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Investigation of Iranian Traditional Drink (Doogh) Characteristics Prepared from Camel Milk Containing *Lactobacillus acidophilus* LA-5

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Abstract

Background and objective: Probiotics offer beneficial impact to human health, including lowering serum cholesterol and decreasing occurrence of intestinal infections as well as conferring anti-carcinogenic activities to foods. The present study assessed probiotic doogh drinks made from camel milk and stability of *Lactobacillus acidophilus* LA-5 (free or microcapsulated) during product shelf life at refrigerated temperatures as well as at simulated gastrointestinal conditions.

Material and methods: Microcapsules of *Lactobacillus acidophilus* LA-5 were produced through coacervation of gelatin and high-methoxy pectin (esterification degree of 70%) or gelatin and Arabic gum as wall materials. Stability of probiotic bacteria in gastrointestinal simulated conditions was assessed. Sensory acceptance of samples was investigated using Hedonic test (9-points) during cold storage for 35 days.

Results and conclusion: After 35 days of cold storage, doogh samples included *Lactobacillus acidophilus* LA-5 microcapsulated by gelatin- pectin and gelatin-Arabic gum had more survival (77.11 and 74.19%, respectively) than free cells (62.34%). As bacteria subjected to simulated gastric juice, the bacterial logarithmic population of free, gelatin-pectin and gelatin-Arabic gum microcapsulated *Lactobacillus acidophilus* LA-5 reached to 4, 6 and 5, respectively. In the case of exposure to simulated intestinal fluid, logarithm of the bacterial population reached to 5.6, 6.5 and 6.2 for free, gelatin-pectin and gelatin-Arabic gum bacterial microcapsules, respectively. Organoleptic assessment showed no significant differences between samples in terms of aroma, appearance and overall acceptance. As a result, doogh produced from camel milk containing gelatin-pectin and gelatin-Arabic gum microcapsules of *Lactobacillus acidophilus* LA-5 included a further cold storage stability, compared to that containing free bacteria. Furthermore, a final probiotic population of more than 10^6 were seen for microcapsules in simulated intestinal fluid.

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1. Introduction

Human intestinal microflora represents a complicate ecosystem, comprising diverse sets of microorganisms which are involved in various functions of the host metabolism. Gut microflora is mainly involved in fermentation of carbohydrates and includes multiple effects on energy homeostasis. The microflora is necessary for the intestinal health; therefore, gut bacteria are recognized as key regulators of the host physiology and pathophysiology [1]. Reports have focused on use of probiotics for the treatment of diseases [2]. Another approach for use of probiotic bacteria is linked to inclusion of probiotic bacteria in food products [3]. Preserving viability of the probiotic bacteria during food processing is a major issue in production of probiotic foods [4]. Several processing parameters such as heat, acidity, oxygen and salt adversely affect viability of probiotic bacteria. In addition to processing parameters, intestinal environment (gastric pH and bile salts) can affect survival of probiotic bacteria. To solve this issue, microencapsulation of probiotic bacteria is a promising approach to enhance stability of probiotic bacteria against food processing conditions as well as intestinal environment to preserve the bacterial viability and functional activity at high levels. In microencapsulation method, wall surrounding probiotic bacteria provides a physical barrier against stress conditions which results in bacteria survival through the gastrointestinal tract [5].

Lactic acid bacteria, especially *Lactobacillus* spp. and *Bifidobacterium* spp., have been described with probiotic characteristics in humans. *Lactobacillus* species, especially *Lactobacillus* (*L.*) *acidophilus*, include good properties such as acid and bile tolerances, which are necessary for production of probiotic foods [6]. Use of *L. acidophilus* LA-5 in food products results in decreases in diarrheas and infections by *Escherichia* (*E.*) *coli*. A source of probiotic bacteria, camel milk, composed of the same nutrients as bovine milk but shows a little difference in compositions. Several studies reported unique therapeutic benefits of camel milk. The molecular basis of anti-diabetic properties of camel milk has been reported [7]. The hypoglycemic effect of camel milk in rats with chemically-induced diabetes is described since consumption of low-temperature processed camel milk contributes to normal serum glucose levels [8]. Anticancer and antioxidant properties of fermented camel milk is reported as well [9]. Camel milk includes distinct characteristics that enhance cholesterol exertion through feces and hence lower deposition of cholesterol in liver [10]. Doogh is a Persian traditional dairy drink produced from mixing yogurt, salt water and sometimes herbals [11]. Studies have focused on use of microencapsulated probiotics in various dairy products [12,13] but a few studies have been carries out on production of probiotic doogh from camel milk with encapsulated probiotics. Therefore, the major aim of the current study was to produce probiotic doogh from camel milk containing free or two various microcapsules of *L. acidophilus* LA-5. Stability of free or microcapsulated probiotic bacteria in simulated gastric environment and cold storage was investigated.

