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Extended Abstract

Evaluation of Survival of *Lactobacillus plantarum* Capsulated in Synbiotic Suspension under Simulated Gastrointestinal Conditions

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Abstract

Nowadays due to the role of nutrition in public health, as well as the use of probiotic bacteria that exposed to acute conditions of food processes and the gastrointestinal tract, is always one of the concerns of researchers in food and pharmaceutical sciences. Therefore, probiotics has been proven as a new technique for the tolerability of food samples coated with biopolymers in beneficial emulsions under gastric and intestinal simulation conditions. In this study, three formulations were prepared for microcoating suspension (a combination of whey protein concentrate, inulin and Persian gum in the range of 14 to 14.3 g per 100 mL). The results showed that microcoating efficiency, resistance to acid and alkali stress and viscosity evaluation, microscopic properties and finally the release rate synthesis, the second formulation (8.5, 5.5 and 0.2 g for whey protein concentrate, inulin and Persian gum, respectively) was considered as a suitable matrix, in the following, the durability characteristics during two months were evaluated at two temperature levels of 25 and 40 °C. The effect of shelf life caused a decrease in the population of *Lactobacillus plantarum* at two temperatures, which was much more severe at a temperature of 40 °C than the ambient temperature. The results of capsule microparticle images obtained by scanning electron microscopy showed that the surface of properties and the effect of wall compositions on the microstructures of the particles at two different temperatures were different. According to the results, the temperature of 40 °C had a much higher surface shrinkage compared to the small particles of the capsule stored at room temperature.

Keywords: Emulsion, Functional matrix, Gastrointestinal system, *Lactobacillus plantarum*, Microencapsulation

Introduction

In recent years, various techniques have been used to increase the protection and stability of probiotics. Nano and micro emulsions can be used as carriers of bioactive compounds such as probiotics. Also, capsules are able to retain the bioactive properties of edible compounds that are almost identical to their natural state when stored in different conditions and increase their bioavailability in the gut due to their smaller size. In symbiotic foods, the presence of prebiotics such as unsaturated and indigestible oligosaccharides such as inulin stimulates the growth and survival of probiotics and has health effects on the host. The main condition for

the effectiveness of food containing probiotics is the viability of that side in the gastrointestinal tract. The microbial strain should be resistant to variety of processing, bile salts in the small intestine, acidic conditions of the stomach too. Numerous studies have been performed on the microencapsulation of probiotic bacteria with various compounds including alginates, chitosan, gums, starches, whey protein and gelatin and their use in various foods such as yogurt, milk, ice cream and cereal-based products. All of these studies showed that the survival of capsulated probiotics against gastric and intestinal stress was significantly increased. The aim of this study was to evaluate the survival and characteristics of microcapsulated *Lactobacillus plantarum* in a synbiotic emulsion under the simulated gastrointestinal conditions during 2 months.

Materials and methods

Activation of strain

Lactobacillus plantarum was cultured in MRS Broth medium at 37 °C for 48 h until 10^{12} CFU/g were formed. New microbial cells were isolated from basal culture medium at 6000 rpm and was washed with sterile 0.1% peptone water (Mokarram, Mortazavi, Najafi, & Shahidi, 2009).

Capsulation technique

The main constituents of the wall are three suspensions containing whey protein, inulin as a prebiotic compound and Persian gum (Table 1). The preparation of capsulation suspension was performed using the method of (Ananta, Volkert, & Knorr, 2005) as well as (Altamirano-Fortoul, Moreno-Terrazas, Quezada-Gallo, & Rosell, 2012).

Table 1. Ratio of suspension compounds for microcapsule formulation

Components of Capsulation	Formulation		
	first	second	third
WPC	7.5	8.5	9.5
Inulin	6.5	5.5	4.5
Persian gum	0.1	0.2	0.3

Preparation of microencapsulated powder

Microcapsulation was performed by spray dryer on a laboratory scale. So that, the temperature of inlet and outlet air the spray dryer was set at 145±2 °C and 50±3 °C respectively. The microbial strain of *Lactobacillus plantarum* in three suspension formulations was fed separately to the main dryer chamber via a peristaltic pump (Burgain, Gaiani, Linder, & Scher, 2011). A sample of the powder dried stored at 25 and 40 °C for 2 months.

