Corn Porous Starch Microencapsulation of Probiotics and Its Survival Rate in Human Gastro-Intestinal Environment

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Abstract

Microencapsulation as one of the most modern methods has considerable effects on probiotic survival. In this study, *Lactobacillus casei* were encapsulated using calcium alginate-gelatinized starch, chitosan coating via emulsion technique, and incubated in simulated gastric juice at pH 1.5 and simulated intestinal juice at pH 8 for 2 hours at 37^{o}C. Microencapsulations were employed via ultrasonification and emulsion technique. Emulsion technique is a method for encapsulation of probiotic bacteria in the capsules smaller than 1mm. In addition, alginate is a natural heteropolysaccharide composed of D-mannuronic and L-guluronic acid residues joined linearly by (1, 4) glycosides linkage. The addition of starch as filler material in the alginate capsules matrix improved the viability of probiotic culture and the polycationic nature of chitosan leads to a strong interaction of carboxylic groups of alginate with the amine groups of chitosan resulting in the formation of a membrane. Not only that, microencapsulation with hydrocolloids as one of the most modern method has remarkable effects on probiotic survival. The results indicated that the survival of microencapsulated probiotic increased significantly in simulated gastro-intestinal condition. Chitosan coating played a significant role in the protection of probiotic bacteria in a simulated gastro-intestinal condition and the diameter of the microcapsules increased with the addition of chitosan coating. In general, this study indicated that microencapsulation with alginate-gelatinized starch coated with chitosan could successfully and significantly protect probiotic bacteria against the adverse condition of a simulated human gastro-intestinal condition.

Keywords: Microencapsulation, Corn Porous Starch, Probiotic,

1. Introduction

Probiotics are live microorganisms that when administered in adequate amounts giving health benefits to the human. Probiotic bacteria are usually used in the production of functional foods and pharmaceutical products. The benefits of this probiotics are it helps in gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, immune system stimulation and improvement in inflammatory bowel disease (Ouwehans, Salminen & Isolauri, 2002). The beneficial effects of the probiotic cells appear when they can be reached and survived in the gastrointestinal environment in high number.

Many reports indicated that there is a poor surviving rate of the probiotic bacteria in the products which contain the free probiotic cells (De Vos, Faas, Spasojevic, Sikkema, 2010). Thus, microencapsulation is an alternative to protect the probiotic bacteria against extreme environments such as low pH and bile salts. Providing probiotic living cells with a physical barrier to resist adverse environmental conditions is, therefore, an approach currently receiving considerable interest (Kailasapathy, 2009). The encapsulation techniques for protection of bacterial cells have resulted in the greatly enhanced viability of these microorganisms in food products as well as in the gastrointestinal tract. The probiotic cells can be incorporated into an encapsulating matrix that can protect the cells from degradation by the extreme environmental factors and release at controlled rates under particular conditions. The materials used for the microencapsulation technique is usually a safe ingredient that can be used in the food applications such as alginate, chitosan, starch, carrageenan, cellulose and pectin.

In conjunction with this, starch which is one of the most widely used natural biopolymers due to its abundant availability, renewability and relatively low cost shows significant benefits in various applications. Besides, when compared to other native starch or modified starch, porous gelatinized starch has an excellent adsorption property due to its large surface area (J.Zhao, Madson, Whistler, 2001). Therefore, it can be used as adsorbents in the areas of food field as well.

Hence, this research mainly focuses on encapsulating the probiotic cells for them to survive in harch environment such as a low pH that it occurs during gastrointestinal transit. Thus, it will increase the surviving rate of probiotic cells in low pH environment.

2. Materials and Methods

2.1 Materials

Cornstarch is purchased from Tesco, with Cap Bintang labelled. Chemicals used were methyl red violet, sodium alginate, calcium chloride, vegetable oil, chitosan, glacial acetic acid, hydrochloric acid, nutrient agar, sodium hydroxide and sodium chloride which obtained from Biochemistry Laboratory, Politeknik Nilai. *Lactobacillus Strain* (ImunoStron Probiotics) and Sterile Saline Solution were purchased from Wise Pharmacy Penang. Other

compounds are Pepsin (Dr. Clark Pepsin Supplement), Pancreatin (NOW Pancreatin) and Bile Salts (Swanson Iron Bile Salt).

