International Food Research Journal 29(4): 879 - 891 (August 2022)
Journal homepage: *http://www.ifrj.upm.edu.my*

Association of carob galactomannans with probiotic bacteria in synbiotic fermented milk and colon targeted-release carrier

[1,3]*Ziar, H., [1]Yahla, I., [1,2]Sadoud, M., [1]Keddar, K., [2]Dilmi-Bouras, A., [1]Riazi, A. and [3]Gérard, P.

[1]*Laboratoire des Micro-Organismes Bénéfiques, des Aliments Fonctionnels et de la Santé (LMBAFS), Abdelhamid Ibn Badis University, Hocine Hamadou Street, Mostaganem 27000, Algeria*
[2]*Laboratoire de Bioressources Naturelles Locales, Hassiba Ben Bouali University, Ouled Fares N19, Chlef 02000, Algeria*
[3]*Micalis Institute, INRAE, AgroParisTech, Paris-Saclay University, 78350 Jouy-en-Josas, France*

Article history

Received:
25 August 2021
Received in revised form:
1 December 2021
Accepted:
16 February 2022

Keywords

raw carob bean gum (LBG), probiotic bacteria, fermentation, microencapsulation, targeted release

Abstract

For the first time, this study presented the use of raw locust bean gum (LBG) as a prebiotic, with one probiotic strain in synbiotic fermented milk or combined with Na-alginate as a biopolymer, for a targeted release of bacteria under colon-like conditions. For this purpose, the fermentative characteristics (biomass, pH), bacterial survival, and developed viscosities of the stored fermented milks were determined. The survival rates of microencapsulated bacteria using the emulsion technique under simulated gastrointestinal conditions (stomach: pH 2 + 0.3% pepsin; colon: pH 6.5 + 1% pancreatin + 0.3% bile) were also evaluated. Results showed that all the tested bacteria maintained better biomass and acidifying activities in the presence of LBG, especially at 2%. During cold storage, the viscosities of the LBG-fermented milks were regulated and better appreciated, especially at 2%. *Lactobacillus rhamnosus* LbRE-LSAS and *Bifidobacterium animalis* subsp. *lactis* Bb12 microencapsulated separately in Ca-alginate-raw carob gum maintained good survival rates (51 - 66%) as compared to free cells (21 - 59%) under simulated digestive conditions, and were released under colon-like conditions. Therefore, the formulation of LBG-enriched fermented milks containing probiotic bacteria could represent a very good candidate for industrial application. Ca-alginate-raw LBG beads for the specific release of probiotics in the colon could benefit consumers with celiac disease or other digestive disorders because LBG is naturally gluten-free.

DOI
https://doi.org/10.47836/ifrj.29.4.15

Introduction

In recent years, the growing popularity of foods that are supposed to be good for our health, also known as functional foods, has led to an explosion of probiotic fermented milk markets worldwide (Ávila *et al.*, 2020). In fact, several studies have been devoted to probiotics, and their results indicate their capacities to improve intestinal balance and prevent certain intestinal and inflammatory diseases (Srutkova *et al.*, 2015; Kerry *et al.*, 2018). At the same time, the application of probiotics in different food products has been widely studied. This interest is partly due to the growing awareness and concern about food allergies and intolerances. Nowadays, probiotics are introduced in a wide range of food products with probiotic claims, starting with mass-market dairy products such as yogurts, cheeses, or infant milk. However, two issues remain largely unresolved in the functional food industry, namely the viability of the probiotics used, and the organoleptic aspect of the product (Maurya *et al.*, 2014).

Loss in probiotic bacteria viability is a negative point that has been the focus of most scientific analyses (Maurya *et al.*, 2014; Champagne *et al.*, 2018; Neffe-Skocinska *et al.*, 2018). In fact, the integrity of microorganisms is subject to severe conditions, particularly the physicochemical treatments performed during the manufacturing of the functional foods. During consumption, the action of digestive enzymes, gastric acidity, and bile significantly reduce the survival rate of probiotic free cells; whereas to exert their beneficial effects,

*Corresponding author.
Email: hasnia.ziar@univ-mosta.dz

probiotics must survive at a minimum number of 10^6 viable cells per gram through the product shelf-life (Neffe-Skocinska *et al.,* 2018).

Several reports have indicated a low survival rate of probiotic bacteria in dairy products containing free cells (De Vos *et al.,* 2010). Therefore, it is essential to consider solutions to address the significant loss of viable cells during and after manufacturing. Microencapsulation of probiotics using biopolymers is one of the most promising and least expensive methods to overcome the problems posed by environmental adversity. This technology can preserve cell viability and metabolism, thus allowing more viable cells to enter the intestine. Recently, various scientific studies have reported using different encapsulation techniques, where emulsification seems to be the best to encapsulate probiotics properly. Alginate is still widely used to produce probiotic microparticles. However, there is increasing interest in its total or partial substitution by other polysaccharides such as gums and prebiotic compounds (Dokoohaki *et al*., 2019).

Probiotic-fermented milk products may exhibit textural defects and taste alterations such as insufficient gel firmness, changes in viscosity, and whey expulsion (syneresis) (Teggatz and Morris, 1990). Appropriately, in many formulations, hydrocolloids thickening and/or gelling agents compensate for the lack of texture due to reducing fat content. They improve consistency (increase viscosity) and reduce syneresis. The agents frequently used are gelatine, pectins, carrageenans, methylcellulose derivatives, gum Arabic, starches, and galactomannans. The latter are reserve polysaccharides found in the translucent endosperm of many legume seeds (Daas *et al.*, 2000) such as guar (*Cyamopsis tetragonoloba* (L.) Taub.), tara (*Caesalpinia spinose* Kuntze*),* and carob (*Ceratonia siliqua* L.).

The Food and Agriculture Organization (FAO) shows that the global production of carob fruit is about 158.61 t/year, produced from about 66.87 hectares, 13% of which is in Africa (FAOSTAT, 2016). The commercial application of carob bean gum is widely used, and many patents have described its use in foods such as jelly foods, baby foods, and others (EFSA, 2017). In addition, researchers have attempted to formulate carob-based milk drinks using pod syrups (Srour *et al.*, 2016).

However, to the best of our knowledge, there are no published data on the use of carob seed galactomannans as prebiotics. At the same time, a few studies have reported the prebiotic effect of fenugreek seed galactomannans (Majeed *et al.,* 2018) and guar beans (Zartl *et al.,* 2018). As a popular natural polymer, carob bean gum can also be used as a biopolymer to control biologically active agents' systemic or local delivery (Jana *et al.*, 2015).

Due to the high concentrations of galactomannans, fibres, and polyphenols in carob seeds, and to promote the use of carob tree products and by-products, raw locust or carob bean gum (LBG) was studied in the present work. First, we produced synbiotic fermented milk containing carob LBG as a prebiotic and associated with probiotic bacteria. Then, the aim was to ensure the effectiveness of the alginate-LBG mixture as a biopolymer for the targeted release of probiotic bacteria in the intestines.

Materials and methods

Bacterial strains

Lactobacillus rhamnosus LbRE-LSAS was a bacterial strain from the collection of beneficial microorganisms, functional foods, and health laboratory (LMBAFS, University of Mostaganem, Algeria). It was isolated from the faeces of a healthy three-week-old baby who had not received any antibiotic therapy, and was exclusively breastfed. *Bifidobacterium animalis* subsp. *lactis* was the probiotic reference strain, commercially known as Bb-12 (Chr. Hansen-Denmark). The yogurt starters used, *Lactobacillus delbrueckii* subsp. *bulgaricus* LB 340 and *Streptococcus thermophilus* TA 040 were purchased from Danisco (France).

