapsules have a spherical shape, and the surface was smooth (Figure 2). The size and shape of microcapsules composed of different encapsulation agents appeared quite uniform with no significant difference (p≥0.05) in sizes. The microcapsule sizes were in the range of 1.830-1.870 mm. Singh et al. [20] suggested that the microcapsules are 50 nm to 2 mm in size, have a spherical shape, consist of a core and shell structure, including particles that are dispersed within a sphere. The important thing for delivery and protection of the probiotics is a relatively quiet uniform in size and shape [17].

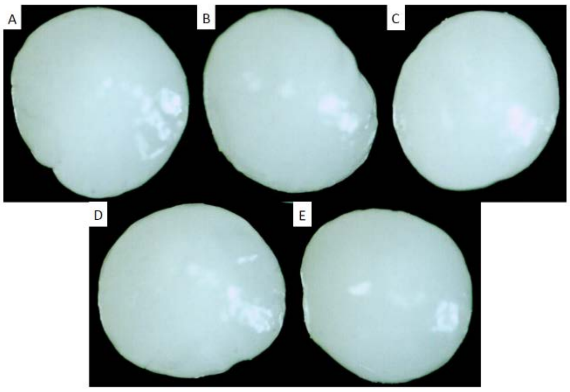

Fig. 2. Stereo microscopy images of microcapsules.
A: AG-C, B: AGC-C, C: AGC-F-C, D: AGC-Pu-C, E: AGC-Y-C.

3.3 Color

In this study, the L* a* and b* values of microcapsules have significant differences ($p<0.05$) in color properties, those were illustrated in Table 2. AGC-Pu-C had the highest L* value (58.217) and AGC-C exhibited the highest negative a* value (-2.653) indicates the degree of greenish. Also, AGC-F-C exhibited the highest positive b* value (6.537), showing a yellow hue. C* represents chroma or color saturation, and h* is the hue angle. The higher the chroma value, the higher the vibrant and pure colors starting from zero. The microcapsules had a low level of color vibrancy, but there were significant differences ($p<0.05$) in color vibrancy according to the composition of the encapsulant used. The hue value was significant difference ($p<0.05$) observed in the microcapsules that had the difference color range. The color analysis results indicated one of the important quality characteristics of microcapsules. The color value is influenced by many factors, for instance the concentration of the encapsulating material and the ratio of encapsulating material, which affect the color of microcapsules.

3.4 Water activity (a_w) and mechanical strength

From the study of water activity of fresh microcapsules, the water activity contents were in the range of 0.976-0.978, which was no significant ($p\geq0.05$) difference. The mechanical strength of microcapsules was evaluated by using a texture analyzer with rupture force (fracturability), which was assigned as a standard of mechanical stability or endurance of the sample against fracture. The microcapsules encapsulated probiotics with different types of encapsulant were shown in Figure 3. It was found that microcapsules from different encapsulants showed significant differences ($p<0.05$) in mechanical strength values. Especially, microcapsules encapsulated with AGC-Pu-C and AGC-C had the greatest mechanical strength with a value of 5.837 and 5.474 N, respectively. However, the microcapsules containing only alginate plus gellan gum and coated with chitosan and a second layer of hyaluronic acid showed the lowest mechanical strength (2.320 N). These results showed that the density of gel structure increased from the addition of composition in encapsulant and different prebiotics.

Table 3. Water activity of microcapsules.

Treatments	Water activity (a_w)^{ns} ± SD
AG-C	0.977 ± 0.001
AGC-C	0.976 ± 0.002
AGC-F-C	0.976 ± 0.002
AGC-Pu-C	0.978 ± 0.001
AGC-Y-C	0.978 ± 0.001

ns: the value was no significant difference ($p\geq0.05$).

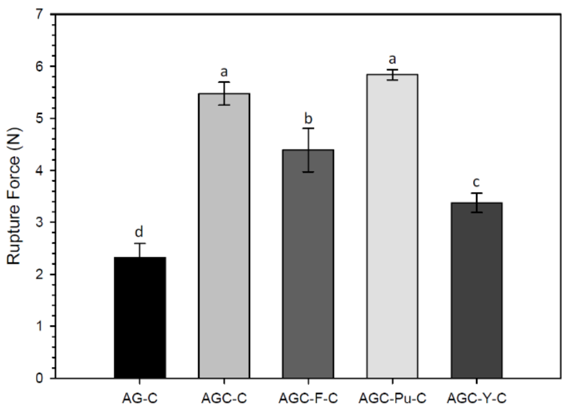

Fig. 3. The mechanical strength of gel microcapsules. Different letters (a-d) on the top of the bar denoted a significant difference ($p<0.05$) between groups.