Microencapsulation efficiency

The number of primary bacteria (N_0) in the culture medium inoculated with the suspension before spray drying on the solid culture medium was counted. Also, the number of live probiotic cells was measured after the drying (N). Finally, the microencapsulation efficiency was evaluated according to Eq. (1) (Michida *et al.*, 2006).

$$EY = (N/N_0) \times 100 \quad (1)$$

Number of bacteria trapped in microcapsules

To evaluate the amount of bacteria trapped in the microcapsule, 1 g of the prepared microencapsulation was added to 9 mL of sterile 0.1% phosphate buffer solution at pH 7. Then, the total number of bacteria released in the phosphate buffer were cultured using MRS

agar for 48 h at 37 °C and the reduced microbial population was calculated after the spray drying (Ananta *et al.*, 2005).

Evaluation of survival of microcapsulated bacteria under gastric and intestinal stress

To simulate the acidic conditions of the stomach, pepsin with 0.5% sodium chloride solution to a concentration of 3 g/L and to simulate the alkaline conditions of the intestine, pancreatic with 0.1 M monopotassium phosphate, to reach a final concentration of 1 g/L with 4.5% bile salt solution was mixed and then the pH was reduced to 2 for the stomach by sterile hydrochloric acid (0.1 M) and to about 8 for the intestine by sterile Sodium Hydroxide (1 M). Finally, the obtained solution was sterilized using a microfilter. Then, in order to evaluate the survival rate of microencapsulated cells at 10, 30, 60, 90 and 120 min, simulated media with 0.8% saline was cultivated in MRS Agar medium for 48 h at 37 °C. Simulated conditions of gastric juice were performed according to the method of (Michida *et al.*, 2006).

Release kinetics of microcapsulated cells in vitro

The percentage of release kinetics rate of *Lactobacillus plantarum* microencapsulated under simulated gastric and intestinal conditions was investigated using (He *et al.*, 2015) method. In summary, 4.5 mL of the combined solution of gastric acid (SGF) and intestinal bile salt was prepared in the form of gastrointestinal solution (SIF). The amounts of 0.5 g of the capsules were homogenous in both media at 37 °C at 150 rpm. In order to evaluate the survival rate of microcapsulated cells in comparison with free cells at 0, 10, 30, 60, 90 and 120 min, the simulated medium with 0.8% saline in MRS Agar superficially cultivated at 37 °C for 48 h. The release of each cell was then adjusted according to Higuchi, Ritter-Peppas, and first order release mathematical models.

Investigation of morphology characteristics of microcapsules and surface topology

To evaluate the microstructure and external topology of the microcapsules at two temperatures, a scanning electron microscope (model LEO 1450 VP, Germany) was used at a certain magnification. (Khosravi Zanjani, Mohammadi, Ahari, Ghiassi Tarzi, & Bakhoda, 2014).

Evaluation of the viability of microcapsulaed bacteria during shelf life

For this purpose, 1 g of each microcapsulated probiotic, which was stored separately at 25 and 40 °C, was mixed in 9 mL of phosphate buffer solution and incubated at 37 °C. Then the logarithm of the number of bacterial colonies that cultured by pour plate method was counted.

Statistical design

The results were statistically analyzed based on a completely randomized design using SAS software and Microsoft Office Excel (Version 2013). The means were compared using the least significant difference test ($P<0.05$).

Results and discussion

The microencapsulation efficiency in the second formulation was at the highest value compared to other formules, ie 10.995%. The results also showed that the effect of microencapsulation on maintaining the probiotic population of the mentioned strain in gastric acid stress was quite significant in comparison with free cells, so that after 120 min acid stress reduced only three logarithmic cycles in microcosm formulation number 2. Population reduction occurred in formulations No. 1 and 3 of the microcoated strain, which showed a decrease of about 9 logarithmic cycles compared to its free cell. In fact, these results confirm the resistance of second formulation in maintaining the microbial population.