All strain cultures were maintained at -70°C using Microbank cryovials (Pro-Lab Diagnostics, UK). Bacteria were serially propagated three times in the appropriate broth (Biomérieux, France; MRS with cysteine-HCl for LbRE-LSAS and Bb12, MRS for LB340, and M17 for TA040) before experimental use. A 1% inoculum was used, and incubation was performed at 37°C for 24 h under anaerobic conditions. In all experiments, cultures of each strain were taken at the exponential growth phase.

Carob seeds

Source of galactomannans, carob seeds, were obtained from pods harvested in the Zenata Forest (Tlemcen City, Algeria). They were stored in opaque, hermetically sealed bags in the dark and away from humidity.

Preparation of locust bean gum (LBG)

Three trials were conducted for the preparation of LBG. Raw and purified gums (in ethanol or isopropanol + acetone) were tested for their efficiency and synergy as prebiotic substrates for probiotic bacteria.

Raw carob bean gum

First, the carob pods were crushed, and the seeds were collected and sorted (Dakia *et al.*, 2008). Next, 100 g of carob seeds (780 seeds) were immersed in 800 mL of distilled water, and heated to 100°C for 1 h in a water bath. The seeds absorbed the water and swelled without destroying the integument envelopes. The seeds were then collected from the water and washed, the envelopes were separated manually, and the germ was removed after the division of the endosperms. The endosperms were dried at 100°C for 1 - 2 h to a constant weight, ground into fine particles, and sieved to obtain the raw gum flour (locust bean gum, LBG).

Purified carob bean gum

Two methods were used to obtain the purified LBG. In the first method, the mixed endosperm in the blender was filtered through a nylon mesh followed by centrifugation at 3,800 *g* (20 min, 20°C). Galactomannan precipitation was performed by adding the supernatant to absolute ethanol (purity 99.7%) in a ratio of 1:2. The ethanol was decanted, and the precipitated galactomannans were dried and stored in a desiccator until use. In the second method (Bouzouita *et al.*, 2007), 1.3 g of crude endosperm was added to 100 mL of distilled water, stirred gently for 2 h at room temperature, and stored overnight at 4°C. Then, the solutions were heated to 80°C in a water bath (30 min, continuous stirring).

The resulting solution was allowed to cool and centrifuged for 1 h at 7,500 *g* (20°C). The supernatant containing solubilised galactomannan was purified by pouring two volumes of isopropanol, and letting the mixture stand for 30 min. The white fibrous material was collected and filtered through a sieve (53 µm), and washed twice with isopropanol and acetone. After 48 h of freeze-drying, the resulting solid was ground into a fine powder. The galactomannan content was 84.7% in raw LBG (water extraction method) and 95% in purified LBG (isopropanol and acetone method). Their respective molecular weights were 523 and 862 KDa, as determined by the intrinsic viscosity method.

Fermentation of LBG by bacteria

For the milk fermentation assays, pasteurised (30 min, 80°C) solutions of raw LBG at final concentrations of 0, 0.1, 0.5, 1, 1.5, or 2% (w/v) were added to skim milk sterilised by tyndallisation. Then, 100 µL of a single 16-h bacterial culture (TA040, LB340, LbRE-LSAS, or Bb12) was added at a final load of 1×10^7 to 5×10^7 cells/mL. The inoculated milks were incubated at 37°C under anaerobic conditions (anaerobic jar with CO_2 generator system; Anaerocult). The fermentative activities of the bacteria were assessed after 24 h of incubation. The number of viable cells, determined three times, was calculated from the colonies obtained after incubation on the appropriate media, and was expressed as log CFU /mL. The acidifying activity developed by each strain in milk was estimated by measuring the pH with a digital pH meter (WTW pH meter 330; Weilheim, Germany).

Determination of bacterial survival in fermented milk stored at 4°C

Bacterial strain survival was determined weekly on a 1 g sample of fermented milk diluted at 1:10 with a sterile diluent solution (0.1% peptone, w/v), incubated on appropriate medium, and the colonies enumerated thereafter.

Measurement and monitoring of fermented skimmed milk viscosity

The viscosity developed in the fermented milk was measured after 1, 7, 14, 21, and 28 days of storage using a viscometer (SNB-1 digital viscometer; Brookfield Engineering Labs. Inc., USA) equipped with four rotating mobiles. The viscosity measurement was performed after the samples were initially stirred by hand for 1 min to obtain homogeneous samples for viscosity measurements. The viscosity was recorded every 60 s for 30 min, and the average of the last 10 measurements was given as the viscosity value of the sample.

Bacterial cell microencapsulation method

Only one concentration of raw LBG gum (among 0.1, 0.5, 1, 1.5, or 2%) based on the optimal micro efficiency value, and the two strains giving the best results with LBG as a prebiotic were tested for specifically targeted release under colon-like conditions. The emulsification technique described by Ziar *et al.* (2012) was followed. Briefly, sterile solutions of 1.8% sodium alginate and 0.1 M $CaCl_2$

were prepared. In a sterile beaker containing 100 mL of vegetable oil (sunflower and soybean), 1 mL of polysorbate 80 was added, and a mixture of bacterial culture (pellet washed once at 10 log CFU/g), sterile LBG solution (by boiling at 80°C/30 min), and sodium alginate solution were prepared. Microdroplets (using a 27.5G needle) were deposited from this mixture on the oil (stirred between 200 and 400 rpm for 20 min). Then, a $CaCl_2$ solution (100 to 200 mL) was added by pouring it on the walls until the emulsion broke. After 15 min of stirring, the formed calcium alginate-LBG microcapsules were separated from the aqueous phase, rinsed twice with sterile saline solution (0.9% NaCl + 5% glycerol), and stored in hermetically sealed sterile tubes at 4°C.

The concentration of sodium alginate was set at 1.8% (w/v) to optimise the concentration of carob bean gum. The microencapsulation process was performed using different concentrations of LBG solution, namely 0.1, 0.5, 1, 1.5, and 2% (w/v). The microencapsulation efficiency was used to determine the optimum concentration of carob bean gum and was calculated using Eq. 1:

$$\text{Microencapsulation efficiency (\%)} = (\text{Log N} / \text{Log N}_0) \times 100 \quad \text{(Eq. 1)}$$

where, N = number of microencapsulated probiotics released from the beads, and N_0 = number of probiotics in the initial microbial solution.

Survival under simulated digestive conditions (simulated gastrointestinal model)

The same protocol described by Ziar *et al.* (2012) was performed with the following modifications: survival of microencapsulated or free bacteria was evaluated in a simulated continuous digestive model at 37°C under anaerobic conditions, conditions similar to those in the stomach and colon. Samples of 0.5 g of free and microencapsulated cells were added to 4.5 mL of simulated gastric juice (SGJ, 37°C). SGJ consisted of a buffered solution of 0.02 M KCl-HCl with 0.3% pepsin, and the pH was adjusted to 2 using 1 M HCl.

Next, 0.5 g of washed bacterial pellets of free and microencapsulated cells that had completed their gastric residence were added to 4.5 mL of simulated intestinal juice (SIJ, 37°C). The SIJ consisted of 0.02 M phosphate buffer (PBS) with 1% porcine pancreatin, 0.3% bile salt, and the pH was adjusted to 6.5 using 1 M NaOH.

