Biotechnological Communication

Biosci. Biotech. Res. Comm. 10(1): 72-77 (2017)

BBRC Bioscience Biotechnology Research Communications

Effects of *Camellia sinensis* on survival of encapsulated *Lactobacillus casei* and *Bifidobacterium lactis* in ice-cream

Negin Noori[1], Leila Khaji[2], and Hassan Gandomi[1]

[1]*Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2]*Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

ABSTRACT

Camellia sinensis is considered as one of the most common beverages with high ant oxidative effects al-around the world. High levels of selenium caused it to be a good prebiotic for enhancement of the survival of probiotic bacteria. The present investigation was done to study the effects of *C. sinesis* extract on the survival of encapsulated *Bifidobacterium lactis* and *Lactobacillus casei* in ice-cream. Aerial parts of the *C. sinesis* were dried and their extract were purified using the distilled water. *B. latis* and *L. casei* strains and also extract of *C. sinesis* were encapsulated using the chitosan-alginate procedure. Encapsulated materials were added to the content of ice-cream in the final stage of procedure at the homemade ice-cream machine. *L. casei* and *B. lactis* strains were decreased through maintenance period. Numbers of free and encapsulated strains of *L. casei* and *B. lactis* during 90 days of maintenance had a range of 7.9 to 30.7 and 21.7 to 38.7 CFU/g and 65.6 to 6.6 and 48.6 to 15.6 CFU/g. Reduction in the numbers of probiotics was entirely lower in encapsulated bacteria. Percent of the survival of probiotics in encapsulated groups was entirely higher than control ($P < 0.05$). Application of chitosan-alginate based microencapsulation along with the using from *C. sinensis* extract is a good way to produce symbiotic ice-cream with high numbers of *L. casei* and *B. lactis* probiotic bacteria.

KEY WORDS: *CAMELLIA SINENSIS*, ENCAPSULATION, *LACTOBACILLUS CASEI*, *BIFIDOBACTERIUM LACTIS*, ICE-CREAM

ARTICLE INFORMATION:

**Corresponding Author: L.kh52834@yahoo.com*

Received 12th Jan, 2017
Accepted after revision 12th March, 2017
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007 CODEN: USA BBRCBA
Thomson Reuters ISI ESC and Crossref Indexed Journal
NAAS Journal Score 2017: 4.31 Cosmos IF : 4.006

Online Contents Available at: http//www.bbrc.in/

INTRODUCTION

Green tea which is derived from the aerial parts of the *Camellia sinensis (C. sinensis)* is considered as one of the most common beverages al-around the world. It is a part of the evergreen family. Leaves of the *C. sinensis* are glossy green with serrated edges. It has a small white flowers with yellow stamens (Moore *et al.*, 2003; Pastore and Fratellone, 2006; Goenka *et al.*, 2013). *C. sinensis is full from* flavonoids with high antimutagenic, antioxidant, anticarcinogenic activities and can prevent from development of cardiovascular and neurodegenerative diseases (Moore *et al.*, 2003; Pastore and Fratellone, 2006; Goenka *et al.*, 2013). This plant can easily growth at the zone of North of Iran and especially Gilan and Mazandaran province, Iran.

Probiotics can be distinct as live microorganisms (bacteria and yeasts) that can bring health benefits to humans' or animals' bodies, usually the maintenance and development of the microbial balance of the intestine environment and inhibition form the substitution of dangerous pathogenic strains into the intestinal tract (Hekmat and McMahon, 1992; Nagpal *et al.*, 2012; Dehkordi *et al.,* 2014). It is essential for most of these live cultures to survive during their shelf life prior being consumed. Therefore, probiotic producing companies have been tried to increase the survival of probiotic bacteria using plant extracts, phenolic compounds and anti-oxidative substances (Hekmat and McMahon, 1992; Nagpal *et al.*, 2012; Dehkordi *et al.*, 2014). Presence of various chemical components in the leaves of *C. sinensis* caused it to be used as a good prebiotic agent for production of symbiotic products (Sourabh *et al.*, 2014).