Table 2. Color of microcapsules.

Treatments	Color ± SD				
	L*	a*	b*	C*	h*
AG-C	54.813 ± 0.031^{d}	-1.870 ± 0.035^{d}	4.383 ± 0.154^{d}	4.770 ± 0.156^{d}	113.123 ± 0.333^{a}
AGC-C	56.967 ± 0.012^{b}	-2.653 ± 0.147^{a}	5.990 ± 0.106^{b}	6.553 ± 0.153^{b}	113.893 ± 0.837^{a}
AGC-F-C	56.557 ± 0.006^{c}	-2.073 ± 0.038^{c}	6.537 ± 0.015^{a}	6.857 ± 0.012^{a}	107.643 ± 0.314^{c}
AGC-Pu-C	58.217 ± 0.040^{a}	-2.447 ± 0.038^{b}	6.460 ± 0.137^{a}	6.910 ± 0.147^{a}	110.707 ± 0.090^{b}
AGC-Y-C	53.393 ± 0.015^{e}	-0.883 ± 0.147^{e}	5.687 ± 0.134^{c}	5.757 ± 0.150^{c}	98.807 ± 1.207^{d}

Superscript letters (a-e) in the same column indicated a significant difference ($p<0.05$) between values.

4 Conclusion

This research showed that the encapsulation of probiotics with use hydrogel and prebiotic pullulan (AGC-Pu-C) enchanted the encapsulation efficiency, resulting in the probiotics surviving up to 10 log CFU/g after the extrusion encapsulation process. The addition of hyaluronic acid to the chitosan coating promotes the spherical shape of the microcapsules. This affects the structure of the microcapsules, resulting in increased strength and efficiency of the encapsulation to protect the probiotics. Furthermore, the mechanical strength was exhibited high. The composition of the encapsulation material that was chosen to use is crucial for developing probiotic encapsulation. Further research should be conducted on the selection of prebiotics that can be effectively utilized by probiotics and promote hydrogel in the microcapsule structure.

