Kafkas Univ Vet Fak Derg
x (x): xxx-xxx, 2019
DOI: 10.9775/kvfd.2018.21274

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Article in Press

Optimization of Entrapment Substances for Microencapsulation of *Lactobacillus plantarum* and *Lactobacillus casei* Shirota Against Gastric Conditions

Emel UNAL TURHAN [1,a]

[1] Osmaniye Korkut Ata University, Kadirli Applied Sciences School, Department of Food Technology, TR-80760 Osmaniye - TURKEY

[a] ORCID ID: 0000-0002-0284-574X

Article Code: KVFD-2018-21274 Received: 30.10.2018 Accepted: 05.02.2019 Published Online: 05.02.2019

How to Cite This Article

Unal Turhan E: Optimization of entrapment substances for microencapsulation of *Lactobacillus plantarum* and *Lactobacillus casei* Shirota against gastric conditions. *Kafkas Univ Vet Fak Derg*, 2019 *(Article in Press)*. DOI: 10.9775/kvfd.2018.21274

Abstract

Microencapsulation is a promising method that has considerable effects on protection of probiotic viability. A variety of coating materials have been utilized to enhance the stability of probiotic microorganisms during the transition through gastrointestinal tract. The aim of this research was to determine optimum coating material combinations for probiotic microencapsulation against gastric conditions. Fructooligosaccharides, peptide, sodium alginate, gelatin and gellan gum were used as entrapment substances to microencapsulate *Lactobacillus plantarum* and *Lactobacillus casei* Shirota with extrusion technique. The response surface technique was applied to detect the optimum proportion of encapsulation substances against gastric condition. Microencapsulation protected probiotic cultures against stress factors such as simulated gastric juice and bile-salt solution. Optimum rate of encapsulation substances varied according to the type of probiotic bacteria. Test results showed that *L. plantarum* should be coated with 1.5% alginate, 0.92% gellan gum, 0.18% gelatin, 0.36% peptide and 1.31% fructooligosaccharides for highest protection. *L. casei* Shirota should also be coated with 2% alginate, 0.98% gellan gum, 0.51% gelatin, 0.86% peptide and 1.98% fructooligosaccharides for highest protection. This research concluded that microencapsulation with encapsulation materials at optimum concentration provided improved protection for the probiotics.

Keywords: *Lactobacillus casei Shirota, Lactobacillus plantarum, Microencapsulation, Response surface method, Extrusion*

Lactobacillus plantarum ve *Lactobacillus casei* Shirota'nın Gastrik Koşullara Karşı Mikroenkapsülasyonu için Kaplama Materyallerinin Optimizasyonu

Öz

Mikroenkapsülasyon, probiyotik canlılığının korunması üzerinde önemli etkileri olan umut verici bir yöntemdir. Probiyotik mikroorganizmaların gastrik koşullara karşı dayanımını arttırmak için çeşitli kaplama materyallerinden yararlanılmıştır. Bu çalışmanın amacı gastrik koşullara karşı probiyotik mikroenkapsülasyonu için ideal kaplama materyali kombinasyonunu belirlemektir. Fruktooligosakkarit, peptit, sodyum aljinat, jelatin ve gellan gam ekstrüzyon tekniği ile *Lactobacillus plantarum* ve *Lactobacillus casei* Shirota'yı mikroenkapsüle etmek için tutuklayıcı maddeler olarak kullanılmıştır. Gastrik koşullara karşı enkapsülasyon materyallerinin ideal oranları cevap yüzey tekniği ile elde edilmiştir. Mikroenkapsülasyon işlemi yapay gastrik su ve safra tuzu çözeltisi gibi stres faktörlerine karşı probiyotik kültürleri korumuştur. Kaplama materyallerinin ideal oranları probiyotik bakteri türüne göre değişmiştir. Test sonuçları yüksek düzeyde koruma için *L. plantarum*'un %1.5 aljinat, %0.92 gellan gam, %0.18 jelatin, %0.36 peptit ve %1.31 FOS ile kaplanması gerektiğini göstermiştir. Yüksek düzeyde koruma için *L. casei* Shirota ise %2 aljinat, %0.98 gellan gam, %0.51 jelatin, %0.86 peptit ve %1.98 FOS ile kaplanmalıdır. Bu araştırma, en uygun konsantrasyonda kaplama materyalleri ile mikroenkapsülasyonun, probiyotiklerin canlılığını iyileştirdiği sonucunu çıkarmıştır.