Combination of lactic acid starters with probiotic (Bifidobacterium and Lactobacillus) is widely used in dairy manufactures (Hekmat and McMahon, 1992; Nagpal *et al.*, 2012; Dehkordi *et al.*, 2014). Two of the most important species of these bacteria are B. lactis and L. casei. L. casei and bifidobacteria are normal inhabitants of the human intestine and numerous health benefits have been reported for them. Several health benefits are attributed to these groups of probiotics, including anticarcinogenic, anti-infection, serum cholesterol reduction, nutritional and antimutagenic effects, stimulation of immune system, and alleviation of lactose intolerance symptoms (Hekmat and McMahon, 1992; Nagpal *et al.*, 2012; Dehkordi *et al.*, 2014). Several investigations revealed that application of B. lactis and L. casei together can cause higher amount of decrease in the acidic pH of dairy products which will cause higher survival of both bacteria in hard conditions and during maintenance of foods (Hekmat and McMahon, 1992; Nagpal *et al.*, 2012; Dehkordi *et al.*, 2014). Alienation of prebiotic components is one of the most common ways to increase the survival of probiotic bacteria in functional foods. Prebiotics are the foods of probiotic bacteria. In the other hand, prebiotics are distinct as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of one or a limited number of bacterial species in the colon, such as bifidobacteria and lactobacilli. Prebiotic components have several types including herbs, fruits, sweet vegetables, fructooligosaccharides and inulin (Patel and Goyal, 2012).

Functional components of the *C. sinensis besides* its high frequency in Iran caused us to carried out the present study in order to investigate the effects of the *C. sinensis* extract on the survival of *L. casei* and *B. lactis* bacteria encapsulated with alginate chitosan in symbiotic ice-cream during 90 days maintenance in freeze conditions.

MATERIAL AND METHODS

Plants

From July to August 2015, aerial parts of the *C. sinensis L.* were collected from the farms of Gilan province, north of Iran. Plants were identified by the experts of the botany of the Faculty of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran. Plants were recorded by the herbarium number of L481-8732.

Preparation and extraction of plant's extract

Plant material was dried in shade for ten days. Then, Plant material was ground to a powder in a mechanical grinder. Five-hundred grams of the dried plants were mixed with 2 liters distilled water. Contents of the previous stage were then filtered using a 0.22 µm membrane. Then, additional 1 liter of the distilled water was added to the non-filtrated contents and shaken for 48 hrs. Contents were filtered in a same condition and finally filtered extracts were dried on a freeze dryer device for 48 hrs. Residual materials were kept at 4 °C.

Preparation of probiotic bacteria

Freeze dried *Lactobaciliu casei* (Lc-01) and *L. casei* (Bb-12) (CHR, Hansen, Denmark) was used in this study. *L. casei* strains were transferred to the Man Rogosa and Sharpe broth media (MRS Broth, Sigma, Uk) and incubated aerobically at 37 °C for 24 hrs. Both bacteria were also cultured on slant MRS agar media and kept at refrigerator.

Bacterial inoculation

One-loop of the MRSA agar media was taken and inoculated to the MRS broth media and incubated anaero-

bically at *37* °C for 48 hrs. bacterial cultures were then centrifuged in a 4000 rpm at 4 °C for 10 min. After deposits of bacteria, the supernatant was evacuated and 2 mL of sterile saline was added to the tube. All tubes were then vortexed. Operations of centrifugation and washing were done 2 times. Spectrophotometer device was set at 600 nm and a cuvette containing sterile saline was used for its calibration. Appropriate amounts of the prepared bacterial suspension were added to a cuvette and its optic absorption was set on the 1 number. The bacterial suspensions were then prepared at a concentration 10 times higher than optical density of 1. An overall of 0.5 ml of the prepared bacterial suspension were taken, and transmitted to a tube contained 4.5 ml of the 0.1% peptone water media (Merck, Germany). Then, the serial dilutions were prepared. Totally, from the 10^{-5} and 10^{-6} dilutions were cultured on the MRS agar media. Colonies were enumerated after 48 hrs of incubation on 37 °C. Numbers of the *L. casei* and *B. lactis* were 1.2×10^{9} and 1×10^{9} CFU/ml.

Bacterial microencapsulation

Microencapsulation procedure was done according to the method described previously by Ahmadi *et al*. (2014) (Ahmadi *et al*., 2014) and Allan-Wojtas *et al*. (2008) (Allan-Wojtas *et al*., 2008). In the encapsulation procedure, bacterial suspension was added to 5 ml of 0.1% peptone water media and then 40 ml of sterile Sodium Alginate and 1% of the *C. sinensis* extract were added to the mixture. In the other hand, encapsulated bacteria were mixed with the extract of *C. sinensis*.