2. Materials and methods

2.1. Materials

Ingredients used for doogh preparation included raw camel milk purchased from local markets (Neyshabur, Iran); cultures of *Streptococcus (S.) thermophiles* and *L. delbrueckii* subsp. bulgaricus (direct starter yogurt YB/Z) provided by Micromilk (Italy); probiotic cultures of *L. acidophilus* LA-5 provided by Chr. Hansen (Denmark); *pancreatin*, bile salts and pectin (70% esterified) purchased from Sigma-Aldrich (USA); and gelatin and Arabic gum from Merck (Germany). Reagents were in analytical grade.

2. 2 Production and characterization of microcapsules containing *L. acidophilus* LA-5

Coacervation was carried out for the production of microcapsules of *L. acidophilus* LA-5. This technique was used for producing microcapsules of gelatin high-methyl pectin; as previously described by Muhoza et al by some modifications. For the complex coacervation of gelatin and high-methyl pectin, studies showed the best coacervation at pH 4.23 and the gelatin high-methyl pectin ratio equal of 3:1 [14]. Therefore, these conditions were chosen as optimum coacervation circumstance. First, pectin and gelatin were dissolved in deionized water (60°C, 2 h). Air bubbles were removed using centrifugation at 1957 ×g for 30 min. For prevention of pre-mix electrostatic attraction, pH was adjusted to 7. A stock solution of gelatin and high-methyl pectin with 3:1 ratio was prepared [14]. Probiotic bacteria were added to gelatin solution. Pectin solution then poured in gelatin solution. Then, mixture was stirred at 300 rpm for 5 min. Adjustment of pH at optimum levels for coacervation (equally 4.23) was carried out using acetic acid (10% v v^{-1}). For completing the coacervation process, mixture was placed in an ice-water bath (15°C, 30 min, 300 rpm). The last step induced formation of gel network via increasing the intra and inter molecular bindings. The coacervation process for gelatin-Arabic gum was carried out according to Yari et al. by some modifications [15]. Microcapsules of gelatin-Arabic gum were prepared by adding probiotic bacteria in aqueous gelatin solution (2% w w^{-1}). Then, Arabic gum (2% w w^{-1}) was added to the mixture. Thereafter, lactose (2% w w^{-1}) (as cryoprotectant) was added. Then, mixture stirred for 5 min and stirring rate of 200rpm. Coacervation was done by adjusting the pH value to 4.0 by using acetic acid solution (50% v v^{-1}). Finally, mixture was cooled down slowly to 4°C. Microcapsules were freeze-dried (-45°C, 0.8 mbar, 24 h; Operon, South Korea). Microcapsules were prepared one day prior to production of doogh and stored at 10°C until use.

2.3 Scanning electron microscopy (SEM)

The SEM (Phenom ProX, USA) used for morphology study of microcapsules at 20 kV.

2.4 Quantification of viable bacteria in microencapsulated probiotics in various wall materials

Briefly, a solution of sodium citrate (0.06 mol l^{-1}) and sodium bicarbonate (0.2 mol l^{-1}) was prepared and mixed with 1 g of microcapsules. This was stirred at 37ºC for 1 h. This step was necessary to release bacteria from capsules. Cell viability of *L. acidophilus* LA-5 was estimated using plate count method after anaerobic incubation at 37ºC for 48 h.

2.5 Production of doogh from camel milk containing free or microcapsulated *L. acidophilus* LA-5

For yogurt preparation, camel milk samples were heated at 80-85ºC for 10 min using water bath and then cooled down to 45ºC. Three mixtures were prepared, including 2.5% v v^{-1} yogurt starter (control sample) and probiotic samples containing yogurt starter along with free or microencapsulated *L. acidophilus* LA-5. These were incubated at 42°C ±1. The pH and titratable acidity were assessed at defined intervals (20 min). When pH reached 4.3 ±0.05, products were cooled down to 4°C ±1. Then, yoghurts were mixed with water (50% w w^{-1}) and salt (0.8%

w w^{-1}) to prepare doogh. Dooghs were stored at 4°C for 35 days [16]. Experiments were replicated thrice.