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References

1. J. Plaza-Diaz, F. J. Ruiz-Ojeda, M. Gil-Campos, A. Gil, Mechanisms of Action of Probiotics. Adv Nutr. **10**, S49–S66 (2019). https://doi.org/10.1093/advances/nmy063
2. G.V. de Melo Pereira, B. de Oliveira Coelho, A.I.M. Júnior, V. Thomaz-Soccol, C.R. Soccol, How to select a probiotic? A review and update of methods and criteria. Biotechnology Advances. **36**, 2060–2076 (2018). https://doi.org/10.1016/j.biotechadv.2018.09.003
3. D. R. Silvaa, J. de Cássia Orlandi Sardib, N. de Souza Pitanguic, S. M. Roquee, A. C. B. da Silvaf, P. L. Rosalena, Probiotics as an alternative antimicrobial therapy: Current reality and future directions. Journal of Functional Foods. **73**, 104080 (2020). https://doi.org/10.1016/j.jff.2020.104080
4. S. Razavi, S. Janfaza, N. Tasnim, D.L. Gibson, M. Hoorfar, Microencapsulating polymers for probiotics delivery systems: Preparation, characterization, and applications. Food Hydrocolloids. **120**, 106882 (2021). https://doi.org/10.1016/j.foodhyd.2021.106882
5. I. A. Neri-Numa, G. M. Pastore, Novel insights into prebiotic properties on human health: A review. Food Research International. **131**, 108973 (2020). https://doi.org/10.1016/j.foodres.2019.108973
6. G. T. C. Delgadoa, W. M. S. C. Tamashirob, Role of prebiotics in regulation of microbiota and prevention of obesity. Food Research International. **113**, 183-188 (2018). https://doi.org/10.1016/j.foodres.2018.07.013
7. M. Cunningham, G. Vinderola, D. Charalampopoulos, S. Lebeer, M. E. Sanders, R. Grimaldi, Applying probiotics and prebiotics in new delivery formats - is the clinical evidence transferable? Trends in Food Science & Technology. **112**, 495-506 (2021). https://doi.org/10.1016/j.tifs.2021.04.009
8. S. Jeznienė, I. Bružaitė, A. Šipailienė, Application of biomacromolecules encapsulation systems for the long-term storage of *Lactobacillus plantarum* F1 and *Lactobacillus reuteri* 182. Heliyon. **10**, e26566 (2024). https://doi.org/10.1016/j.heliyon.2024.e26566
9. A. Kistaubayeva, M. Abdulzhanova, S. Zhantlessova, I. Savitskaya, T. Karpenyuk, A. Goncharova, Y. Sinyavskiy, The Effect of Encapsulating a Prebiotic-Based Biopolymer Delivery System for Enhanced Probiotic Survival. Polymers. **15**, 1752 (2023). https://doi.org/10.3390/polym15071752
10. P. Apiwattanasiri, R. Charoen, S. Rittisak, K. Phattayakorn, S. Jantrasee, W. Savedboworn, Co-encapsulation efficiency of silk sericin-alginate-prebiotics and the effectiveness of silk sericin coating layer on the survival of probiotic *Lactobacillus casei*. Food Bioscience. **46**, 101576 (2022). https://doi.org/10.1016/j.fbio.2022.101576
11. S. Chew, C. Tan, L. Pui, P. Chong, B. Gunasekaran, N.K. Lin, Encapsulation Technologies: A Tool for Functional Foods Development. IJITEE. **8** (5S) (2019)
12. W. Y. Koh, X. X. Lim, T. Tan, R. Kobun, B. Rasti, Encapsulated Probiotics: Potential Techniques and Coating Materials for Non-Dairy Food Applications. Appl. Sci. **12**, 10005 (2022). https://doi.org/10.3390/app121910005
13. S. Agriopoulou, S. Smaoui, M. Chaari, T. Varzakas, A. C. Karaca, S. M. Jafari, Encapsulation of Probiotics within Double/Multiple Layer Beads/Carriers : A Concise Review. Molecules. **29**, 2431 (2024). https://doi.org/10.3390/molecules29112431
14. A. N. Kovács, N. Varga, Á. Juhász, E. Csapó, Serum protein-hyaluronic acid complex nanocarriers: Structural characterisation and encapsulation possibilities. Carbohydrate Polymers. **251**, 117047 (2021). https://doi.org/10.1016/j.carbpol.2020.117047
15. J. Ma, C. Xu, H. Yu, Z. Feng, W. Yu, L. Gu, Z. Liu, L. Chen, Z. Jiang, J. Hou, Electro-encapsulation of probiotics in gum Arabic-pullulan blend nanofibres using electrospinning technology. Food Hydrocolloids. **111**, 106381 (2021). https://doi.org/10.1016/j.foodhyd.2020.106381
16. S. Kraithong, A. Theppawong, Y. Liu, P. Sangsawad, R. Huang, Fucoidan as an Encapsulant material: Applications and advantages in active agents and probiotic encapsulations. Food Chemistry. **477**, 143570 (2025). https://doi.org/10.1016/j.foodchem.2025.143570
17. K. Thinkohkaew, N. Aumphaiphensiri, T. Tangamornsiri, N. Niamsiri, P. Potiyaraj, I. Suppavorasatit, Inulin extracted from burdock root

(Arctium lappa L.) incorporated alginate/chitosan hydrogel beads for probiotics encapsulation. Journal of Agriculture and Food Research. **18**, 101405 (2024). https://doi.org/10.1016/j.jafr.2024.101405

18. K. Thinkohkaew, V. Jonjaroen, N. Niamsiri, A. Panya, I. Suppavorasatit, P. Potiyaraj, Microencapsulation of probiotics in chitosan-coated alginate/gellan gum : Optimization for viability and stability enhancement. Food Hydrocolloids. **151**, 109788 (2024). https://doi.org/10.1016/j.foodhyd.2024.109788
19. M. Ahmadi, F. Hashempour-Baltork, S. Shojaee-Aliabadi, M. J. Marand, F. Shahraz, A. M. Mortazavian, S. M. Hosseini, Effect of yeast cell wall and β-glucan on survival of *Lactobacillus acidophilus* LA-5 encapsulated in spray-dried cross-linked alginate microcapsules. LWT. **222**, 117662 (2025). https://doi.org/10.1016/j.lwt.2025.117662
20. M. N. Singh, K. S. Y. Hemant, M. Ram, H. G. Shivakumar, Microencapsulation: a promising technique for controlled drug delivery. RPS. **5**, 65-77 (2010)