Anahtar sözcükler: *L. casei Shirota, L. plantarum, Mikroenkapsülasyon, Cevap yüzey tekniği, Ekstrüzyon*

INTRODUCTION

Probiotics have numerous useful properties on human health. Because of their beneficial effects, probiotic cultures often used in several functional food products [1]. As a matter of fact, there is a recent trend towards consumption

İletişim (Correspondence)
+90 328 8880090 Fax: +90 328 8880091
emelunalturhan@gmail.com

of functional foods worldwide [2-4]. World Health Organization (WHO) reported that probiotics are live microorganisms that, "when administered in sufficient amounts, confer a health benefit on the host" [5]. *Lactobacillus* and *Bifidobacterium* species have been the most commonly known probiotics and play an important role in the function and integrity of the intestinal ecosystem and the immune system [6-8]. Especially, *L. plantarum* and *L. casei* Shirota are the most popular and often performed probiotics in food production.

Probiotic bacteria have to survive during gastric transit and have positive effects on health of the host. Maintenance of probiotic viability at the time of consumption and ensuring of sufficient probiotic amounts are challenges on probiotic manufacturers [9,10]. Especially stress factors such as low pH, enzymes and bile salts in gastrointestinal system lead to a negative impact on probiotic robustness and performance. Inhibitory activity of stomach acid and bile salt should be overcome to retain probiotic viability and functionality [9,11]. For the therapeutic effects of probiotics, viable cell counts should be higher than or equal to10^7 CFU/g or mL of product and probiotic bacteria should be able to survive under gastrointestinal conditions [12,13]. In this case, microencapsulation technique is an alternative and effective strategy to protect survival of probiotics against hard conditions. The promised health benefits of probiotics were achieved with microencapsulation [14,15]. Two methods often used for microencapsulation are emulsion and extrusion. Extrusion method has many advantages that it is simple and inexpensive method with gentle operations, does not involve deleterious solvents, does not cause probiotic cell injuries and can be done under aerobic and anaerobic conditions [15,16]. In extrusion technique, probiotic bacteria are added into the hydrocolloid solution (mostly alginate) for entrapping in the gel matrix and then the cell suspension is passed through the syringe needle to form droplets, which free-fall into the solidification solution [8].

There are differences in the characteristics of probiotic strains and the right encapsulation method should be selected for each probiotic [13]. The physicochemical properties of the capsules have a significant impact on the viability of microencapsulated probiotic bacteria. Efficiency of microencapsulation can show differences depending on the kind and the concentration of the encapsulation substances, particle dimensions, initial viable cell counts and microbial strains. As a matter of fact, choose of capsule materials plays an important role in the bacterial cell protection against environmental stresses and affect release of probiotic cells as available and metabolically active state in gastrointestinal system. The appropriate encapsulation substances act as protective agent and may offer the highest robustness of the probiotics in microcapsules during transport from digestive tract of host and/or during exposure to adverse conditions from food matrices [17]. Alginate, gelatin and gellan gum are the most often used polymers for microencapsulation of probiotic bacteria due to their simplicity, non-toxicity, biocompatibility, excellent membrane-forming ability and low cost [18,19,20].

The other approach utilized to achieve sufficiently high numbers of probiotics in intestinal systems is the use of "prebiotics" [21]. Prebiotics were used to refer non digestible food substances that induce the growth or activity of beneficial microorganisms in the gastrointestinal tract of host [12,22,23]. As a matter of fact, food industry and researchers showed a major concern in the use of prebiotics because of synergistic effects between probiotics and prebiotics [2]. Fructo-oligosaccharides (FOS) are the most commonly used prebiotics and nowadays peptides are used as growth promoter [21]. However, research on the use of prebiotic in microcapsules is scarce and more work is needed to measure the stability of these capsules system in gastric conditions.

In the present study, determination of optimum entrapment substances combinations for probiotic microencapsulation against gastric conditions and enhancement of probiotic survival were aimed.

MATERIAL and METHODS

Bacterial Strains and Culture Conditions

Probiotic strains used in this research are *L. plantarum* (Blessing-Biotech GmbH-Stuttgart/Germany) and *L. casei* Shirota (Yakult-RIUM/The Netherlands).

Probiotic cultures were grown in de Man, Rogosa Sharpe (MRS) broth (Merck, Germany) at 37°C for 24 h. After incubation, cells were removed by centrifugation (3000×g, 10 min at 4°C), washed and resuspended twice in saline solution. The final cell concentrations of probiotic cultures were adjusted to 10^{10} CFU/mL for microencapsulation.

