



Effect of encapsulated probiotic in Inulin-Maltodextrin-Sodium alginate matrix on the viability of *Enterococcus mundtii* SRBG1 and the rheological parameters of fermented milk

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ARTICLE INFO

Handling Editor; Xing Chen

Keywords:

Spray dry
Inulin
Maltodextrin
Sodium alginate
Milk fermentation
Rheological parameters

ABSTRACT

In the current research, *Enterococcus mundtii* SRBG1 newly isolated from Bat guano was encapsulated using spray drying technique to create a probiotic powder using six combinations of inulin, maltodextrin and sodium alginate. The encapsulation yield, moisture content, physical characteristics, and shape were investigated. Microcapsules yields ranged from 67 to 85 percent, which is consistent with typical B-290 spray-drier yields. The moisture content showed to increase ($4 \pm 0.15\%$) with the addition of sodium alginate to inulin and maltodextrin. In the gastrointestinal conditions (simulated gastric juice and bile salts), it was shown that the viability of probiotic cells in capsules was higher than that of free cells. This demonstrated the effectiveness of combining inulin and maltodextrin to encapsulate substances in surviving in gastro-intestinal conditions. Additionally, we evaluated the non-encapsulated and encapsulated SRBG1 by assessing their impact on the rheological parameters of fermented milk. The results showed that in the absence of sodium alginate the viscosity of milk was lower than with the other protectors, which was confirmed by the quick acidification of the fermented milk by microcapsules containing sodium alginate.

1. Introduction

Functional foods play a crucial role in human health by delivering antioxidants, essential oils, minerals, vitamins, and beneficial microorganisms (probiotics). Probiotics are microorganisms that provide the host health advantages (Barajas-Álvarez et al., 2022).

Products made with free probiotic cells had a low survival rate. They must be protected by a physical barrier to endure harmful

environmental, processing, storage, and intestinal conditions (Arepally and Goswami, 2019). One of the alternative approaches for protecting probiotic cells from harmful environmental conditions is microencapsulation (Schell and Beermann, 2014). Extrusion, spray drying, emulsion, fluidized bed drying, and freeze drying are some of the several encapsulation techniques (Arepally and Goswami, 2019).

When compared to other encapsulation technologies for bioactive microorganisms, spray drying is widely used, because it offers small

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<https://doi.org/10.1016/j.crfs.2022.09.027>

Received 9 August 2022; Received in revised form 24 September 2022; Accepted 29 September 2022

Available online 30 September 2022

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capsules with typical diameters of less than 100 μm at similarly low costs (Pinto et al., 2015). This facilitates a larger contact surface for the nutrient's availability. The food industry uses spray drying because of its rapid processing and drying times, low water activity (aw), low energy requirements, simplicity of transport, flexibility, high process yield, simple storage, homogeneous distribution throughout the product, and favourable applications in the production of functional foods (Sharma et al., 2022).

Over the recent two decades, extensive research has been conducted on the encapsulation of various probiotic strains using different food grade polymers (Chitosan, sodium alginate, inulin, gum Arabic, maltodextrin ...) due to their non-toxicity, strong biocompatibility, and capacity to form gels during encapsulation (Arepally and Goswami, 2019; Yonekura et al., 2014). Thermal and oxidative damage are avoided during storage by using polymer carbohydrate with a high activation energy. Maltodextrin (MD) is a popular coating material because of its non-toxicity, low cost, good solubility, low viscosities even at high solid content, and ease of availability. Maltodextrin also has a minor prebiotic effect (Anekella and Orsat, 2013). In the same context, inulin is a prebiotic mainly composed of fructose units with (2–1) linkages to glucose at the end of the chain, and it is an interesting encapsulating agent as a maltodextrin (Fernandes et al., 2014).

The concept of symbiotic bacteria is derived from the synergistic combination of probiotics and prebiotics. Several studies found that when symbiotic microcapsules were exposed to simulated gastrointestinal tract, they were more effective (Silva et al., 2018).

Several studies performed on *Enterococcus mundtii* from several sources such as soybeans, fermented sourdough, and others, presented the strain as a potential probiotic (Campos et al., 2006; Pingitore et al., 2012). *Enterococcus mundtii* ST4SA has been shown to have an excellent adhesion property and displace *Clostridium sporogenes* and *Enterococcus faecalis* in competitive exclusion experiments (Van Zyl et al., 2016).