For viable biomass studies, aliquots at different gastric exposure times were taken from the vessel's bottom using a selected laboratory tool (diameter = 3.5 mm). Counting was performed on MRS-cys agar after lysis in sodium phosphate buffer (PBS, pH = 7, 0.2 M, vigorous stirring for 20 min at 4°C).

Another count was performed on MRS-cys agar without lysis in PBS to estimate the integrity of the beads. For SIJ samples, the bacterial pellet was collected. A count was performed on MRS-cys agar without lysis in PBS. The survival rates were determined using Eq. 2:

$$\text{Survival rate (\%)} = (\text{Initial load of entrapped bacteria CFU/g} - \text{load of entrapped bacteria at T Time level of exposure}) / \text{Initial load of entrapped bacteria CFU/g} \times 100 \quad \text{(Eq. 2)}$$

Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA) using STATISTICA software (Data Analysis Software System, v. 8.0; StatSoft Inc., Tulsa, OK, USA). Difference was considered significant at $p < 0.05$. The main sources of variation, which were considered statistically significant, were compared using the LSD (least significant difference) test. All results represent the means of three independent experiments ($n = 9$).

Results and discussion

Fermentative activities of bacteria in the presence of LBG

Both purified LBG failed to fulfil the prebiotic property since other bacteria could use them as the sole carbon source. With LBG extracted from water, our results (unpublished results) revealed an inhibitory effect against pathogens. However, this inhibitory effect was moderate for lactic acid bacteria of dairy origin, and absent for probiotic bacteria isolated from the intestines. The objective of the present work was achieved with raw LBG and not with purified LBG.

Results for the fermentative activities of *Lactobacillus rhamnosus* LbRE-LSAS, *Bifidobacterium animalis* subsp. *lactis* Bb12, *Lactobacillus delbrueckii* subsp. *bulgaricus* LB340, and *Streptococcus thermophilus* TA040 were expressed in terms of biomass (log CFU /mL) (Figure 1) and pH (Figure 2) recorded after 24 h at 37°C under

anaerobic conditions. After 24 h in the presence of LBG, all strains showed an increase in biomass (Figure 1) where +0.25, +0.64, +1.26, and +1.24 log units were monitored in control fermented milks with LbRE-LSAS, Bb12, TA040, or LB340 strain, respectively.

With 0.1% LBG (Figure 1), LbRE-LSAS reached a high biomass ($p < 0.05$) of 8.35 log CFU /mL (an increase of +0.57 log unit) as compared to Bb12, TA 040, and LB340; where 8.19, 7.94, and 8.05 log CFU /mL were recorded, respectively. By increasing the concentration of added LBG to 0.5% (Figure 1), LbRE-LSAS and Bb12 strains showed better biomasses ($p < 0.05$) of 8.06 and 8.14 log CFU /mL, respectively.

However, TA040 and LB340 were attenuated (-0.22 and -1.05 log cycle, respectively). The moderate biomasses accumulated with *S. thermophilus* and *L. bulgaricus* monocultures enriched with 0.5% LBG could be related to the prolonged lag phase (as compared to their respective control cultures) observed for both yogurt starters. Milk coagulation occurred after up to 11 h of fermentation. Since the prebiotic used in the present work was raw gum, we suggest that yogurt bacteria's lactic acid production might be related to their preferred protein assimilation from both origins, milk and raw LBG (4.8%), rather than sugar fermentation.

With 1% LBG (Figure 1), Bb12 and TA040 reached the highest biomasses ($p > 0.05$), 8.29 and 8.15 log CFU /mL, respectively, while LbRE-LSAS showed high biomass similar to that recorded with 0.1% LBG. The *Lactobacillus* LB340 starter appeared to be slightly ($p > 0.05$) attenuated (-0.03 log unit). One reason for this behaviour could be the composition of the raw carob flour which is rich in total polyphenols (19 mg/g fresh matter), condensed (2.75 mg/g), and water-soluble tannins (0.5 mg/g). These compounds are known for their antimicrobial activity (Avallone *et al.,* 1997).

With the 1.5% LBG concentration (Figure 1), strains LbRE-LSAS, Bb12, TA040, and LB340 showed an increase ($p < 0.05$) of +0.18, +1.12, +0.74, and +0.65 log units, respectively (from 0 to 24 h). With 2% LBG (Figure 1), the increase in biomass was approximately +0.5 log units regardless of strain ($p < 0.05$), despite a slight decrease of -0.13 log cycles in the biomass of TA040. This result reflected the consumption of LBG used as a prebiotic.

The best-known prebiotics on the market are inulin and fructooligosaccharides, but legumes are interesting sources of prebiotics, which can be used by bifidobacteria and lactobacilli. Carob seeds are composed mainly of galactomannans, highly polydisperse linear polysaccharides consisting of a β-(l-4)-mannosyl backbone with a single D-galactopyranosyl unit attached via α-(l-6) linkages as a side branch. Galactomannan polysaccharides can be obtained from carob beans by aqueous or aqueous alkaline extraction. Their content in the seeds can reach 85% (Dakia *et al.,* 2008). In the present work, the ratio of protein, crude fibre, fat, and galactomannan in carob bean gum powder was 4.8%:1.17%:0.43%:84.7%, respectively.

Galactomannans are complex polysaccharides not easily fermented by common bacteria. However, some probiotic bacteria possess the enzymatic ability to break down galactomannans. In a recent study by Zartl *et al.* (2018), seven out of 20 selected probiotic strains could metabolise guar galactomannans. In fact, *S. salivarius* subsp. *thermophilus* metabolised all tested oligosaccharides well, including guar oligosaccharides. In the same study, *L. rhamnosus* GG and *B. animalis* subsp. *lactis* Bb12 showed the highest fermentation rate of guar oligosaccharides. Similar observations were obtained in the present work, while in a previous study (Ziar *et al.*, 2014), we demonstrated that our strains could ferment many different prebiotic carbohydrates.

The pH results recorded after 24 h of fermentation of LBG-enriched milks are shown in Figure 2. The recorded values showed a decrease ($p < 0.05$) in pH proportional to the increase in LBG concentration in all the formulated fermented milks. The milk acidification indicated that all the strains retained their acidifying activities in the presence of LBG as compared to their optimal pH reference values of 4.2 to 5.8, recorded after 24 h in MRS-glucose (0.5%) as the sole carbon source (Ziar *et al.*, 2014).

Despite this, the most acidic values were recorded with the probiotic strain Bb 12, which showed very low pH values ($p < 0.05$) below 5 (from 4.88 to 4.76), when LBG was added at a concentration greater than or equal to 1% (Figure 2). As compared to the rest of the strains tested, Bb12 appeared to retain ($p > 0.05$) the same acidifying activity when the LBG concentration was increased from 1 to 1.5% (Figure 2).