Optimization of Entrapment Substances for Probiotic Cultures

The kind and proportion of the entrapment substances have effect on the stability of probiotic strains. The detection of entrapment substances in optimum compositions is crucial for highest protection [24]. For this reason, response surface technique was performed for optimization of entrapment substances [25]. Modelling of this experiment was based on variables (coating materials) and responses (probiotic cell viability). Modelling results from the response surface technique was detected with Design expert 6.02 software *(Table 1)*. Alginate, gelatin, gellan gum, FOS and peptide were selected as entrapment substances. Also, responses in this experiment were based on viable cell counts of probiotics in simulated gastric fluid (SGF) and bile-salt solution (BSS) were evaluated.

Table 1. *Variables of experiment*

Combination	Alginate (%)	Gellan Gum (%)	Gelatin (%)	Peptide (%)	FOS (%)
1	2.00	1.00	0.00	1.00	0.00
2	2.00	0.00	0.00	1.00	2.00
3	0.50	0.00	0.00	0.00	0.00
4	0.50	0.00	1.00	1.00	2.00
5	0.50	1.00	1.00	0.00	2.00
6	1.25	0.50	0.50	0.50	1.00
7	1.25	0.50	0.50	0.50	2.00
8	0.50	1.00	0.00	1.00	2.00
9	1.25	0.50	0.50	1.00	1.00
10	1.25	1.00	0.50	0.50	1.00
11	2.00	1.00	1.00	0.00	0.00
12	1.25	0.50	0.00	0.50	1.00
13	1.25	0.50	0.50	0.50	1.00
14	2.00	0.00	1.00	1.00	0.00
15	1.25	0.50	0.50	0.00	1.00
16	0.50	1.00	1.00	1.00	0.00
17	1.25	0.00	0.50	0.50	1.00
18	1.25	0.50	0.50	0.50	0.00
19	2.00	0.00	1.00	0.00	2.00
20	1.25	0.50	1.00	0.50	1.00
21	2.00	1.00	0.00	0.00	2.00
22	0.50	0.50	0.50	0.50	1.00
23	2.00	0.50	0.50	0.50	1.00
24	1.25	0.50	0.50	0.50	1.00
25	1.25	0.50	0.50	0.50	1.00
26	1.25	0.50	0.50	0.50	1.00

Microencapsulation of Probiotic Cultures

Fructo-oligosaccharides and peptide are prebiotics-promoting probiotic growth and often used for synbiotic effect from synergy between probiotics and prebiotics. Previous researchers were mainly applied calcium alginate, gelatin, and gellan gum as coating materials because these entrapment substances provide better protection for probiotics in food and in the intestinal tract. The proper selection of probiotic strains, prebiotics and coating materials is crucial in obtaining a therapeutic effect [2,19,25,26]. On this sense, microencapsulation in this study were performed with entrapment substances supporting probiotic growth and protection. Probiotic cultures *(L. plantarum* and *L. casei* Shirota*)* were microencapsulated with entrapment substances consisting of 26 different combinations *(Table 1)* according to extrusion technique. As a preliminary, 26 different solutions containing sodium alginate (0.5-2%), gelatin (0-1%), gellan gum (0-1%), FOS (0-2%) and peptide (0-1%) were sterilized by autoclaving (121°C for 15 min) and cooled to 40°C. For microencapsulation by extrusion technique, probiotic cell suspension including *L. plantarum* or *L. casei* Shirota (10^{10} CFU/mL) was added into 50 mL of this sterile coating material solution to yield a final concentration of 1% (V/V). This mixture was placed in a syringe with 0.11 mm needle and injected into sterilized gelling solution (0.1 M $CaCl_2$). The capsules, 0.5 mm in diameter were retained for 1 h for solidification and then aseptically transferred into a sterile petri dishes [19,24]. Probiotic microcapsules obtained in this study were showed in *Fig. 1*.

Resistance of Entrapped Probiotic Strains to SGF and BSS

A solution consisted of 0.5% sodium chloride and 0.3% pepsin was adjusted to pH 2 with 1 N HCl and was used for the determination of resistance to SGF. The microencapsulated probiotic bacteria (1 g) were added into SGF solution (10 mL) in flask and incubated in shaking water bath (100 rpm) at 25°C for 1 h. To determine the resistance to BSS, microencapsulated probiotics (1 g) were inoculated into solution of 2% ox gall powder (Sigma, USA) and incubated in shaking water bath (100 rpm) at 25°C for 1 h [24,26].