2.2 Preparation of Corn Porous Starch

30 gram of cornstarch powder was weighed using the weighing balance and placed into a 250 ml beaker. Then, the cornstarch powder was suspended in 100 ml of deionized water and stirred until the powders were completely diluted. (K.Zanjani & B.Tarzi & A.Sharifan & N. Mohammadi 2014)

2.3 Ultrasonification of Corn Starch Solution

The diluted cornstarch solution was further treated with ultrasounds for 30 minutes at 20^{o}C by using ultrasonicator homogenizer at a frequency of 20kHz and power 170watt. Then, the starch solution was centrifuged at 4000 rpm for 10 minutes and left to dry inside the falcon tube for 24 hours at room temperature. Finally, starch samples were stored in a sealed plastic container at room temperature for further use (K. Zanjani & B. Tarzi & A. Sharifan & N. Mohammadi 2014).

2.4 Measurement of Adsorption Capacity

2.00gram of corn porous starch were added to 50ml of methyl violet solution (0.02 g/L) and stirred for 5 hours. Then, the suspension was centrifuged at 10 000 rpm for 10 minutes. The amount of non-adsorbed methyl violet was quantified by determining the absorbance of the supernatant with UV/vis spectroscopy at 583nm. The adsorption ratio was calculated using the following equation:

$$\text{Adsorption Ratio} = Co - C1 \,/\, Co \times 100\%$$

Co and C1 are the concentration of methyl violet solution before and after adsorption, respectively (K.Zanjani & B.Tarzi & A.Sharifan & N. Mohammadi 2014).

2.5 Preparation of Cell Suspension

Lactobacillus strain, lyophilized cells were inoculated in nutrient agar for 24 hours under aerobic conditions at 37°C, respectively and biomasses were then harvested by centrifuging at 4000 rpm for 10 minutes at 4°C. The cultures were then washed twice with the sterile saline solution (0.9%) and used in the microencapsulation process (K.Zanjani & B.Tarzi & A .Sharifan & N. Mohammadi 2014).

2.6 Method For Encapsulation

Briefly, 2 gram of cornstarch was added into 100 ml of distilled water and boiled until it formed a gel, then 1% of Sodium Alginate was added and stirred until they were dissolved and dispersed. Then pure probiotic cultures were transferred to the solutions while stirring under sterile conditions to ensure uniform distribution of the cells. The final mixture was suspended in 500 ml of vegetable oil containing 0.2% tween 80 and mixed at 350 rpm for 20 minutes until they appeared creamy. Capsules were prepared by adding 200ml Calcium Chloride 0.1 M into a mixture, the phase separation of oil and water emulsion occurred. The mixtures were allowed to stand for 30 minutes to settle Calcium Alginate capsules in the bottom of beaker at the Calcium Chloride layer. The oil layer was drained and capsules in Calcium Chloride solution were harvested by low speed centrifuge at 350 gram for 10 minutes and kept in 0.1% peptone solution at 4°C. Next, chitosan aqueous solutions were prepared. The mixtures were filtered through filter paper and autoclaved at 121°C for 15 minutes. Then 20 gram of washed microcapsules alginate-gelatinized starch was immersed in 100ml of chitosan solution and shake at 100 rpm for 40 minutes on an orbital shaker for coating. The chitosan-coated microcapsule were washed with peptone solution and kept in 0.1% peptone solution at 4°C (K .Zanjani & B. Tarzi & A. Sharifan & N. Mohammadi 2014).