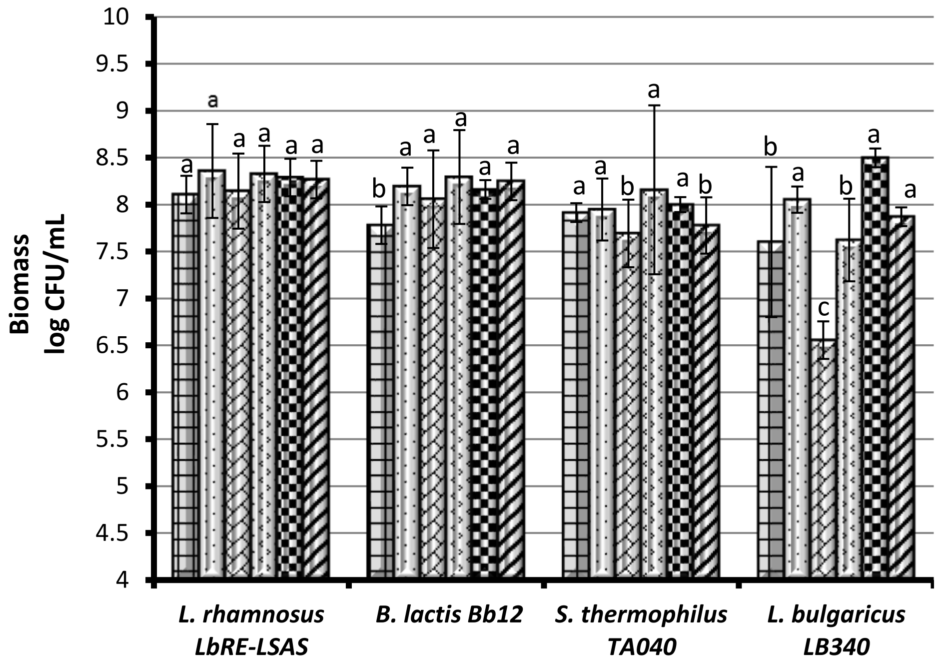

Figure 1. Biomass (log CFU/mL, 24 h/37°C) registered in milks fermented with *L. rhamnosus* LbRE-LSAS, *B. lactis* Bb12, *S. thermophilus* TA040, or *L. bulgaricus* LB340, containing raw carob bean gum (LBG) at final concentrations (w/v) of 0 (▦), 0.1 (⬚), 0.5 (▩), 1 (⬚), 1.5 (▩), or 2% (▨). a-cStatistically different according to LSD test.

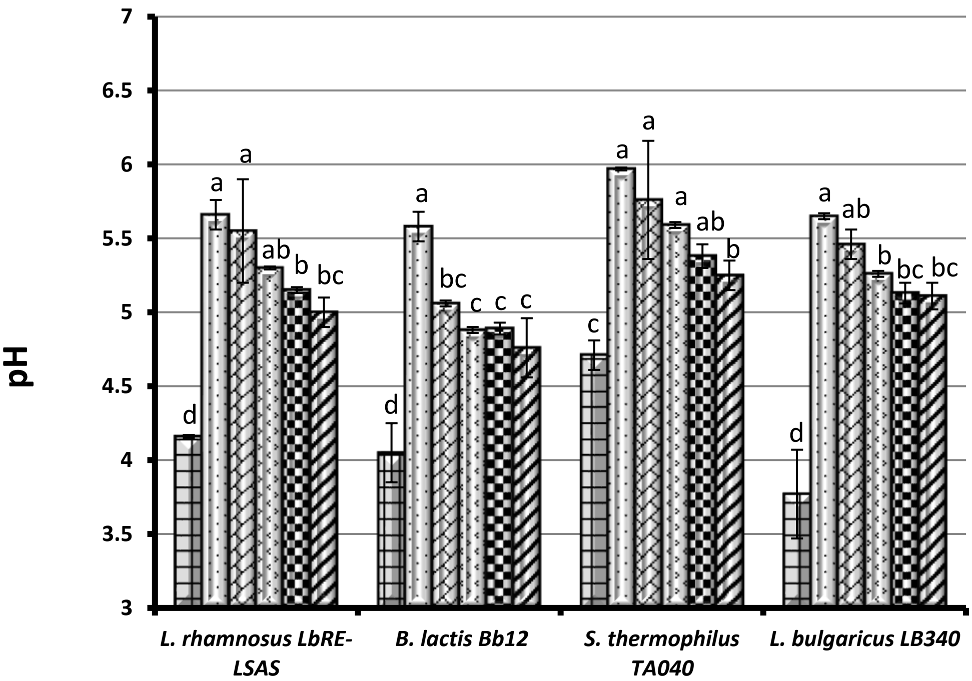

Figure 2. pH recorded after 24 h fermentation of milks with *L. rhamnosus* LbRE-LSAS, *B. lactis* Bb12, *S. thermophilus* TA040, or *L. bulgaricus* LB340, containing raw carob bean gum (LBG) at final concentrations (w/v) of 0 (▦), 0.1 (⬚), 0.5 (▩), 1 (⬚), 1.5 (▩), or 2% (▨). a-dStatistically different according to LSD test.

The acidifying activity of all strains reached its maximum ($p < 0.05$) in the presence of 2% LBG, but remained less pronounced as compared to the controls where the lowest pH value was recorded with the bifidobacterial strain Bb12 (4.76; 2% LBG). The recorded pH values could be due to the buffering action caused by the carob proteins, and the increase in solids from the carob gum supplementation (Al-Dabbas *et al.*, 2010). Jrad *et al.* (2020) reported that camel yogurt with 2% carob powder had the highest pH value as compared to plain yogurt. These results corroborate our results, where control fermented milks with Bb 12, LbRE-LSAS, LB340, or TA040 showed better acidifying activities ($p < 0.05$) than LBG-enriched fermented milks.

Viability

The viability of bacteria assessed in stored fermented milks containing LBG is presented in Figure 3 as viable log CFU/g. A gradual decrease in the number of viable cells was recorded week by week throughout the cold storage for all strains. However, it is recognised that for probiotic bacteria, a minimum number of 10^7 viable cells per gram of product is required to exert their health benefits (Knorr, 1998).

On the first day of storage, and in the absence of LBG, the beneficial strain Bb12 recorded a loss of viability of -0.66 log cycles (7.12 log CFU/g viable cells). This loss gradually increased during the storage period, and the level of viable cells remained satisfactory until the 7th day. After that, it decreased significantly to 4.26 log CFU/g on the 28th day of storage (Figure 3).

In the present work, carob galactomannans significantly improved ($p < 0.05$) the viability of Bb12 in cold-stored fermented milks. Indeed, the loss of viable cells was slightly increased in the presence of LBG. At the end of storage, we recorded 4.66, 5.57, 6.49, 6.81, and 7.21 log CFU/g of viable cells in the presence of 0.1, 0.5, 1, 1.5, and 2% LBG, respectively (Figure 3).

In general, the viability of Bb12 at the end of storage was 57, 69.2, 78.3, 83.4, and 87.5% recorded in the presence of 0.1, 0.5, 1, 1.5, and 2% LBG, respectively, while it was only 54.76% in the absence of this polymer. The same observations were also highlighted in the fermented milks containing LbRE-LSAS. On the other hand, it maintained higher viability rates ($p < 0.05$) as compared to the bifidobacterial strain. By the last week of refrigerated storage (28th day), the viability of the LbRE-LSAS strain still exceeded 90% in the presence of 2% LBG. We also calculated improvement rates of 14 - 28% (depending on the concentration of added LBG) in the remaining enriched fermented milks as compared to the control. Similar trends were also reported in a recent study by Pereira *et al.* (2020), where probiotic bacteria *L. acidophilus* LA-5 and *B. lactis* Bb12 showed high viability during 28 days of storage, with counts above 6 log CFU/g in 5% inulin hydrolysed fermented milks.