Preparation of ice-cream

Formulation of ice-cream was consisted of 10% fat (cream and fluid milk), 11% milk solid not-fat (MSNF, Mashhad, Iran), 15% sucrose, 0.1% vanilla (Polar Bear Brand, China), 0.15% emulsifier (Puratus, Belgium), and 0.35% stabilizer. Milk and cream (fat) samples were mixed and heated until the temperature of mixture reached to 40 °C. Then, sucrose (270 g), vanilla (0.9 g) and stabilizer (10 g) were added to the mixture and heated at 80 °C for 20 min. Then, mixture was transferred to the homemade ice-cream machine (Elegant BL 1380) and 1% of encapsulated and also free (control group) bacteria were added to them and process was continuing for 20 min. Samples of ice-creams were then packaged in the 50 g plastic containers and kept at -18 °C.

Enumeration and study the survival of bacteria in ice-cream

Two-hundred milliliters of the 0.1 molar sodium citrate were added to the 50 grams of ice-creams in the polyethylene bags. Polyethylene bags were then settled on the bag mixer. After 10 min remaining at the laboratory, the bag mixer device was used 3 times for their well mixing. Then, 0.5 ml of the contents of the bag mixture was added to the tubes containing 4.5 ml of 0.1 peptone water. After well vortex of tubes, 10^{-5} dilutions were prepared. Then, 100 microliters of each diluent were taken and superficially cultured on the MRS agar media. Plates were then incubated on the aerobic (for *L. casei*) and anaerobic (for *B. lactis*) conditions at 37 °C for 48 hrs. Finally, bacterial colonies were encountered.

Statistical analysis

All statistical analyses were carried out using the SPSS statistical software (version 20, USA). One-way ANOVA and Tukey tests were used to study an any statistical differences between groups of the investigation. In all tests, $P < 0.05$ was considered as a significant level.

RESULTS AND DISCUSSION

The present investigation was done to study the effects of the *C. sinensis* extract on the survival of *L. casei* and *B. lactis* bacteria encapsulated with alginate chitosan in symbiotic ice-cream during 90 days maintenance in freeze conditions. Table 1 represents the results of the enumeration of free and encapsulated *L. casei* in symbiotic ice-cream during 90 days. Results showed that numbers of free strains of *L. casei* had a range of 7.9 to 30.7 CFU/g. We found that the numbers of *L. casei* strains were decreased through maintenance period in symbiotic ice-cream. In the encapsulated *L. casei* strains with *C. sinensis* extract, reduction in the numbers of bacteria through the maintenance period had a slower tilt. Ranges of encapsulated *L. casei* strains were 21.7 to 38.7 CFU/g. Statistically significant differences were found for the numbers of bacteria of both groups in the days of 0, 30, 45, 75 and 90 of maintenance. Percent of the survival of the free and encapsulated *L. casei* strains during 90 days maintenance period is shown in table 2. Significant statistical differences were seen between the percent of free and encapsulated *L. casei* strains ($P < 0.05$).

Table 3 represents the results of the enumeration of free and encapsulated *B. lactis* in symbiotic ice-cream during 90 days. Results showed that numbers of free strains of *B. lactis* had a range of 65.6 to 6.6 CFU/g. We found that the numbers of *B. lactis* strains were decreased through maintenance period in symbiotic ice-cream. In the encapsulated *B. lactis* strains with *C. sinensis* extract, reduction in the numbers of bacteria through the maintenance period had a slower tilt. Ranges of encapsulated *B. lactis* strains were 48.6 to 15.6 CFU/g. Statistically significant differences were found for the

Table 1. Average log of the numbers of free and encapsulated *L. casei* in symbiotic ice-cream during 90 days of maintenance.

Types of bacteria	Numbers of bacteria (Log CFU/g) in various days						
	0	15	30	45	60	75	90
Free *L. casei*	30.7±0.46a*	26.7±0.61a	25.7±0.72b	22.7±0.58b	21.7±0.73a	15.7±0.62b	7.9±0.57b
Encapsulated *L. casei* with *C. sinensis*	38.7±0.4b	30.7±0.33a	33.7±0.71a	30.7±0.53a	27.7±0.29a	26.7±0.64a	21.7±0.36a

*Dissimilar leather in each column represent the significant difference about P <0.05.

Table 2. Percent of the survival of free and encapsulated *L. casei* in symbiotic ice-cream during 90 days of maintenance.

Types of bacteria	Survival (%) in various days					
	15	30	45	60	75	90
Free *L. casei*	91.40a*	90.40a	86.60a	83a	70.90a	62.20a
Encapsulated *L. casei* with *C. sinensis*	90.20a	83.80b	84.30a	78.60b	77.40a	69.10a

*Dissimilar leather in each column represent the significant difference about P <0.05.

Table 3. Average log of the numbers of free and encapsulated *B. lactis* in symbiotic ice-cream during 90 days of maintenance.