2.7 Preparation of Simulated Gastric And Intestinal Juices And Inoculation of Cells

Simulated intestinal juices were prepared by suspending pancreatin in sterile sodium chloride solution (0.5%, weight over volume) to a final concentration of 1g/L, with 4.5% bile salts and adjusting the pH to 8.0 with sterile 0.1M NaOH. Both solutions were filtered for sterilization through a 0.22 µm membrane. The probiotic bacteria were inoculated to the simulated gastrointestinal juice individually in two different forms, non-encapsulated and capsulated calcium alginate-gelatinized starch coated with chitosan. Then 1 gram of freshly encapsulated bacteria samples was gently mixed with 10ml of sterile simulated intestinal juice and incubated at 37°C for 30, 60, 90, and 120 minutes. Surviving bacteria were enumerated by pour plate counts in nutrient agar aerobically incubated at 37°C for 2 days (K. Zanjani & B. Tarzi & A. Sharifan & N. Mohammadi 2014).

2.8 Analysis for Encapsulation yield

Encapsulation yield (EY) is the number of bacterial cells that survived the process and encapsulated inside the microcapsules were calculated as follows:

$$\text{Encapsulation Yield (EY)} = (N / No) \times 100$$

Where No is the number of viable bacteria in CFU/mL of culture and N is the number of viable bacteria in CFU/g of microcapsules (K .Zanjani & B. Tarzi & A. Sharifan & N. Mohammadi 2014).

3. Result and Discussion

3.1 Adsorption capacity of corn porous gelatinized starch

The result obtained from table 1 indicated that the adsorption capacity of corn porous starch increased at 6.6% from the proposed value in the early

study. The suitability of corn porous gelatinized starch as an adsorbent was tested. Based on the study, it was found that the corn porous gelatinized starch has greater adsorption capacity than native starch. The potential application of the porous starch was evaluated using methyl violet as an adsorbed model. The adsorption capacity was optimized by stirring the methyl violet solution continuously for a few hours. The test indicates the average reading of 44.7% of adsorption capacity which is 6.6% higher than the expected value. The results suggest that the porous starch has more excellent adsorption capacity than the native starch.26

3.2 Appearance of microencapsulated probiotic bacteria

Figure 4 showed the physical appearance of microencapsulated probiotic bacteria by using corn porous gelatinized starch as the wall material and chitosan as a binding agent. A microcapsule consists of a semipermeable or non-permeable, spherical, thin and strong membrane-surrounding a solid or liquid core. These microcapsules have the diameter range from a few microns to 1mm. The wall material used in this study derived from corn starch. Natural polymers such as corn starch are preferred due to their biodegradability, compatibility, food grade nature and wide availability. Chitosan is used as the coating material and binding agent for improving activity. Addition of microcapsule, however, does not affect the sensory properties of the probiotic bacteria.

3.3 Survival rate of bacteria in simulated gastric juice

Several studies have demonstrated differences among strains of probiotic bacteria with regard to their survival in the acid environment. Probiotics must survive in gastric acids to reach the small intestine and colonize the host for appropriate prevention and management of several gastrointestinal diseases. One of the research objectives is to review the effect of microencapsulation on the survival of probiotics in an *in vitro* model simulating gastric transit. In our study, corn porous gelatinized starch used as wall material for the microencapsulation process of probiotic bacteria. The microencapsulation process is done to protect the probiotic bacteria and deliver them safely to the intestinal tract.

Based on the result, there is no release of probiotics within the early incubation period in the simulated gastric juice. Only after two hours, most of the probiotics widespread in the simulated environment. Corn porous

gelatinized starch has been successfully utilized as wall material for the microencapsulation process, to increase the resistance of these sensitive microorganisms against the gastric condition. Therefore, to improve the survival rates of probiotic microorganisms during gastric transit, the microencapsulation is considered to be a promising process. The effectiveness of microencapsulation process was evaluated from the result in figure 4.3. It can be seen that there is no growth of bacteria during the first 30 to 90 minutes of the incubation period.

3.4 Survival rate of bacteria in simulated intestinal juice

Based on the recent study, probiotics must survive in the acidic gastric environment if they are to reach the small intestine and colonize the host, thereby imparting their benefit. The ability of corn porous gelatinized starch to enhance probiotic survival by providing energy and metabolic precursor has been extensively studied. The data obtained from the result are available to describe the effects of the microencapsulation technique and its underlying mechanism of action for enhancing survival of probiotic bacteria. In simulated intestinal juice, most of the bacteria successfully released to the environment within 30 to 90 minutes of the incubation period.