The effect of microencapsulation on maintaining the probiotic population of the mentioned strain in intestinal alkaline conditions was quite evident in comparison with free bacterial cells. About 4 cycles of population reduction in formulations 1 and 3 were fine-grained, which was reduced by about 9 logarithmic cycles compared to its free cell. Therefore, the No. 2 microcapsulation emulsion performed far better in maintaining the microbial population than the other two matrices.

Evaluation of viscosity of three microcoating formulations of *Lactobacillus plantarum* showed that with increasing shear stress, the shear rate in model of the law of power at constant temperature (25 °C) had an increasing trend. In fact, formulation 2 had a more proportional viscosity process compared to the other two formulations.

The results of the synthetic percentage of probiotic bacterial release showed that the effect of time and direct contact with acid-alkali conditions on the release of microcellular cells in the second matrix was much more than the other two matrices and finally compared to the control sample.

After the study based on characteristics such as microcoating efficiency, resistance to acid stress and alkalinity, viscosity evaluation, microscopic properties and finally the release rate, the second formulation was considered as a suitable matrix and then the durability properties for two months at 25 and 40 °C were investigated.

The results of microencapsulates images obtained by SEM showed that the surface properties and microstructures of the particles at two temperatures were different. In fact, the microcapsule made in the second formulation, which was stored at 40 °C, had a much higher surface shrinkage compared to the microcapsule stored at 25 °C.

Finally, the shelf life caused a decrease in the number of microbial populations at both storage temperatures, which was much more severe at 40 °C. The results also showed that at the beginning of the first week of the second month, two logarithmic cycles of the population have decreased compared to the first weeks in the first month. During the storage period in the second month, especially in the third and fourth weeks, the resistance of the biopolymer matrix was significantly different compared to the previous storage weeks.

Conclusions

Overall, the results indicated that the second formulation, as a prebiotic compound, could provide the best properties in terms of efficiency, 120 min resistance to simulated gastrointestinal environments, good release, and acceptable viscosity for *Lactobacillus plantarum* microencapsulation. In the continuation of the performed studies, the morphology of the micro encapsulated side with the second formulation during storage at 25 °C showed better surface properties and longer survival over time.

References

Altamirano-Fortoul, R., Moreno-Terrazas, R., Quezada-Gallo, A., & Rosell, C. M. (2012). Viability of some probiotic coatings in bread and its effect on the crust mechanical properties. *Food Hydrocolloids, 29*(1), 166-174. doi:https://doi.org/10.1016/j.foodhyd.2012.02.015

Ananta, E., Volkert, M., & Knorr, D. (2005). Cellular injuries and storage stability of spray-dried Lactobacillus rhamnosus GG. *International dairy journal, 15*(4), 399-409. doi:https://doi.org/10.1016/j.idairyj.2004.08.004

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of food engineering, 104*(4), 467-483. doi:https://doi.org/10.1016/j.jfoodeng.2010.12.031

He, Y., Wu, Z., Tu, L., Han, Y., Zhang, G., & Li, C. (2015). Encapsulation and characterization of slow-release microbial fertilizer from the composites of bentonite and alginate. *Applied Clay Science, 109-110*, 68-75. doi:https://doi.org/10.1016/j.clay.2015.02.001

Khosravi Zanjani, M., Mohammadi, N., Ahari, H., Ghiassi Tarzi, B., & Bakhoda, H. (2014). Effect of microencapsulation with chitosan coating on survival of Lactobacillus casei and Bifidobacterium bifidum in ice cream. *Iranian Journal of Nutrition Sciences & Food Technology, 8*(4), 125-134.

Michida, H., Tamalampudi, S., Pandiella, S. S., Webb, C., Fukuda, H., & Kondo, A. (2006). Effect of cereal extracts and cereal fiber on viability of Lactobacillus plantarum under gastrointestinal tract conditions. *Biochemical engineering journal, 28*(1), 73-78. doi:https://doi.org/10.1016/j.bej.2005.09.004

Mokarram, R., Mortazavi, S., Najafi, M. H., & Shahidi, F. (2009). The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. *Food Research International, 42*(8), 1040-1045. doi:https://doi.org/10.1016/j.foodres.2009.04.023

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