Types of bacteria	Numbers of bacteria (Log CFU/g) in various days						
	0	15	30	45	60	75	90
Free *B. lactis*	65.6±0.33a	45.6±0.54a	34.6±0.81a	18.6±0.19a	11.6±0.22b	8.6±0.51b	6.6±0.25b
Encapsulated *B. lactis* with *C. sinensis*	48.6±0.28b	30.6±0.39b	30.6±0.64a	18.6±0.44a	17.6±0.36a	17.6±0.75a	15.6±0.61a

*Dissimilar leather in each column represent the significant difference about P <0.05.

numbers of bacteria of both groups in the days of 0, 15, 60, 75 and 90 of maintenance. Table 4 shows the percent of survival for the free and encapsulated *B. lactis* strains during 90 days of maintenance. Significant statistical differences were seen between the percent of free and encapsulated *B. lactis* strains (P <0.05).

Microencapsulation is a novel method to protect from probiotic bacteria like lactobacillus and bifidobacterium species and also increase their survival in hard conditions and also the maintenance period. In keeping with the hard conditions of acidic foods, protection of probiotic bacteria from the acidic conditions of stomach is another important reason for using from microencapsulation procedure.

To date, several methods have been applied to protect from the probiotic microorganisms and increase their survival in food science. As far as we know, the present investigation is the first prevalence report of the application of the extract of *C. sinensis* on the survival of encapsulated *L. casei* and *B. lactis* on the symbiotic ice-cream during the 90 days of maintenance. Our results represented that application of *C. sinensis* can protect from the numbers of the *L. casei* and *B. lactis* during the period of maintenance.

Table 4. Percent of the survival of free and encapsulated *B. lactis* in symbiotic ice-cream during 90 days of maintenance.

Types of bacteria	Survival (%) in various days					
	15	30	45	60	75	90
Free *B. lactis*	66.70a	50a	33.90b	28.90b	28.30b	26.70
Encapsulated *B. lactis* with *C. sinensis*	76.30a	66.70a	50a	49.20a	50.80a	40

*Dissimilar leather in each column represent the significant difference about P <0.05.

Several investigations have been conducted in this field. Akin *et al.* (2007) (Akin *et al.*, 2007) showed that ice-cream is a good dairy-based food product for transmission of probiotic bacteria to customers, while Kailasapathy and Sultana (2003) (Kailasapathy and Sultana, 2003) mentioned that decrease in the numbers of probiotic bacteria during the freezing stages in ice-creams is an inhibitory factor for survival of bacteria. Similar to the results of the Akin *et al.* (2007) (Akin *et al.*, 2007), we found that ice-cream has a high capacity for transmission of probiotics to human. We found that the survival percent of free and encapsulated *L. casei* and *B. lastics* probiotics in ice-creams were 62.20% and 69.10% and 26.70% and 40%, respectively which was acceptable. Reduction in the numbers of probiotic bacteria is neither a surprising finding neither in ice-cream and nor in any other types of dairy products. It a common finding in all researches and even in all factory's products. An important matter is the fact that *C. sinensis* extract can increase the viability of *L. casei* and *B. lastics* probiotics in ice-creams. Shah and Ravula (2000) (Shah and Ravula, 2000) represented that the microencapsulation procedure is a good and functional procedure to increase the viability of probiotic bacteria in frozen desserts which was similar to our findings. Krasaekoopt *et al.* (2004) (Krasaekoopt *et al.*, 2004) approved that the microencapsulation using the alginate and chitosan is the best practical method to increase the viability of probiotics in dairy products which support the basic idea of our study.

Survival of probiotics in ice-cream has been impacted in unappropriated environmental conditions such as presence of oxygen, freezing procedure and its bad effects due to the creation of ice crystals, mechanical stress of the production procedures and its storage at low temperature. The main reason for the higher decrease in the numbers of *B. lactis* strains in compare to the *L. casei* is the fact that bifidobacteria are mainly anaerobic and aeration process which is necessary for formation of ice-creams can enter high amounts of oxygen into the ice-cream which is resulted in the destruction of bifidobacteria.

Picot and Lacroix (2003) (Picot and Lacroix, 2003) reported that the extract of *C. sinensis* has a high degree of selenium which can facilitate and improve the growth of *L. casei* and *B. lactis* bacteria in functional foods. They also stated that high antioxidant and antiradical effects of the *C. sinensis* extract cause its strong protection on probiotic bacteria. Another study which was conducted by Molan *et al.* (2009) (Molan *et al.*, 2009) showed that the survival of *B. lactis* and *L. acidophilus* probiotics in ice-cream was increased using 1% inulin powder. They showed that the numbers of probiotic bacteria through 90 days maintenance was decreased from 10^7 to 10^6 log CFU/g, while in the control group was decreased to 10^5 log CFU/g.