Fig 1. Microencapsulated probiotic cells

Enumeration of Probiotic Strains in Microcapsules

One gram of microencapsulated probiotic bacteria samples were diluted with 9 mL of sterile phosphate buffer solution (0.1 M, pH 7.0) and allowed to homogenize for 15 min. Probiotic bacteria (CFU/g) were plated on de Man, Rogosa Sharpe Agar (Merck, Germany) and incubated at anaerobic conditions (Anaerocult A, Merck) for 48 h at 30°C [19,27].

RESULTS

Viable cell counts in microcapsules containing probiotic strains *(L. plantarum* or *L. casei* Shirota) were measured before and after treatment to SGF and BSS conditions and this measurement results were given in *Table 2*. Additionally, reduction in viable cell counts of probiotic strains after treatment of SGF and BSS for each combination of coating materials (from 1 to 26) was calculated from results in

***Table 2.** Responses of experiment (log CFU/g)*

LP Counts Before SGF/BSS	LP Counts After SGF	LP Reduction After SGF	LP Counts After BSS	LP Reduction in BSS	LC Counts Before SGF/ BSS	LC Counts After SGF	LC Reduction in SGF	LC Counts After BSS	LC Reduction BSS
9.04	7.54	1.50	7.53	1.51	9.20	7.00	2.20	7.79	1.41
9.87	8.02	1.85	7.82	2.05	9.14	7.73	1.41	7.04	2.10
9.00	6.84	2.16	7.86	1.14	9.04	7.00	2.04	8.41	0.63
9.23	6.47	2.76	7.69	1.54	9.63	7.04	2.59	8.00	1.63
9.79	6.60	3.19	8.60	1.19	9.11	7.36	1.75	8.14	0.97
9.11	7.00	2.11	8.34	0.77	9.61	7.17	2.44	7.56	2.05
9.85	6.60	3.25	8.20	1.51	9.95	7.04	2.91	8.34	1.61
9.90	8.07	1.83	7.80	2.10	9.07	7.20	1.87	7.47	1.60
9.97	7.90	2.07	8.43	1.54	9.04	7.93	1.11	7.69	1.35
9.88	8.07	1.81	7.85	2.03	9.14	7.73	1.41	7.03	2.11
9.96	7.73	2.23	7.20	2.76	9.95	5.60	4.35	8.27	1.68
9.96	7.07	2.89	8.34	1.62	9.07	6.60	2.47	8.14	0.93
9.49	7.60	1.89	7.60	1.89	9.04	7.17	1.87	7.30	1.74
9.97	7.91	2.06	8.23	1.74	9.14	7.93	1.21	7.65	1.49
9.36	8.04	1.32	8.32	1.04	9.07	7.82	1.25	8.00	1.07
9.07	6.47	2.60	7.77	1.30	9.69	7.32	2.37	8.00	1.69
9.00	5.00	3.00	6.69	2.31	9.07	5.30	3.77	6.11	2.96
9.04	7.77	1.27	8.00	1.04	9.04	7.11	1.93	8.00	1.04
9.67	7.11	2.56	8.04	1.93	9.17	7.43	1.74	8.20	0.97
9.88	8.03	1.85	7.83	2.05	9.11	7.71	1.40	7.00	2.11
9.96	8.14	1.82	8.17	1.79	9.00	7.32	1.68	8.00	1.00
9.85	8.02	1.83	7.84	2.01	9.14	7.72	1.42	7.02	2.12
9.88	8.04	1.84	7.83	2.05	9.14	7.72	1.42	7.00	2.14
9.30	8.36	0.94	7.60	1.70	9.50	7.74	1.76	7.66	1.84
9.84	9.07	0.77	8.60	1.24	9.62	5.60	4.02	8.20	1.42
9.88	8.04	1.84	7.84	2.04	9.14	7.72	1.42	7.04	2.10

***LP:** L. plantarum, **LC:** L. casei Shirota, **SGF:** simulated gastric fluid, **BSS:** bile-salt solution, **Reduction:** Difference between probiotic viable cell counts before SGF or BSS and after SGF or BSS*