Hence, microencapsulation technology helps in maintaining the viability of probiotic bacteria. Microencapsulation keeps the probiotic active through the gastrointestinal tract before been released in the targeted environment. The effectiveness of microencapsulated process was evaluated from the result in figure 7. It can be seen that there is the maximum growth of bacteria during the first 30 to 90 minutes of the incubation period.

3.5 Questionnaire (SPSS) on product testing

A recent survey has been conducted to evaluate the perception of respondents towards microencapsulated probiotic bacteria in term of its physical appearances and attributes. The finding of this survey analyzed using SPSS software, which is the most common analysis instrument. As for the feel and texture of the product, 90% of the respondent agrees that the surface of the product is either soft and squishy or gentle and smooth. The surface of the product has been successfully developed to meet the expectation of consumers, as it gone through the oil phase separation-

emulsion process. Overall, the respondents suggest that the microencapsulated probiotic bacteria derived from corn starch as wall material is better in terms of texture, smell, surface and size compared to the chemical-based microencapsulated probiotic product already existed in the market.

4. Conclusion

In conclusion, corn porous gelatinized starch was prepared by using ultrasonification modification in order to increase the surface area and enlarge the pore volume as compared to the native starch. The maximum adsorption ratio of adsorbing with methyl violet obtained was 47.7%, which scientifically gave an increase up to 6.6% of absorptivity after being modified using ultrasonificator homogenizer. Therefore, the results showed that corn porous gelatinized starch possess excellent adsorption capacity. On top of that, microencapsulation of *L. casei* in calcium alginate-gelatinized starch with chitosan coating resulted in better survival of cells after simulated gastro-intestinal condition, as compared to free cells.

Therefore, the applied approach in this study might prove beneficial for the delivery of probiotic cultures to the simulated human gastrointestinal tract. In the nutshell, microencapsulating the probiotics culture using starch as the coating material and chitosan as a binding agent thus, gives the best combination by increasing the survival rate of probiotics in gastric juice up to 100% protecting the probiotic culture from adverse conditions.

References

Anal, A.K., & Singh, H. (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology*, 18(5): 240-251.

De Vos, P., Faas, M. M., Spasojevic, M., & Sikkema, J. (2010) Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal*, 20: 292-302.

J.Zhao, M.A., Madson, R.L., & Whistler. (2001) Cavities in Porous Corn Starch Provide a Large Storage Space. *Cereal Chem.* 73 379-380.

Kailasapathy, K. (2009). Encapsulation technologies for functional foods and nutraceutical product development. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 4(6).

Khosravi Zanjani, M.A., Ghiassi Tarzi, B., Sharifan, A., & Mohammadi, N. (2014). Microencapsulation of Probiotics by Calcium Alginate-gelatinized Starch with Chitosan Coating and Evaluation of Survival in Simulated Human Gastro-intestinal Condition. *Iranian Journal of Pharmaceutical Research: IJPR*, 13(3), 843 – 852.

Ouwehand, A.C., Salminen, S., Isolauri, E. (2002) Probiotics: an overview of beneficial effects. Antonie Van Leeuwenhoek, 82: 279–289.

Appendices

Figure 1: Corn starch powder were weighed and diluted with deionized water into a solution

Figure 2: Ultrasonification of corn starch was carried out using Ultrasonicator homogenizer.