CONCLUSION

This research project showed that application of the *C. sinensis* extract can improve the survival of *L. casei* and *B. lactis* bacteria in ice-cream. We recommended the application of chitosan-alginate microencapsulation procedure along with the application of *C. sinensis* extract to production of symbiotic ice-cream with high numbers of *L. casei* and *B. lactis* probiotic bacteria even after 90 days of maintenance in freeze temperature.

ACKNOWLEDGEMENTS

Authors want to thank Prof. Afshin Akhondzadeh Basti and Prof. Ali Misaghi at the Department of Food Safety, University of Tehran, Tehran, Iran for their significant practical provision. This work was supported by the University of Tehran.

REFERENCES

Ahmadi, A., Milani, E., Madadlou, A., Mortazavi, S.A., Mokarram, R.R. and Salarbashi, D. (2014). Synbiotic yogurt-ice cream produced via incorporation of microencapsulated lactobacillus acidophilus (la-5) and fructo oligosaccharide. Journal of Food Science and Technology, Vol. 51, No. 8, pp. 1568-1574.

Akin, M.B., Akin, M.S. and Kirmaci, Z. (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream. Food Chemistry, Vol. 104, No. 1, pp. 53–59.

Allan-Wojtas, P., Truelstrup, H.L. and Paulson, A.T. (2008). Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. LWT-Food Science and Technology, Vol. 41, No. 1, pp. 101–108.

Dehkordi, F.S., Yazdani, F., Mozafari, J. and Valizadeh, Y. (2014). Virulence factors, serogroups and antimicrobial resistance properties of *Escherichia coli* strains in fermented dairy products. BMC Research Notes, Vol. 7, pp. 217.

Goenka, P., Sarawgi, A., Karun, V., Nigam, A.G., Dutta, S. and Marwah, N. (2013). Camellia sinensis (Tea): Implications and role in preventing dental decay. Pharmacognosy Review, Vol. 7, No. 14, pp. 152-6.

Hekmat, S. and McMahon, D.J. (1992). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. Journal of Dairy Science, Vol. 75, No. 6, pp. 1415-22.

Kailasapathy, K. and Sultana, k. (2003). Survival and b-D-galactosidase activity of encapsulated and free *Lactobacillus acidophilus* and *Bifidobacterium lactis* in ice cream. Australian Journal of Dairy Technology, Vol. 58, No. 3, pp. 223–227.

Krasaekoopt, W., Bhandari, B. and Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. International Dairy Journal, Vol. 13, No. 1, pp. 3-13.

Molan, A.L., Flanagan, J., Wei, W. and Moughan, P.J. (2009). Selenium-containing green tea has higher antioxidant and prebiotic activities than regular green tea. Food Chemistry, Vol. 114, No. 3, pp. 82–87.

Moore, R.J., Jackson, K.G. and Minihane, A.M. (2009). Green tea (*Camellia sinensis*) catechins and vascular function. British Journal of Nutrition, Vol. 102, No. 12, pp. 1790-802.

Nagpal, R., Kumar, A., Kumar, M., Behare, P.V., Jain, S. and Yadav, H. (2012). Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiology Letters, Vol. 334, No. 1, pp. 1-15.

Pastore, R.L. and Fratellone, P. (2006). Potential health benefits of green tea (*Camellia sinensis*): a narrative review. Explore (NY), Vol. 2, No. 6, pp. 531-9.

Patel, S. and Goyal, A. (2012). The current trends and future perspectives of prebiotics research: a review. 3 Biotechnology, Vol. 2, No. 2, pp. 115–125.

Picot, A. and Lacroix, C. (2003). Effects of micronization on viability and thermotolerance of probiotic freeze-dried cultures. International Dairy Journal, Vol. 13, No. 6, pp. 78-84.

Shah, N.P. and Ravula, R.R. (2000). Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. Australian Journal of Dairy Technology, Vol. 55, No. 3, pp. 139–144.

Sourabh, A, Kanwar, S.S, Sud, R.G, Ghabru, A. and Sharma, O.P. (2014). Influence of phenolic compounds of Kangra tea, *Camellia sinensis* (L) O Kuntze] on bacterial pathogens and indigenous bacterial probiotics of Western Himalayas. Brazilian Journal of Microbiology, Vol. 44, No. 3, pp. 709-15.