For yogurt starters, the number of viable cells at the end of storage was 5.13 and 7.17 log CFU/g for *L. bulgaricus*, 5.23 and 7.19 CFU/g for *S. thermophilus*, recorded in the presence of 0.5 and 2% LBG, respectively (Figure 3), as compared to only 5.28 and 5.46 log CFU/g in their respective control fermented milks without LBG addition. Similar results were observed in a recent study (Jrad *et al.*, 2020), where *S. thermophilus* maintained appropriate levels (8 log CFU/g) in ultrafiltered camel yogurt with 2% carob pod powder for three weeks at 4°C.

From our results, we noted that the viability of bacteria improved ($p < 0.05$) by the addition of 1 - 2% galactomannans from carob seeds, maintaining a number of viable cells exceeding the FAO/WHO required level of 6 log CFU/g after four weeks of refrigerated storage (Neffe-Skocinska *et al.*, 2018).

Effect of carob galactomannans on viscosity of fermented milks

The changes in viscosity of the fermented milks during 28 days of storage at 4°C are listed in Table 1. The initial viscosity of the fermented milk (1 day) depended on the LBG content added to the skim milk. It ranged from 1014 to 1080 mPa.s in the absence of LBG, and reached 1014 to 1044, 1015 to 1075, 1088 to 1195 ($p < 0.05$), 1322 to 1520 ($p < 0.05$), and 1399 to 1582 ($p < 0.05$) mPa.s, followed in fermented milk containing 0.1, 0.5, 1, 1.5, and 2% LBG, respectively (Table 1).

The variation in viscosity during the storage period can be attributed to syneresis, protein hydration, and exopolysaccharide production (Aliakbarian *et al.*, 2016). In the present work, viscosity values increased ($p < 0.05$) in fermented milks containing strain TA040 and 0, 0.1, or 0.5% LBG from the second week. However, fermented milks containing the same strain and 1, 1.5, or 2% LBG had higher viscosity values until the 14th day, and decreased thereafter (Table 1).

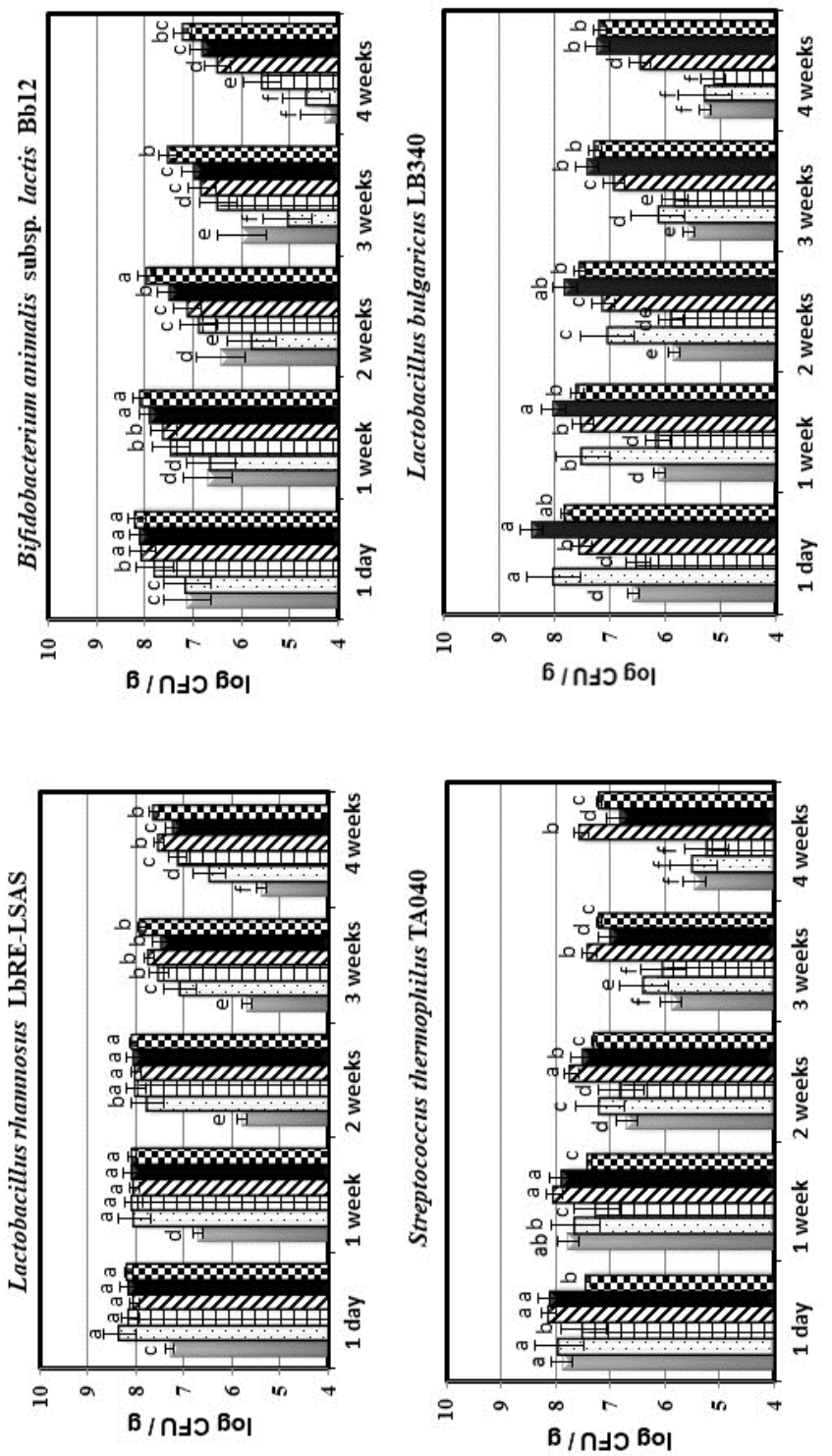

Figure 3. Viability (log CFU/g, 4°C) of *L. rhamnosus* LbRE-LSAS, *B. lactis* Bb12, *S. thermophilus* TA040, or *L. bulgaricus* LB340 in stored fermented milks containing raw carob bean gum (LBG) at final concentrations (w/v) of 0 (), 0.1 (), 0.5 (), 1 (), 1.5 (), or 2% (). [a-f]Statistically different according to LSD test.

Table 1. Evolution of the viscosity values (mPa.s) in LBG-enriched fermented milks stored at 4°C.