2. 6 Estimation survival of *L. acidophilus* LA-5 at various circumstances

Viable cells of *L. acidophilus* LA-5 (free or microencapsulated) were assessed as follows. To break capsules, the microencapsulated bacteria were poured into 2% w v^{-1} of sodium citrate solution (pH 7.0) and stirred for 5 min [17]. Then, serial dilutions were prepared in 0.1% w v^{-1} of peptone water and transferred to MRS agar using pour plate method. Plates were incubated at 37°C for 72 h under anaerobic condition [18]. For enumeration of free probiotic bacteria, serial diluting, culturing on MRS agar and anaerobically incubating at 37°C for 72 h were used. To count microbial cells during cold storage, samples were analyzed after 1, 7, 14, 21, 28 and 35 days according to the mentioned method. To estimate the stability of *L. acidophilus* LA-5 in gastrointestinal conditions, simulated gastric juice was prepared according to Vecchinoe et al. [19]. Briefly, 200 µl of doogh samples were inoculated into 5 ml of the simulated gastric juice and incubated at 37°C for 0, 30, 60, 90 and 120 min. Other steps were carried out based on the previous descriptions. To estimate microbial stability under intestinal condition, simulated intestinal fluid was used. Simulated intestinal fluid was prepared by dissolving bile salts (0.3% w v^{-1}; Sigma-Aldrich, USA) and pancreatin (0.1% w v^{-1}; Sigma-Aldrich, USA) in 0.85% NaCl solution and adjusting pH to 8. Then, 200 µl of doogh samples were inoculated into 5 ml of the simulated intestinal fluid and incubated at 37°C for 0, 30, 60, 90 and 120 min. viable cells were enumerated as previously described [19].

2. 7 Assessment of organoleptic properties

To assess sensory characteristics of doogh samples, Hedonic test (9-points) was used with help of seven trained panelists. At the beginning and between the tests, panelist rinsed their mouth with 20°C water and ate biscuit (with no salts). Tests were carried out in special rooms, free of odors and colors and equipped with fluorescent lights and air conditioners. Each test was carried out by giving 15 ml of the sample in bottle (free of odors and colors) to each panelist. On each bottle, a 3-digit code selected from random tables was labeled [20]. The highest and lowest acceptance scores included 9 and 1, respectively.

2.8 Statistical analysis

Data were assessed using analysis of variance and Duncan test at 5% significance level for the comparison between means using Statistica Software v.13.3.0 (TIBCO, USA). All experiments were carried out thrice.

3. Results and discussion

3.1 Survival of *L. acidophilus* LA-5 after microencapsulation process and during cold storage

Results showed that encapsulation process included no detrimental effects on *L. acidophilus* LA-5 in both walls used. The viable cells in gelatin-pectin included 1.4×10^8 and in gelatin-Arabic gum included 1.3×10^8. The first population of free *L. acidophilus* LA-5 included 1.52×10^8. This showed that encapsulation process was gentle and encapsulation wall material was compatible with probiotic bacteria [12]. The high viability of probiotic bacteria, verifies appropriation of selected encapsulation materials and processes. Generally, effects of processes depend on probiotic bacteria and coacervation wall materials [21].

Results for probiotic stability cold storage are shown in Table 1. The initial cell counts of the probiotic bacteria in three tested samples of doogh were similar with no significant differences. During storage, a decrease in probiotic population was seen in all samples. Doogh samples composed of free probiotic bacteria showed a faster decrease rate than that other samples did (at the end of cold storage) since probiotic bacteria reached lower critical levels recommended by FAO/WHO [22]. This could be attributed to protective effects of capsules against acidity and salt content of doogh samples. In this respect, capsules produced from gelatin-pectin showed a better performance at the end of day 35.

Table 1. Viability of *Lactobacillus acidophilus* LA-5 (log^{10} CFU ml^{-1}) during 35 days of refrigerated storage of probiotic doogh samples (mean ±SD, n = 3).