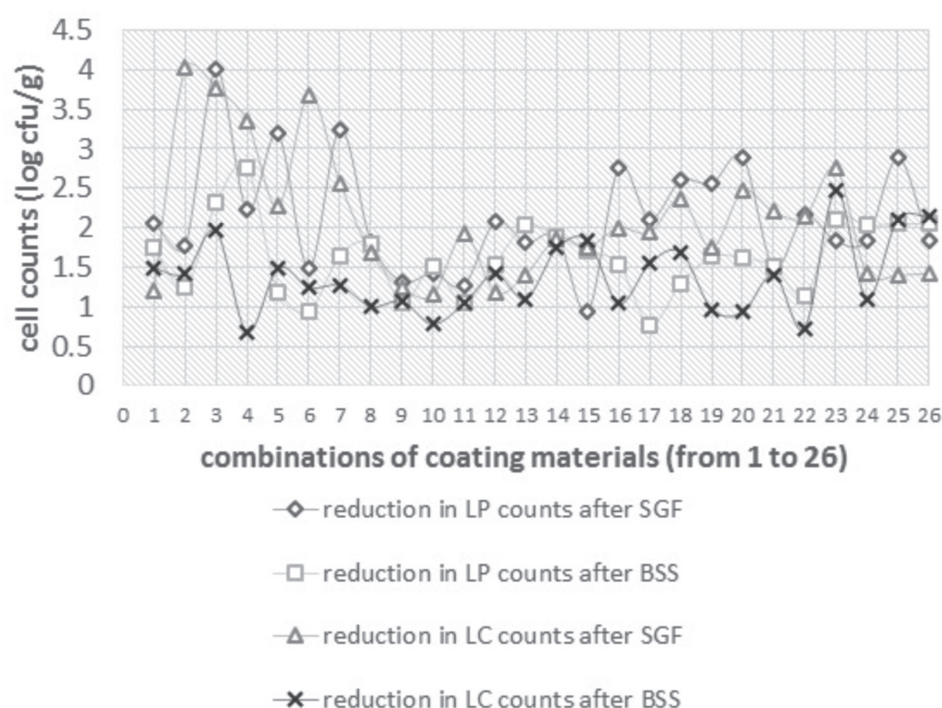

Fig 2. Reduction in viable cell counts of probiotic strains after treatment of SGF and BSS

Table 2 and this reduction was shown in *Fig. 2*.

As observed from results of this research, probiotic viable cell counts in microcapsules changed between 9.04 and 9.97 log CFU/g before exposure to SGF and BSS, while probiotic viable cell counts in microcapsules ranged from 5.0 to 8.60 log CFU/g after exposure to SGF and BSS.

Simulated gastric fluid conditions caused a drop from 0.77 to 3.25 log CFU/g in *L. plantarum* counts and from 1.11 to 4.35 log CFU/g in *L. casei* Shirota counts, respectively. After BSS condition, a reduction in *L. plantarum* and *L. casei* Shirota counts varied from 0.77 to 2.76 log CFU/g and from 0.63 to 2.96 log CFU/g, respectively.

Optimum concentrations of 5 different entrapment substances were predicted through the model established with response surface methodology. As seen in *Table 1*, concentrations of entrapment substances tested in this study were adjusted between 0.5-2% for alginate, 0-1% for gellan gum, 0-1% for gelatin, 0-1% for peptide and 0-2% for FOS. The encapsulation material composition and concentration providing the highest probiotic cell viability were calculated by using prediction model according to results obtained in *Table 1*. The optimum values for the obtainment of microcapsules with highest probiotic robustness were found as the mix of 1.5% alginate, 0.92% gellan gum, 0.18% gelatin, 0.36% peptide and 1.31% FOS for *L. plantarum* and as the mix of 2% alginate, 0.98% gellan gum, 0.51% gelatin, 0.86% peptide and 1.98% FOS for *L. casei* Shirota.

DISCUSSION

It is known from literature works that free cells of probiotic strains are more susceptible than microencapsulated cells under gastrointestinal conditions. As a matter of fact, several researchers reported that microencapsulation provided additional protection to probiotic cells with a physical barrier against stress factors in intestinal system and exhibited more robustness during gastric transit than their free cell [28,29,30,31]. Based on previous studies, there was no need to test the viability of free probiotic strains after exposure to SGF and BSS because encapsulation enhances the viability of probiotic strains. Microencapsulation technique is required to ensure survival or stability of probiotics bacteria during the passage to digestive tract of host. However, coating materials used in microencapsulation had differently effect on protection of probiotic against adverse factors [32]. In accordance with this, the present results showed that resistance in probiotic viability changed according to coating material combinations (26 different microcapsule) after SGF and BSS. The use of prebiotic materials (peptide and FOS) in addition to gelling agents such as sodium alginate and gelatin for microencapsulation provides a better protection to probiotic bacteria. These prebiotic agents may act as a supporter of probiotic viability. As a matter of fact, various coating material combinations with regard to their compositions and concentrations have caused different levels of probiotic resistance against gastric conditions according to earlier studies [18,19,26,33]. Similarly, the present study confirmed this different effect of coating material combinations on probiotic resistance. Additionally, statistical analysis showed that microencapsulation with different coating material combinations had effect on resistance of probiotic strains against SGF at the 95% confidence level.