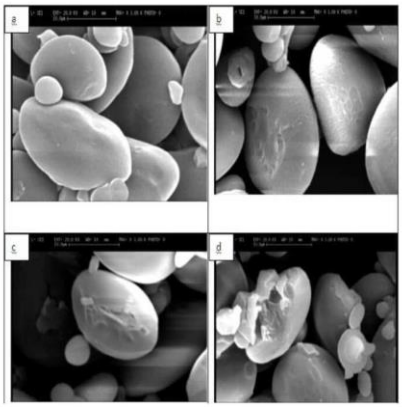

Figure 3: Composition of Ultrasonicated Starch

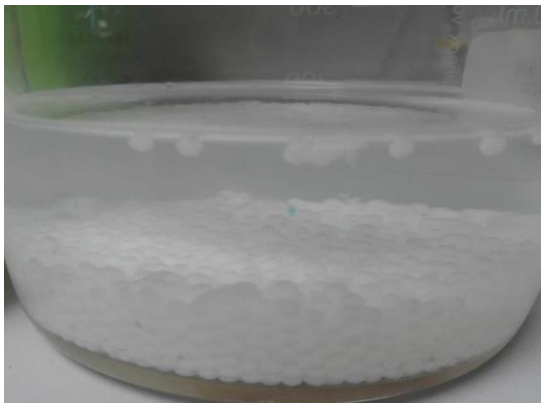

Figure 4: Overview of Microencapsulated Probiotic Bacteria

Table 1: Reading of Adsorption Capacity

Sample Number	Adsorption capacity
1st Reading	32.9%
2nd Reading	42.1%
3rd Reading	59.2%
Average Reading	44.7%

Table 2: Frequency of Respondents

	Frequency	Percent	Valid Percent	Cumulative Percent
Soft and squishy	4	40.0	40.0	40.0
Hard and brittle	1	10.0	10.0	10.0
Gentle and smooth	5	50.0	50.0	50.0
Total	10	100.0	100.0	100.0