Storage day	Doogh with free *L. acidophilus* LA-5	Doogh with microencapsulated *L. acidophilus* LA-5 in gelatin-pectin	Doogh with microencapsulated *L. acidophilus* LA-5 in gelatin-Arabic gum
1	8.18 ± 0.2^{Aa}	8.17 ± 0.21^{Aa}	8.10 ± 0.24^{Aa}
7	7.8 ± 0.1^{Ba}	8.13 ± 0.19^{Aa}	8.0 ± 0.22^{ABa}
14	7.3 ± 0.12^{Cb}	7.75 ± 0.15^{Ba}	7.71 ± 0.21^{BCa}
21	6.1 ± 0.15^{Db}	7.65 ± 0.1^{Ba}	7.55 ± 0.17^{Ca}
28	5.7 ± 0.1^{Ec}	6.8 ± 0.1^{Ca}	6.5 ± 0.15^{Db}
35	5.1 ± 0.1^{Fc}	6.3 ± 0.1^{Da}	6.01 ± 0.11^{Eb}

Means with different uppercase letters in the same column show significance (P≤0.05)
Means with different lowercase letters in the same row show significance (P≤0.05)
L. acidophilus LA-5=*Lactobacillus acidophilus* LA-5

The protective effect of microencapsulation on probiotic bacteria has been reported by other researchers. Xanthan-chitosan microcapsules of *Bifidobacterium bifidum* BB01 exhibited better survival during three storage weeks of pure milk, compared to free probiotic bacteria [23]. Furthermore, a further probiotic survival of pectin-whey protein microcapsules of *L. acidophilus* LA-5 (62%) was reported, compared to free bacteria (10%) [13]. Results of the present study showed the survival rates of free and microencapsulated *L. acidophilus* LA-5 (gelatin-pectin and gelatin-Arabic gum) bacteria as 62.34 and 77.1 and 74.19%, respectively. Nature of doogh (high acidity and salt content) could affect stability of probiotic bacteria in this product, especially in free probiotic bacteria. Salinity decreases bacterial activity through decreasing esterase activity of the bacterial cells. This effect was mostly significant at 3.5% concentration of NaCl at the end of Day 7 of storage [24].

3.2 Scanning electron microscopy (SEM) of the microcapsules

Morphology of the microcapsules was studied using SEM (Figure 1). The SEM images verified that the encapsulation of probiotic bacteria was successful. No holes or cracks were observed in the two microcapsules. Gelatin-Arabic gum microcapsule structure was porous, compared to gelatin-pectin microcapsules. It could explain further stability of gelatin-pectin microcapsules subjected to simulated intestinal fluid, compared to gelatin-Arabic gum microcapsules. Due to the further porous structure of gelatin-Arabic gum microcapsules, further penetration of simulated gastric juice or intestinal fluid might occur and hence further damages to probiotic bacteria might observe (Figures 2 and 3).

3.3 Stability of the two encapsulated *L. acidophilus* LA-5 in simulated gastric juice

For the probiotic activity preservation, bacteria must survive gastric environment and reach intestines at adequate numbers (10^6-10^8 CFU g^{-1}). Therefore, it is necessary to ensure the cell viability in gastric simulation conditions [13]. In the current study, two various microcapsules were assessed in simulated gastric juice (Figure 2).

Both microencapsulated cells in gelatin-pectin and gelatin-Arabic gum exhibited better cell stability in simulated gastric juice, compared to free probiotic cells. Moreover, gelatin-pectin microcapsules showed more positive effects on viability of encapsulated bacteria than that gelatin-Arabic gum microcapsules did, especially at longer times ($P<0.05$). It could be due to the changes occurred in microcapsule structures at low pH, resulting in penetration of acidic media into microcapsules. Gelatin-pectin microcapsules showed further stability in low pH conditions than that gelatin-Arabic gum microcapsules did. Similarly, microcapsules made from xanthan-chitosan at initial population of 10^9 included protective activity against acidity and cell viability of encapsulates included 3.77 log rather than 1.14 log for free cells [25]. Encapsulation of *L. rhamnosus* with chitosan-coated alginate capsules with Lactobacillus resulted in a 92% survival rate in gastric condition (pH 3) within 60 min [26].