LBG (w/v, %)	Fermented skim milk containing one strain	1 day	7 days	14 days	21 days	28 days
0	*S. thermophilus* TA040	1080 ± 0.1^{cA}	1100 ± 0.91^{bA}	1300 ± 0.1^{bB}	1400 ± 0.3^{cC}	1500 ± 0.4^{cD}
	L. bulgaricus LB340	1060 ± 0.01^{bA}	1060 ± 0.21^{aA}	1080 ± 0.01^{aB}	1067 ± 0.22^{bA}	1068 ± 0.03^{bA}
	B. lactis Bb12	1014 ± 0.2^{aA}	1057 ± 0.3^{aB}	1066 ± 0.02^{aB}	1047 ± 0.02^{aB}	1028 ± 0.01^{aA}
	L. rhamnosus LbRE-LSAS	1041 ± 0.14^{bA}	1071 ± 0.4^{aB}	1078 ± 0.02^{aB}	1043 ± 0.11^{aA}	1019 ± 0.02^{aA}
0.1	*S. thermophilus* TA040	1047 ± 0.21^{bA}	1119 ± 0.7^{cB}	1170 ± 0.1^{bC}	1337 ± 0.3^{bD}	1432 ± 0.02^{cE}
	L. bulgaricus LB340	1014 ± 0.7^{aA}	1032 ± 0.4^{aA}	1160 ± 0.11^{bB}	1320 ± 0.1^{bC}	1360 ± 0.06^{bD}
	B. lactis Bb12	1020 ± 0.4^{aA}	1051 ± 0.1^{aB}	1070 ± 0.02^{aB}	1039 ± 0.24^{aA}	1020 ± 0.02^{aA}
	L. rhamnosus LbRE-LSAS	1044 ± 0.11^{bA}	1080 ± 0.1^{bB}	1064 ± 0.02^{aB}	1041 ± 0.12^{aA}	1022 ± 0.07^{aA}
0.5	*S. thermophilus* TA040	1054 ± 0.02^{bA}	1120 ± 0.1^{cB}	1200 ± 0.5^{cC}	1340 ± 0.5^{bD}	1440 ± 0.03^{cE}
	L. bulgaricus LB340	1015 ± 0.1^{aA}	1040 ± 0.2^{aB}	1180 ± 0.14^{cC}	1330 ± 0.33^{bD}	1380 ± 0.13^{bE}
	B. lactis Bb12	1045 ± 0.87^{bA}	1075 ± 0.2^{bB}	1066 ± 0.7^{aAB}	1055 ± 0.4^{aA}	1041 ± 0.4^{aA}
	L. rhamnosus LbRE-LSAS	1075 ± 0.44^{cA}	1145 ± 0.3^{cC}	1102 ± 0.21^{bB}	1075 ± 0.2^{aA}	1014 ± 0.22^{aA}
1	*S. thermophilus* TA040	1195 ± 0.65^{cB}	1171 ± 0.22^{cA}	1166 ± 0.22^{cA}	1161 ± 0.04^{cA}	1152 ± 0.06^{cA}
	L. bulgaricus LB340	1088 ± 0.12^{aB}	1089 ± 0.14^{aB}	1071 ± 0.04^{aB}	1032 ± 0.04^{aA}	1034 ± 0.02^{aA}
	B. lactis Bb12	1143 ± 0.7^{bB}	1149 ± 0.11^{bB}	1129 ± 0.05^{bA}	1126 ± 0.06^{bA}	$1137 \pm 0.2b^{cAB}$
	L. rhamnosus LbRE-LSAS	1135 ± 0.33^{bAB}	1144 ± 0.1^{bB}	1124 ± 0.07^{bA}	1121 ± 0.06^{bA}	1113 ± 0.1^{bA}
1.5	*S. thermophilus* TA040	1322 ± 0.66^{aAB}	1334 ± 0.08^{aB}	1323 ± 0.04^{bA}	1321 ± 0.03^{bA}	1308 ± 0.05^{bA}
	L. bulgaricus LB340	1337 ± 0.1^{aB}	1327 ± 0.3^{aB}	1301 ± 0.01^{aA}	1280 ± 0.01^{aA}	1278 ± 0.03^{aA}
	B. lactis Bb12	1322 ± 0.1^{aB}	1376 ± 0.1^{bC}	1342 ± 0.04^{bB}	1318 ± 0.1^{bB}	1248 ± 0.02^{aA}
	L. rhamnosus LbRE-LSAS	1520 ± 0.12^{bC}	1525 ± 0.1^{cC}	1488 ± 0.03^{cB}	1457 ± 0.01^{cB}	1367 ± 0.02^{cA}
2	*S. thermophilus* TA040	1572 ± 0.11^{bA}	1575 ± 0.1^{bA}	1573 ± 0.02^{bA}	1562 ± 0.4^{cA}	1558 ± 0.01^{cA}
	L. bulgaricus LB340	1402 ± 0.2^{aB}	1415 ± 0.2^{aB}	1400 ± 0.02^{aB}	1376 ± 0.1^{aA}	1360 ± 0.01^{aA}
	B. lactis Bb12	1399 ± 0.3^{aA}	1423 ± 0.2^{aA}	1429 ± 0.02^{aA}	1427 ± 0.5^{bA}	1422 ± 0.11^{bA}
	L. rhamnosus LbRE-LSAS	1582 ± 0.2^{bA}	1586 ± 0.3^{bA}	1554 ± 0.4^{bA}	1546 ± 0.2^{cA}	1539 ± 0.02^{cA}

[a-c] Comparisons were made between strains in the same column for a particular LBG concentration at T time of storage. [A-E] Comparisons were made for each strain in the same row for a particular LBG concentration at different T times of storage.

On the 28th day of cold storage, we recorded a decrease ($p < 0.05$) in viscosity values in all LBG-enriched fermented milks containing LbRE-LSAS or Bb12 (except for a non-significant increase ($p > 0.05$) observed with 1% LBG) as compared to the viscosity values recorded on the 21st day. Fermented milks containing Bb12 or LbRE-LSAS and 0, 0.1, or 0.5% LBG had the lowest viscosity values at the end of storage ($p < 0.05$) (Table 1). Ünal *et al.* (2003) reported that cold storage could significantly influence the viscosity and syneresis of LBG-enriched fermented dairy products. In fact, in the present work, viscosity decreased when LBG concentration was higher than 2%, while syneresis increased.

In the present work, the 2% LBG-enriched fermented milks containing *S. thermophilus* TA040 showed the highest ($p < 0.05$) viscosity value (> 1500 mPa.s) after 28 days of storage. A similar value ($p > 0.05$) was also recorded in the 2% LBG-enriched fermented milks containing the LbRE-LSAS strain (Table 1). Jrad *et al.* (2020) pointed out that 2% carob pod powder provided lower syneresis values in ultrafiltered dromedary yogurt throughout 21 days of storage.

In general, the presence of carob galactomannans had a very positive effect on the viscosity of fermented milk. The firmness remained stable during storage (unpublished results). This could be attributed to protein aggregates formed by the interaction via intermolecular disulphide bonds of casein micelles with denatured whey proteins. Park *et al.* (2019) demonstrated that the water absorption capacity increased with the addition of carob bean gum. The authors stated that the xanthan and carob supplemented yogurt for the experimental goat yogurt

showed significantly ($p < 0.05$) better consistency than the control and other gum added yogurts. Interestingly, according to Koksoy and Kilic (2004), locust bean gum has synergistic effects with other stabilisers in decreasing serum separation and increasing viscosity.

In the present work, adding this polysaccharide did not contribute to any undesirable flavour or taste to the finished product (unpublished results). This result is in good agreement with the work of Teles and Flores (2007). The authors reported that, unlike locust bean gum, a high concentration of xanthan or guar gum reduces the organoleptic acceptability of the product.

Optimisation of carob bean gum concentration and choice of strains for microencapsulation

In summary, with regard to fermentation abilities and viscosity test, LbRE-LSAS and Bb 12 strains gave the best results ($p < 0.05$) with 2% LBG, which are interesting as compared to yogurt starters (LB340 and TA040). Moreover, the results suggested that fermented milks containing carob gum could be sufficiently complete and rich in nutrients to allow the strains' good growth and metabolic activity. All these combined parameters favoured the selection of *L. rhamnosus* LbRE-LSAS and *B. animalis* subsp. *lactis* Bb 12 as excellent candidates for microencapsulation with LBG.