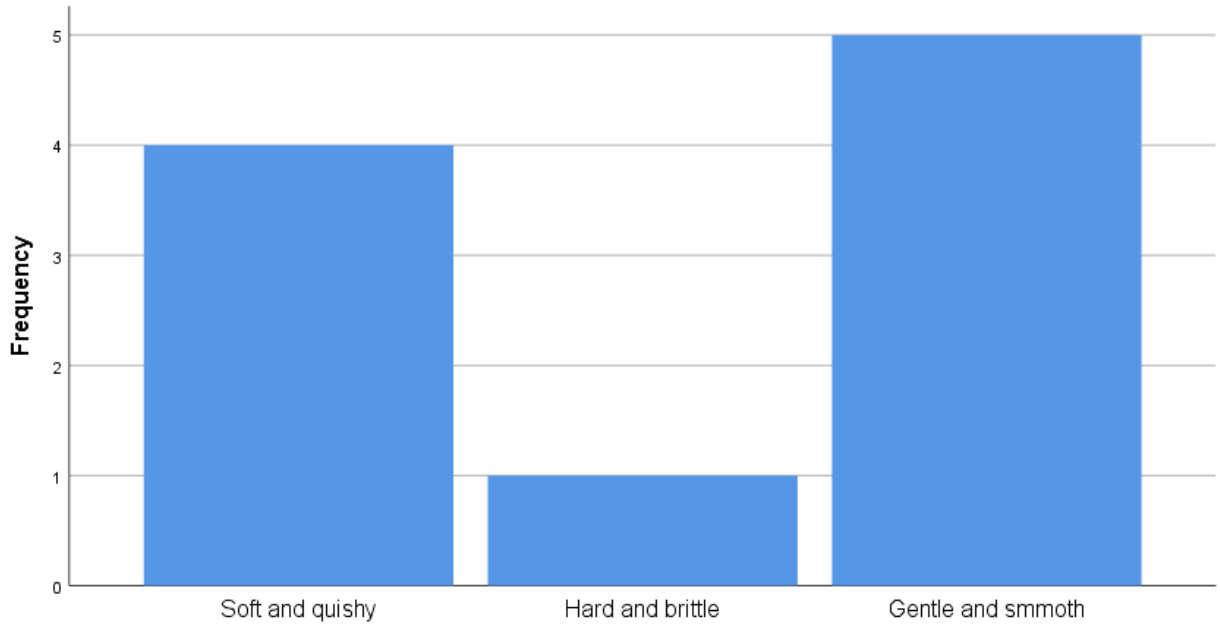

Figure 5: Physical Appearance of Product

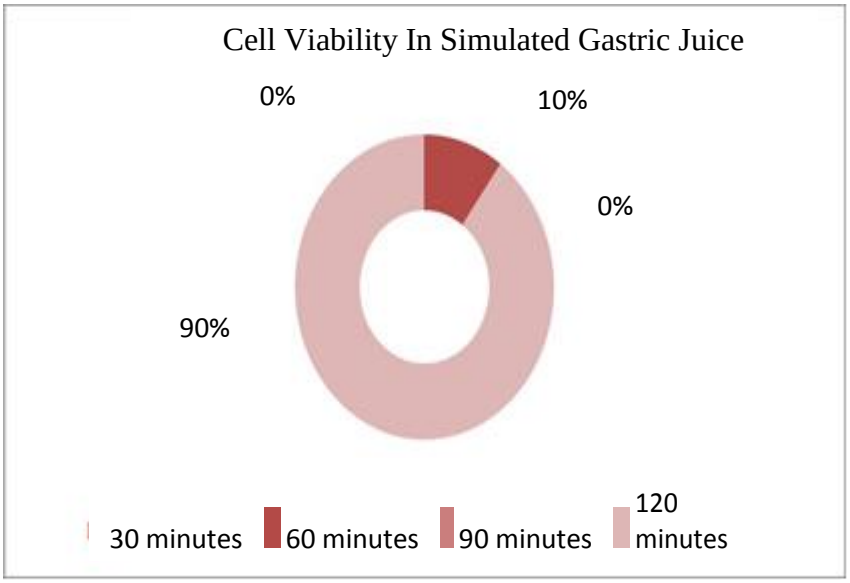

Figure 6: Cell Viability in Simulated Gastric Juice

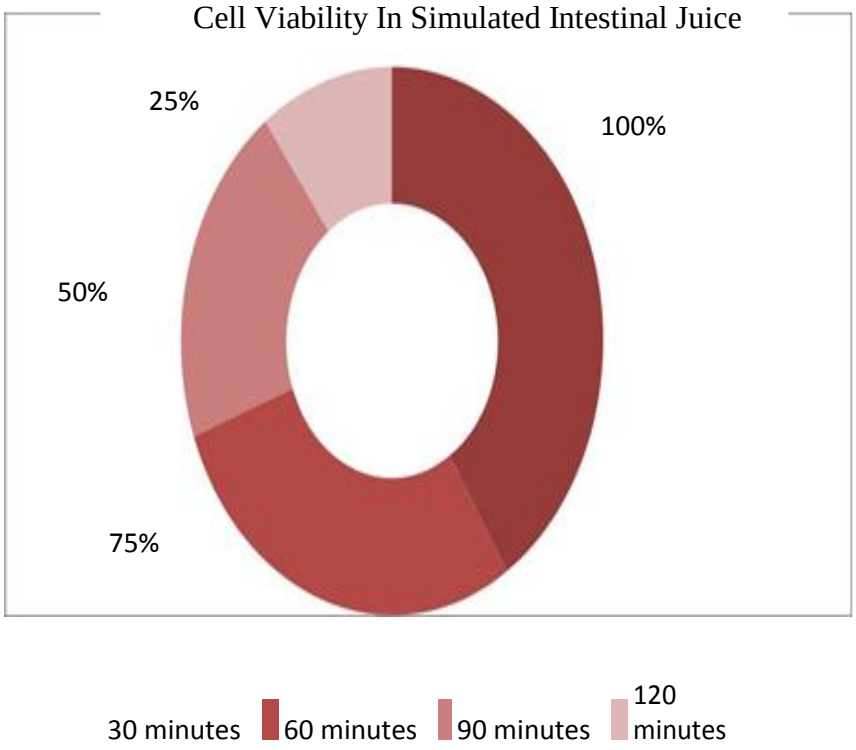

Figure 7: Cell Viability in Intestinal Juice