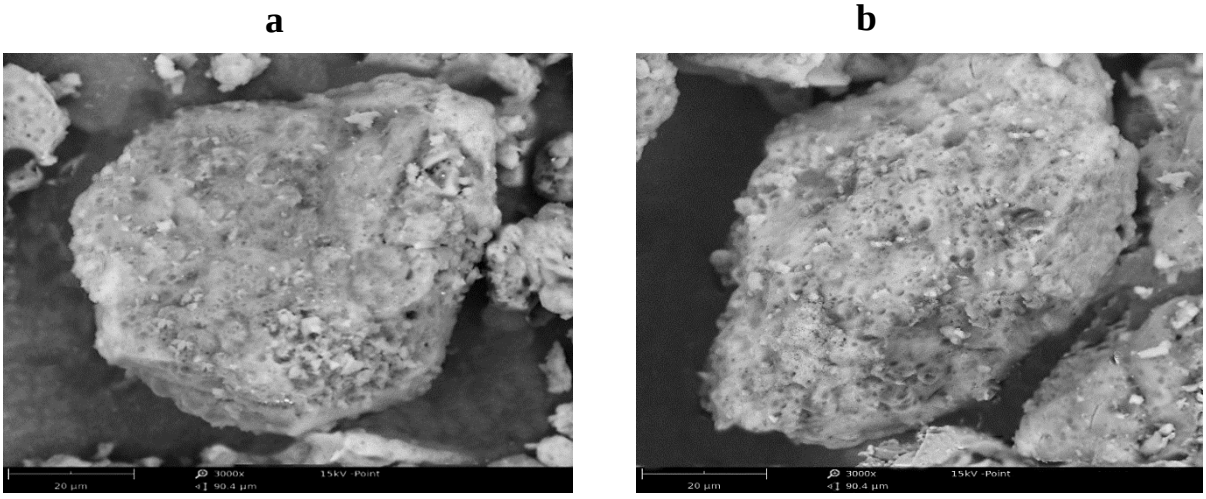

Figure 1. Scanning electron microscopy of both microcapsules at 3000× magnification. Microcapsules produced using gelation-pectin coacervation with. *Lactobacillus acidophilus* LA-5 (a). Microcapsules produced using gelation-Arabic gum coacervation with *Lactobacillus acidophilus* LA-5 (b)

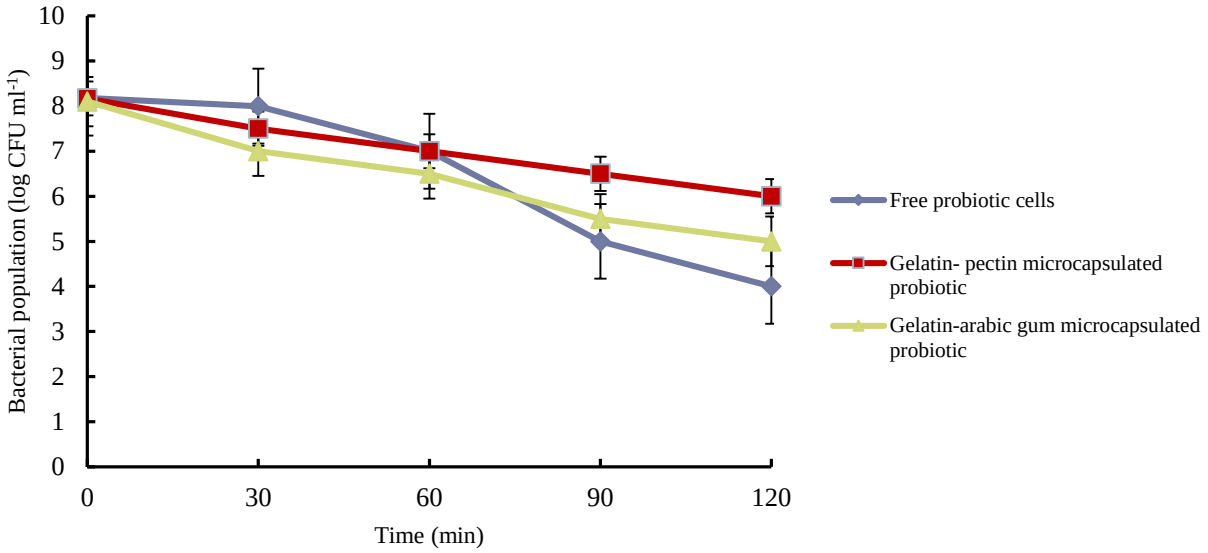

Figure 2. Cell survival of free suspended and encapsulated cells in simulated gastric juice

3. 4 Stability of free or microcapsules *L. acidophilus* LA-5 in simulated intestinal fluid