It was found that when the concentration of carob bean gum increased from 0.5 to 1.5% (w/v), the microencapsulation efficiency of the beads decreased from 94.98 to 90.37%. However, from 1.5 to 2% (w/v) LBG, the microencapsulation efficiency of beads increased from 90.37 to 97.22% ($p < 0.05$). Thus, the 2% carob bean gum was chosen to prepare the Ca-alginate-LBG bio-carrier of probiotic bacteria (bead size = 329 µm on average). Gul and Dervisoglu (2017) reported that the microencapsulation efficiency ranged from 86.71 to 95.25%, and increased with the alginate concentration in the emulsification method. The authors also concluded that 2% alginate could effectively encapsulate the probiotic *Lactobacillus casei* Shirota (LCS).

Survival rates of microencapsulated probiotic bacteria under continuous simulated gastrointestinal conditions

Under gastric like-conditions

The effects of simulated gastric conditions on bacterial survival rates were evaluated at 0, 30, 60, and 120 min residence time intervals. LbRE-LSAS and Bb12 strains (microencapsulated or free) were subjected to pepsin (3 g/L) combined with pH 2 acidity simulating gastric conditions (Table 2). At 0 min of exposure, the viable load of LbRE-LSAS was more affected in the free state than in the microencapsulated state, with respective survival rates of 79.5 and 96% (Table 2). After 30 min, the viability of microencapsulated LbRE-LSAS further decreased by one log unit, and the survival rates remained almost the same until 60 min of exposure. For free cells, however, biomass was virtually stable ($p > 0.05$) until 30 min of exposure (77.8%), after which we noticed a sharp ($p < 0.05$) drop in cell viability (51.1%) when the stay was extended to 60 min. Subsequently, 67 and 49.1% survival rates were recorded in microencapsulated and free LbRE-LSAS cells, respectively, at the end of 120 min of residence in simulated gastric conditions (Table 2).

Table 2. Survival rates (%) of *B. lactis* Bb12 and *L. rhamnosus* LbRE-LSAS under continuous digestive simulated conditions.

Time of residence	*B. lactis* Bb12		*L. rhamnosus* LbRE-LSAS	
	Free	Microencapsulated	Free	Microencapsulated
Gastric simulated condition (pH 2 + pepsin)				
0 min	90.68^{bD}	94.66^{bC}	79.5^{aC}	96.06^{cD}
30 min	82.28^{bC}	93.77^{cC}	77.81^{aC}	86.25^{bC}
60 min	80.15^{bC}	82.54^{bB}	51.1^{aB}	83.23^{bC}
120 min	74.53^{bB}	82.44^{cB}	49.14^{aB}	67^{bB}
Intestinal simulated condition (0.3% bile + pancreatin)				
16 h	59.4^{cA}	66.13^{dA}	21.28^{aA}	50.68^{bA}

Means followed by different superscripts differ statistically at $p < 0.05$; $^{a-d}$statistically different in the same row; $^{A-D}$statistically different in the same column.

With strain Bb12 (Table 2) and from pepsin and gastric acid exposure (0 min), 94.6 and 90.6% ($p > 0.05$) viable cells were recorded for microencapsulated and free Bb12, respectively. At 30 min, microencapsulated biomass remained stable (93.7%), while that of free cells dropped ($p < 0.05$) (82.3%). At 120 min, the survival rate of microencapsulated cells was about 82.4%, while that in the free state did not exceed 75%. Therefore, the mixed Ca-alginate-galactomannan gel seems to effectively mitigate the harmful effects of our similar gastric conditions, managing to maintain an acceptable and high level of viable cells.

Ding and Shah (2009) performed microencapsulation of several probiotic bacteria with carob gum as a matrix. The probiotic strain Howaru *rhamnosus* showed a biomass loss in the simulated gastric medium (120 min at pH 2), corresponding to 5.91 log units as compared to the initial biomass. Using a mixed Ca-alginate-galactomannan gel, our microencapsulation technique allowed a loss of only 2.9 log units in *L. rhamnosus* LbRE-LSAS. These results reflected the effectiveness of the protective aspect of galactomannans and the integrity of the microencapsulated cells.

Under intestinal like-conditions

After the gastric conditions, the bacteria were transferred to simulated intestinal conditions under the action of bile (0.3%, w/v) and 1% pancreatin (w/v) for 16 h. At this level, LbRE-LSAS free cells drastically lost viable biomass ($p < 0.05$). The survival rate was about 21.3%, calculated against the viable biomass persisting at the end of the gastric stay. On the other hand, the viable biomass of microencapsulated LbRE-LSAS cells remained higher, with a survival rate of 50.7% (Table 2). The Bb12 strain showed a similar decrease in viability ($p > 0.05$) for free and microencapsulated cells. The survival rates were 59.4 and 66.1%, respectively, calculated against the viable biomass recorded after 120 min of gastric stay (Table 2).

In general, the microencapsulated cells were more resistant ($p < 0.05$) to digestive hostility than the free state. Furthermore, the probiotic strain Bb12 appeared to be more resistant ($p < 0.05$) to these hostile conditions than the isolated strain LbRE-LSAS. The biomass required for a probiotic strain to produce a beneficial effect must be greater than 6 log CFU/mL. In the present work, only strain Bb12 met this requirement when microencapsulated in raw LBG-Ca-alginate beads, and was therefore considered capable of producing a beneficial effect. In our previous work, both Bb12 and LbRE-LSAS strains displayed more than 7.5 log CFU/mL at the end of the simulated gastrointestinal conditions. However, this could be due to the buffering effect generated by the simulated food bolus on the microcapsules used (Ziar *et al.,* 2012).

In the present work, all microcapsules containing bacteria dissolved after 16 h under intestinal conditions. Moreover, β-mannanase and other enzymes are present in the human colon which could lead to *in vivo* carob bean gum degradation (Jain *et al.,* 2007).

Conclusion

As a natural product, carob is not only beneficial to human health, but also of great economic and environmental importance, and more attention should be paid to it. The synergistic combination of probiotic bacteria and LBG gum as a prebiotic is still little explored. The present work investigated the prebiotic quality of raw carob gum with two yogurt starters and two probiotic strains with approved cholesterol assimilation activities. All fermented carob milks containing one strain showed improved growth abilities ($p < 0.05$). Probiotic counts in 1 or 2% raw LBG stored fermented milks were above the recommended level (6 log CFU/g), and significantly ($p < 0.05$) higher than controls. Similarly, viscosity measurements are related to survival counts in LBG-enriched yogurt, and thus can be developed as an effective functional food by improving physicochemical characteristics such as apparent viscosity.

We also used raw LBG in combination with sodium alginate to generate a protective vehicle for the colon. Under simulated digestive conditions, our results indicated that 67 - 82% of viable probiotic cells were recorded after gastric transit, and 51 - 66% in the colon when protected in raw LBG-Ca-alginate beads. The LBG constituents of carob seeds will be further characterised in our future research. Clinical studies on *in vivo* physiological benefits are also needed to confirm a prebiotic status. The present work is considered a preliminary evaluation, and may also provide evidence for formulating synbiotic fermented milk containing carob seed

galactomannans and the appropriate probiotic strain. Due to the low cost and simplicity of the process, our suggested emulsion technique could be suitable for large-scale production of microcapsules in the food and medical industries, and potentially for delivery of probiotics to the gut.

Acknowledgement

The present work is the first part of a bigger project registered under the grant "PRFU" (grant no.: D00L01UN270120190003) funded by the Algerian Ministry of Higher Education and Scientific Research.

References

Al-Dabbas, M. M., Al-Ismail, K., Abou Taleb, R. and Ibrahim, S. 2010. Acid-base buffering properties of five legumes and selected food *in vitro*. American Journal of Agricultural and Biological Sciences 5: 154-160.