A reduction in *L. plantarum* counts and *L. casei* Shirota counts changed approximately between 1 and 4 log CFU/g after SGF and between 1 and 2 log CFU/g after BSS. This situation detected that these probiotic bacteria were more resistant to BSS than SGF. Chen et al.[19] reported that that probiotic strains exhibited higher resistance to acidic conditions than to bile salts. However, in another study, probiotic *L. rhamnosus* were found more resistant to bile salts than to acid [34]. This situation considered that resistance of probiotics against SGF and BSS conditions could change according to strains.

SGF conditions caused a drop from 0.94 to 4 log CFU/g in *L. plantarum* counts and from 1.15 to 4.02 log CFU/g in *L. casei* Shirota counts, respectively. After BSS condition, a reduction in *L. plantarum* and *L. casei* Shirota counts varied from 0.77 to 2.76 log CFU/g and from 0.68 to 2.48 log CFU/g, respectively. As reported in Chen et al.[25], our results indicated that coating material combinations had different effect on resistance of probiotic against SGF and BSS conditions.

As mentioned above, optimum rate of 5 different entrapment substances for microencapsulation of each probiotic cell were calculated from optimization model obtained by using response surface methodology. Concentrations of encapsulation agents changing between 0.5-2% for alginate, 0-1% for gellan gum, 0-1% for gelatin, 0-1% for peptide and 0-2% for FOS were tested. The reason for choosing these concentration ranges in this study is suggestions from previous researchers [28]. Entrapment substances at different type and concentrations were evaluated with regard to the protection of cell viability. Probiotic microcapsules with entrapment substances at 26 different combinations were prepared according to the experimental design shown in *Table 1*. Formulation of optimization model from 26 coating material combinations detected optimum proportion of entrapment substances for each probiotic strain. The viability by the best combination were also tested and found higher than other combinations. These results confirmed our hypothesis that optimum combination of encapsulation materials provide highest protection against gastric conditions and give the highest cell viability [35,36].

Concentrations of alginate used for gelling change between 1.5 and 2.5%. However when alginate was used with other gelatinization agents, concentrations of alginate were tested between 0.5 and 2% [19]. As known from literature, peptides as nitrogen sources improve viability of probiotics [19,25]. The present study confirmed that peptides with their prebiotic effect have synergistic activity on probiotic viability. Incorporation of microcapsules with extra coating materials supported additional protecting of the probiotic bacteria [37,38]. Our results confirmed that. Some researcher reported that microencapsulation applications such as incorporation of different coating materials and the double emulsion protected probiotic strains against simulated gastrointestinal tract conditions [18,34]. Similarly, our study showed that extra coating improved survival of probiotic.

In conclusion, selection of optimum or appropriate coating materials used for microcapsules may improve the survival of probiotic strains in functional food products. Microcapsules with prebiotic may be safely used as protective delivery vehicle for the passage from gastrointestinal tract of probiotic strains. Moreover, the present study results may attract the attention of other researchers to investigate innovative entrapment substances. On this sense, further modification and improvement in microencapsulation technique is necessary for resistance of probiotics against gastric conditions.

REFERENCES

1. Tomar O, Akarca G, Beykaya M, Çağlar A: Some characteristics of Erzincan tulum cheese produced using different probiotic cultures and packaging material. *Kafkas Univ Vet Fak Derg*, 24 (5): 647-654, 2018. DOI: 10.9775/kvfd.2018.19596

2. Saad N, Delattre C, Urdaci M, Schmitter JM, Bressollier P: An overview of the last advances in probiotic and prebiotic field. *LWT - Food Sci Technol*, 50 (1): 1-16, 2013. DOI: 10.1016/j.lwt.2012.05.014

3. Khan MI, Arshad MS, Anjum FM, Sameen A, Rehman A, Gill WT: Meat as a functional food with special reference to probiotic sausages. *Food Res Int*, 44 (10): 3125-3133, 2011. DOI: 10.1016/j.foodres.2011.07.033

4. Doğan M, Özpinar H: Investigation of probiotic features of bacteria isolated from some food products. *Kafkas Univ Vet Fak Derg*, 23 (4): 555-562, 2017. DOI: 10.9775/kvfd.2016.17273

5. FAO/WHO: Probiotics in Food. Health and Nutritional Properties and Guidelines for Evaluation, vol. 85. FAO/WHO, Rome-Italy, 2003.