Figure 3 illustrates survival percentage of free and microencapsulated probiotic bacteria subjected to simulated intestinal fluid. After 1 h of incubation, viable cells encapsulated in gelatin-pectin decreased from 8.17 to 7.3 log CFU ml^{-1} and this decrease continued to 6.5 log CFU ml^{-1} after 2 h. For gelatin-Arabic gum microcapsules, cell counts of *L. acidophilus* LA-5 included 7.1 and 6.2 log CFU ml^{-1} after 1 and 2 h, respectively. It could be concluded that the latter microcapsules showed lower protection, compared to gelatin-pectin microcapsules. Both microcapsules included preservation activity of probiotic bacteria against simulated intestinal fluid, compared to free bacteria ($P<0.05$). Barrier forming due to encapsulation of *Bifidobacterium bifidum* F-35 into whey protein and protecting role of resulted microcapsules against bile salt and simulated intestine fluid damages has been reported [27].

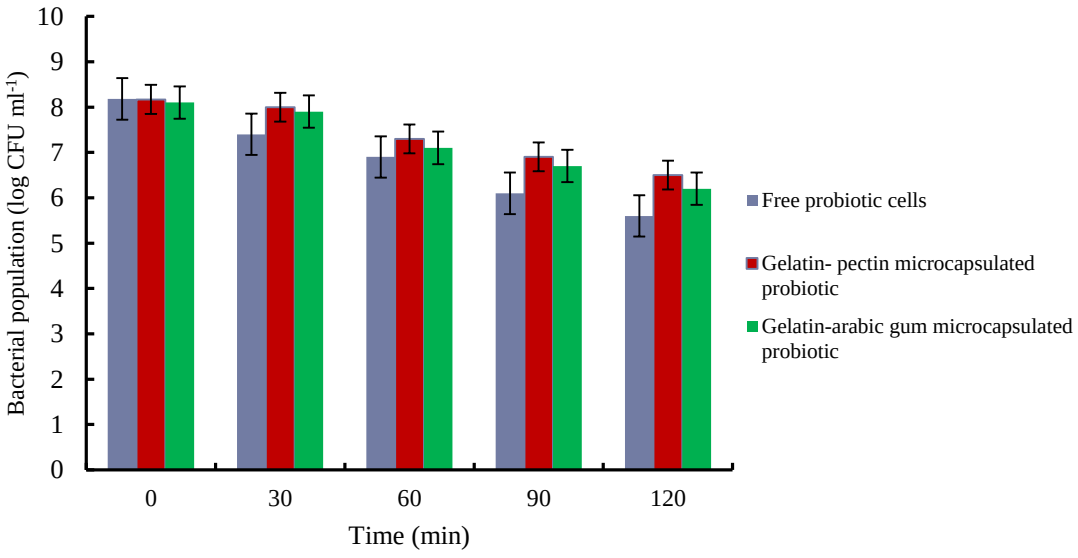

Figure 3. Cell survival of free suspended and encapsulated cells after treatment in simulated intestinal juice

3.5 Organoleptic assessment

Sensory characteristics of doogh samples were investigated after 1 and 35 days of cold storage (after confirming the product safety according to analysis methods for doogh published by the Iranian National Standards Organization [19]. As shown in Figure 4, no significant differences were seen between overall acceptability of the three doogh samples in Day 1 as well as between the samples after Day 35 of storage. Although, all doogh samples included lower scores after 35 days of storage than 1 day of storage; possibly due to losses in freshness of the product after a long storage. These results could contribute to sharp taste of camel milk, compared cow milk. No differences were reported between the samples at similar storage day.

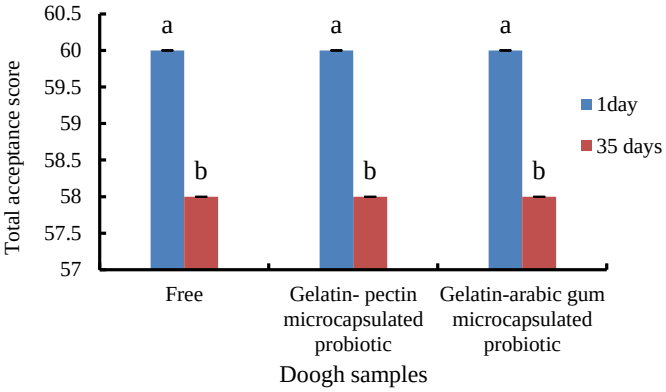

Figure 4. Sensory characteristics of doogh samples produced from camel milk after 1 and 35 days of refrigerated storage