Aliakbarian, B., Bagnasco, L., Perego, P., Leardi, R. and Casale, M. 2016. UV-VIS spectroscopy for monitoring yogurt stability during storage time. Analytical Methods 8: 5962-5969.

Avallone, R., Plessi, M., Baraldi, M. and Monzani, A. 1997. Determination of chemical composition of carob (*Ceratonia siliqua*): Protein, fat, carbohydrates, and tannins. Journal of Food Composition and Analysis 10: 166-172.

Ávila, B. P., da Rosa, P. P., Fernandes, T. A., Chesini, R. G., Sedrez, P. A., de Oliveira, A. P. T., ... and Buttow Roll, V. F. 2020. Analysis of the perception and behaviour of consumers regarding probiotic dairy products. International Dairy Journal 106: article ID 104703.

Bouzouita, N., Khaldi, A., Zgoulli, S., Chebil, R., Chekki, R., Chaabouni, M. M. and Thonard, P. 2007. The analysis of crude and purified locust bean gum: A comparison of samples from different carob tree populations in Tunisia. Food Chemistry 101: 1508-1515.

Champagne, C. P., Gomes da Cruz, A. and Daga, M. 2018. Strategies to improve the functionality of probiotics in supplements and foods. Current Opinion in Food Science 22: 160-166.

Daas, P. J. H., Schols, H. A. and de Jongh, H. H. J. 2000. On the galactosyl distribution of commercial galactomannans. Carbohydrate Research 329: 609-619.

Dakia, P. A., Blecker, C., Robert, C., Wathelet, B. and Paquot, M. 2008. Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. Food Hydrocolloids 5: 807-818.

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

Ding, W. K. and Shah, N. P. 2009. An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. Journal of Food Science 74: M53-M61.

Dokoohaki, Z. N., Sekhavatizadeh, S. S. and Hosseinzadeh, S. 2019. Dairy dessert containing microencapsulated *Lactobacillus rhamnosus* (ATCC 53103) with quince seed mucilage as a coating material. LWT - Food Science and Technology 115: article ID 108429.

European Food Safety Authority (EFSA). 2017. Re-evaluation of locust bean gum (E 410) as a food additive. EFSA Journal 15(1): 4646.

Food and Agriculture Organization Corporate Statistical Database (FAOSTAT). 2016. Carobs. Rome: Food and Agriculture Organization (FAO).

Gul, O. and Dervisoglu, M. 2017. Application of multicriteria decision technique to determine optimum sodium alginate concentration for microencapsulation of *Lactobacillus casei* Shirota by extrusion and emulsification. Journal of Food Process Engineering 40(3): article ID e12481.

Jain, A., Gupta, Y. and Jain, S. K. 2007. Perspectives of biodegradable natural polysaccharides for site-specific drug delivery to the colon. Journal of Pharmacy and Pharmaceutical Sciences 10: 86-128.

Jana, S., Gandhi, A., Sheet, S. and Sen, K. K. 2015. Metal ion-induced alginate-locust bean gum IPN microspheres for sustained oral delivery of aceclofenac. International Journal of Biological Macromolecules 72: 47-53.

Jrad, Z., Oussaief, O., Zaidi, S., Khorchani, T. and El-Hatmi, H. 2020. Co-fermentation process

strongly affect the nutritional, texture, syneresis, fatty acids and aromatic compounds of dromedary UF-yogurt. Journal of Food Science and Technology 58: 1727-1739.

Kerry, R. G., Patra, J. K., Gouda, S., Park, Y., Shin, H. S. and Das, G. 2018. Benefaction of probiotics for human health: A review. Journal of Food and Drug Analysis 26: 927-939.

Knorr, D. 1998. Technology aspects related to microorganisms in functional foods. Trends in Food Science and Technology 9: 295-306.

Koksoy, A. and Kilic, M. 2004. Use of hydrocolloids in textural stabilization of a yoghurt drink, ayran. Food Hydrocolloids 18: 593-600.

Majeed, M., Majeed, S., Nagabhushanam, K., Arumugam, S., Natarajan, S., Beede, K. and Ali, F. 2018. Galactomannan from *Trigonella foenum-graecum* L. seed: Prebiotic application and its fermentation by the probiotic *Bacillus coagulans* strain MTCC 5856. Food Science and Nutrition 6: 666-673.

Maurya, P., Mogra, R. and Bajpai, P. 2014. Probiotics: An approach towards health and disease. Trends in Biosciences 7: 3107-3113.

Neffe-Skocińska, K., Rzepkowska, A., Szydłowska, A. and Kołożyn-Krajewska, D. 2018. Trends and possibilities of the use of probiotics in food production. In Holban, A. M. and Grumezescu, A. M. (eds). Alternative and Replacement Foods, p. 65-94. United States: Academic Press.

Park, Y. W., Oglesby, J., Hayek, S. A., Aljaloud, S. O., Gyawali, R. and Ibrahim, S. A. 2019. Impact of different gums on textural and microbial properties of goat milk yogurts during refrigerated storage. Foods 8(5): article no. 169.

Pereira, J. A., Pinto, S. S., Dias, C. O., Vieira, M. P., Ribeiro, D. H., Amboni, R. D. and Fritzen-Freire, C. B. 2020. Potentially symbiotic fermented milk: A preliminary approach using lactose-free milk. LWT - Food Science and Technology 118: article ID 108847.

Srour, N., Daroub, H., Toufeili, I. and Olabi, A. 2016. Developing a carob-based milk beverage using different varieties of carob pods and two roasting treatments and assessing their effect on quality characteristics. Journal of the Science of Food and Agriculture 96: 3047-3057.

Srutkova, D., Schwarzer, M., Hudcovic, T., Zakostelska, Z., Drab, V., Spanova, A., ... and Schabussova, I. 2015. *Bifidobacterium longum* CCM 7952 promotes epithelial barrier function and prevents acute DSS-induced colitis in strictly strain-specific manner. PLoS One 10: article ID e0134050.

Teggatz, J. A. and Morris, H. A. 1990. Changes in the rheology and microstructure of ropy yogurt during shearing. Food Structure 9(2): article no. 9.

Teles, C. D. and Flores, S. H. 2007. The influence of additives on the rheological and sensory properties of nonfat yogurt. International Journal of Dairy Technology 60: 270-276.

Ünal, B., Metin, S. and Işıklı, N. D. 2003. Use of response surface methodology to describe the combined effect of storage time, locust bean gum and dry matter of milk on the physical properties of low-fat set yoghurt. International Dairy Journal 13: 909-916.

Zartl, B., Silberbauer, K., Loeppert, R., Viernstein, H., Praznik, W. and Mueller, M. 2018. Fermentation of non-digestible raffinose family oligosaccharides and galactomannans by probiotics. Food and Function 9: 1638-1646.

Ziar, H., Gérard, P. and Riazi, A. 2012. Calcium alginate-resistant starch mixed gel improved the survival of *Bifidobacterium animalis* subsp. *lactis* Bb12 and *Lactobacillus rhamnosus* LBRE-LSAS in yogurt and simulated gastrointestinal conditions. International Journal of Food Science and Technology 47: 1421-1429.

Ziar, H., Gérard, P. and Riazi, A. 2014. Effect of prebiotic carbohydrates on growth, bile survival and cholesterol uptake abilities of dairy-related bacteria. Journal of Science of Food and Agriculture 94: 1184-1190.