6. Klindt-Toldam S, Larsen SK, Saaby L, Olsen LR, Sivenstrub G, Mullertz A, Knochel S, Heimdal H, Nielsen DS, Zielinska D: Survival of *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* HN019 encapsulated in chocolate during in vitro simulated passage of the upper gastrointestinal tract. *LWT-Food Sci Technol*, 74, 404-410, 2016. DOI: 10.1016/j.lwt.2016.07.053

7. Bernat N, Chafer M, Gonzalez-Martinez C, Rodriguez-Garcia J, Chiralt A: Optimization of at milk formulation to obtain fermented derivatives by using probiotic *Lactobacillus reuteri* microorganisms. *Food Sci Technol Int*, 21 (2): 145-157, 2014. DOI: 10.1177/1082013213518936

8. Succi M, Tremonte P, Pannella G, Tipaldi L, Cozzolino A, Coppola R, Sorrentino E: Survival of commercial probiotic strains in dark chocolate with high cocoa and phenols content during the storage and in a static in vitro digestion model. *J Funct Food*, 35, 60-67, 2017. DOI: 10.1016/j.jff.2017.05.019

9. Horáčková S, Žaludová K, Plocková M: Stability of selected *Lactobacilli* in the conditions simulating those in the gastrointestinal tract. *Czech J Food Sci*, 29, S30-S35, 2011. DOI: 10.17221/283/2011-CJFS

10. Makinen K, Berger B, Bel-Rhlid R, Ananta E: Science and technology for the mastership of probiotic applications in food products. *J Biotechnol*, 162 (4): 356-365, 2012. DOI: 10.1016/j.jbiotec.2012.07.006

11. Doherty SB, Gee VL, Ross RP, Stanton C, Fitzgerald GF, Brodkorb A: Development and characterization of whey protein micro-beads as potential matrices for probiotic protection. *Food Hydrocoll*, 25, 1604-1617, 2011. DOI: 10.1016/j.foodhyd.2010.12.012

12. Hernandez-Hernandez O, Muthaiyan A, Moreno FJ, Montilla A, Sanz ML, Ricke SC: Effect of prebiotic carbohydrates on the growth and tolerance of *Lactobacillus*. *Food Microbiol*, 30, 355-361, 2012. DOI: 10.1016/j.fm.2011.12.022

13. Šipailienė A, Petraitytė S: Encapsulation of probiotics: Proper selection of the probiotic strain and the influence of encapsulation

technology and materials on the viability of encapsulated microorganisms. *Probiotics Antimicrob Proteins*, 10 (1): 1-10, 2017. DOI: 10.1007/s12602-017-9347-x

14. Sidira M, Kandylis P, Kanellaki M, Kourkoutas Y: Effect of immobilized *Lactobacillus casei* on the evolution of flavor compounds in probiotic dry-fermented sausages during ripening. *Meat Sci*, 100, 41-51, 2015. DOI: 10.1016/j.meatsci.2014.09.011

15. Kalkan S, Öztürk D, Selimoglu BS: Determining some of the quality characteristics of probiotic yogurts manufactured by using microencapsulated *Saccharomyces cerevisiae* var. *boulardii*. *Turk J Vet Anim Sci*, 42, 617-623, 2018. DOI: 10.3906/vet-1804-5

16. Tee WF, Nazaruddin R, Tan YN, Ayob MK: Effects of encapsulation on the viability of potential probiotic *Lactobacillus plantarum* exposed to high acidity condition and presence of bile salts. *Food Sci Technol Int*, 20 (6): 399-404, 2013. DOI: 10.1177/1082013213488775

17. Burgain J, Gaiani C, Linder M, Scher J: Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *J Food Eng,* 104, 467-483, 2011. DOI: 10.1016/j.jfoodeng.2010.12.031

18. Li XY, Chen XG, Liu CS, Liu CG, Xue YP: Preparation of alginate-gelatin capsules and its properties. *Front Mater Sci China*, 2 (3): 253-260, 2008. DOI: 10.1007/s11706-008-0042-4

19. Chen MJ, Chen KN, Kuo YT: Optimum thermotolerance of *Bifidobacterium bifidum* in gellan-alginate microparticles. *Biotechnol Bioeng,* 98 (2): 411-419, 2007. DOI: 10.1002/bit.21450

20. Ünal E, Erginkaya Z: Probiyotik mikroorganizmaların mikro-enkapsülasyonu. *Gıda,* 35 (4): 297-304, 2010.