4. Conclusion

In this study, microcapsules containing *L. acidophilus* LA-5 probiotic bacteria with two various wall materials (gelatin-pectin and gelatin-Arabic gum) were prepared. Results verified successful encapsulation of the probiotic bacteria based on high survival rate after encapsulation. Furthermore, results showed that embedding *L. acidophilus* LA-5 in microcapsules was an effective approach to improve the survival rate of probiotic cells under simulated gastric juice and intestinal fluid. In addition, doogh samples including encapsulated probiotic bacteria preserved functional properties during cold storage for 35 days. In conclusion, encapsulation with highlighted materials can be an efficient approach for the production of probiotic doogh from camel milk with no significant changes in sensory attributes.

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6. Conflict of Interest

None declared.

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بررسی ویژگی های نوشیدنی سنتی ایرانی (دوغ) حاصل از شیر شتر حاوی *لاکتوباسیلوس اسیدوفیلوس LA-5*

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چکیده

سابقه و هدف: زیست‌یارها اثرات مفید بر سلامتی انسان دارند، از جمله پایین آوردن کلسترول سرم، کاهش شیوع عفونت روده ای و فعالیت ضد سرطانی مواد غذایی. هدف از این مطالعه، بررسی خصوصیات نوشیدنی دوغ زیست‌یار تهیه شده از شیر شتر و پایداری *لاکتوباسیلوس اسیدوفیلوس LA-5* در طی زمان نگهداری در یخچال و همچنین در شرایط شبیه سازی دستگاه هاضمه بود.

مواد و روش ها: *لاکتوباسیلوس اسیدوفیلوس LA-5* با ژلاتین و پکتین حاوی تعداد زیادی گروه متوکسی[1](با درجه استری ٪۷۰) یا ژلاتین و صمغ عربی به عنوان ماده دیواره‌ای و به روش هم انباشتگی[2] ریزپوشانی شد. پایداری باکتری‌های زیست‌یار[3] در شرایط شبیه سازی شده معده‌ای-روده‌ای بررسی شد. پذیرش حسی نمونه‌ها با آزمون هدونیک (۹ امتیازی) در هنگام نگهداری سرد به مدت ۳۵ روز انجام شد.

یافته‌ها و نتیجه‌گیری: پس از ۳۵ روز نگهداری در یخچال، نمونه‌های دوغ حاوی *لاکتوباسیلوس اسیدوفیلوس LA-5* ریزپوشانی شده میزان زنده مانی بیشتری (به ترتیب ۷۷/۱۱ و ۷۴/۱۹ برای پکتین-ژلاتین و صمغ عربی-ژلاتین) نسبت به دوغ حاوی باکتری آزاد پروبیوتیک (۶۲/۳۴ درصد) داشتند. در شرایط تماس با مایع شبیه سازی شده معدی، لگاریتم جمعیت باکتریایی به ترتیب برای باکتری های آزاد، ریز پوشانی شده با پکتین- ژلاتین و صمغ عربی- ژلاتین به ۴، ۶ و ۵ رسید. در مورد تماس با مایع شبیه‌سازی شده روده‌ای، لگاریتم جمعیت باکتریایی به ترتیب برای باکتری‌های آزاد، ریزپوشانی شده با پکتین-ژلاتین، و صمغ عربی- ژلاتین به ۵/۶، ۶/۵ و ۶/۲ رسید. در ارزیابی حسی تفاوت معنی داری بین نمونه‌ها از نظر بو، ظاهر و پذیرش کلی نشان داده نشد. در نتیجه، دوغ تولید شده از شیر شتر حاوی *لاکتوباسیلوس اسیدوفیلوس LA-5* ریزپوشانی شده با پکتین-ژلاتین و صمغ عربی-ژلاتین در مقایسه با نمونه حاوی باکتری آزاد پایداری بیشتری در شرایط نگهداری سرد دارد. علاوه بر این، جمعیت نهایی زیست‌یار ریزپوشانی شده در مایع شبیه سازی شده روده‌ای بیشتر از 10^6 بود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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واژگان کلیدی

- شیر شتر
- *لاکتوباسیلوس اسیدوفیلوس*
- ریزپوشانی
- زیست‌یارها

***نویسنده مسئول**

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[1] high-methoxy pectin
[2] coacervation
[3] probiotic bacteria