21. Özer D, Akin S, Özer B: Effect of inulin and lactulose on survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-02 in acidophilus-bifidus yoghurt. *Food Sci Tech Int*, 11 (1): 19-24, 2005. DOI: 10.1177/1082013205051275

22. Licht TR, Ebersbach T, Frøkiær H: Prebiotics for prevention of gut infections. *Trends Food Sci Techol*, 23, 70-82, 2012. DOI: 10.1016/j.tifs.2011.08.011

23. Unal Turhan E, Erginkaya Z, Uney MH, Ozer EA: Inactivation effect of probiotic biofilms on growth of *Listeria monocytogenes*. *Kafkas Univ Vet Fak Derg*, 23 (4): 541-546, 2017. DOI: 10.9775/kvfd.2016.17253

24. Unal Turhan E, Erginkaya Z, Polat S, Özer EA: Design of probiotic dry fermented sausage (sucuk) production with microencapsulated and free cells of *Lactobacillus rhamnosus. Turk J Vet Anim Sci,* 41, 598-603, 2017. DOI: 10.3906/vet-1701-76

25. Chen KN, Chen MJ, Liu J, Lin CW, Chiu HY: Optimization of incorporated prebiotics as coating materials for probiotic micro-encapsulation. *J Food Sci,* 70 (5): M260-M266, 2005. DOI: 10.1111/j.1365-2621.2005.tb09981.x

26. Chen KN, Chen MJ, Lin CW: Optimum combination of the encapsulating materials for probiotic microcapsules and its experimental verification (R1). *J Food Eng,* 76, 313-320, 2006. DOI: 10.1016/j.jfoodeng.2005.05.036

27. Harrigan WF: Laboratory Methods in Food Microbiology. 3rd edn., Academic Press Ltd., USA, 1998.

28. Lacroix C, Yildirim S: Fermentation technologies for the production of probiotics with high viability and functionality. *Curr Opin Biotechnol*, 18, 176-183, 2007. DOI: 10.1016/j.copbio.2007.02.002

29. Khosravi Zanjani MA, Ghiassi Tarzi B, Sharifana A, Mohammadi N: Microencapsulation of probiotics by calcium alginate-gelatinized starch with chitosan coating and evaluation of survival in simulated human gastro-intestinal condition. *Iran J Pharm Res*, 13 (3): 843-852, 2014.

30. Martin MJ, Lara-Villoslada F, Ruiz MA, Morales ME: Micro-encapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innov Food Sci Emerg Technol*, 27, 15-25, 2015. DOI: 10.1016/j.ifset.2014.09.010

31. Sarao LK, Arora M: Probiotics, prebiotics, and microencapsulation: A review. *Crit Rev Food Sci Nutr*, 57 (2): 344-371, 2017. DOI: 10.1080/10408398.2014.887055

32. Ramos PE, Cerqueira MA, Teixeira JA, Vicente AA: Physiological protection of probiotic microcapsules by coatings. *Crit Rev Food Sci Nutr*, 58 (11): 1864-1877, 2018. DOI: 10.1080/10408398.2017.1289148

33. Nag A, Han KS, Singh H: Microencapsulation of probiotic bacteria using pH-induced gelation of sodium caseinate and gellan gum. *Int Dairy J*, 21, 247-253, 2011. DOI: 10.1016/j.idairyj.2010.11.002

34. González-Fernández C, Santos EM, Jaime I, Rovira J: Influence of starter cultures and sugar concentrations on biogenic amine contents in chorizo dry sausage. *Food Microbiol*, 20, 275-284, 2003. DOI: 10.1016/S0740-0020(02)00157-0

35. Sathyabama S, Ranjith Kumar M, Bruntha P, Vijayabharathi R, Brindha V: Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. *LWT-Food Sci Technol*, 57 (1): 419-425, 2014. DOI: 10.1016/j.lwt.2013.12.024

36. Shori AB: Microencapsulation improved probiotics survival during gastric transit. *HAYATI J Biosci*, 24, 1-5, 2017. DOI: 10.1016/j.hjb.2016.12.008

37. Ding WK, Shah NP: Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *Int Food Res J*, 15 (2), 219-232, 2008.

38. Ye Q, Georges N, Selomulya C: Microencapsulation of active ingredients in functional foods: From research stage to commercial food products. *Trends Food Sci Techol,* 78, 167-179, 2018. DOI: 10.1016/j.tifs.2018.05.025