In this study, *Enterococcus mundtii* SRBG1 was newly isolated from bat guano and encapsulated by spray drying using different combinations of inulin, maltodextrin with or without sodium alginate. The six matrixes were evaluated for their capacity to protect the bacteria. The encapsulated bacteria were also used as a starter culture for milk fermentation, to assess the effect the different microcapsules on the rheological parameters of fermented milk.

2. Materiel and methods

2.1. Guano sampling and isolation of strains

Fresh guano was harvested in sterile tubes from a cave located in *Errachidia* Morocco called "Kef aziza" and transported at 4°C to the laboratory. By using a serial dilution plate count, the isolation of strains was accomplished by transferring 2.0 g of fresh guano to a dilution tube containing 9.0 mL of sterilized saline solution and shaking the tube for 10 min. The dilution tubes were shaken, then left idle for 30 min to allow the suspension to stabilize. After shaking for 10 min and allowing to stabilize for 30 min, 1.0 mL of the bacterial suspension from the 10¹ dilution was transferred to a second dilution tube containing 9.0 mL of sterilized water (10²). Pour-plating on de Mann Rogosa Sharpe (MRS Agar) was done and incubated for 72 h at 37°C.

2.2. DNA extraction and PCR

Among 20 isolates, one isolate showed previously to have the most probiotic properties (Sakoui et al., 2022). This isolate was selected for this study and identified using molecular methods. The Maxwell® RSC Cell DNA kit (RefAS1370 Promega) was used in accordance with the manufacturer's instructions for the total DNA extraction. Then, the 16 S ribosomal DNA was amplified by PCR using the extracted DNA as a template. The highly conserved universal primers Fd1 5'-AGAGT TTGATCTGGCTCAG-3' and RP2 5'-ACGGCTACCTGTACGACTT-3'

were used for the amplification. Each PCR reaction contained approximately 100 ng of genomic DNA, 10 mM dNTP (Promega), 5X buffer (Promega), 25 mM MgCl₂ (Promega), 10 mM primer, and Taq polymerase 5U. The PCR reactions were performed using a DLAB TC1000-G thermocycler (Promega). 95 °C, 2'/(95 °C, 40' - 55 °C, 40' - 72 °C, 1') 35; 72 °C/5' /4 °C was the PCR thermic profile.

2.3. Gene sequencing

ExoSAP-IT purification kit from G.E. Healthcare was used to purify the PCR products before proceeding to the sequencing. The sequencing was carried out using the BigDye Terminator Kit version 1.0 (Applied Biosystems). The obtained isolate's gene sequence was examined using BLAST implemented in Geneious Prime Software (2022.2). BioEdit 7.2 was used to estimate the sequence identity values.

2.4. Preparation of the different matrix

In the presence study, six carriers were tested containing inulin and/or maltodextrin combined or not with sodium alginate (Table 1). Inulin and/or maltodextrin were added to a preheated sodium alginate solution for a total dissolution.

2.5. Spray dry process

500 ml of the bacterial biomass suspension and the sterile encapsulant solution were combined before the drying process. The mixture was stirred using a magnetic stirrer. The material was then spray-dried. In a Buchi mini spray-dryer B-290 (GEA, Germany), mixtures of the bacterial biomass and the protectants were dried. Samples were processed at a constant feed rate (pump feed rate 28%), 100% aspirator setting and at 120°C air inlet temperature.

2.6. Determination of encapsulated SRBG1 viable cells

The viable cell count was conducted using a modified version of the methodology described previously by Okuro et al. (2013). 3g of powder was added to 27 mL of a saline solution and the mixture was then homogenized using a vortex. The samples were next serially diluted in the same saline solution before being plated on MRS agar. The plates were incubated for 48 h at 37°C. After incubation, live probiotic cells were counted and the results were expressed as log CFU/g.

2.7. Encapsulation yield (EY)

As suggested by Xavier dos Santos et al. (2019), the encapsulation yield (EY), was calculated following equation (1):

$$\text{Encapsulation yield } EY (\%) = M/M_0 \times 100 \quad (1)$$

Where M₀ is the mass of solids in the processed suspension and M is the mass of spray drying powder recovered from the spray dryer.

2.8. Moisture content (MC)

Moisture content was estimated following the protocol described by Xavier dos Santos et al. (2019). Briefly, the MC was calculated based on the difference in weight between before and after drying the capsules at

Table 1
The composition of the different encapsulant agent used (g/100 mL).

	C1	C2	C3	C4	C5	C6
Inulin	10%	10%	-	-	5%	5%
Maltodextrin	-	-	10%	10%	5%	5%
Sodium Alginate	-	2%	-	2%	-	2%

105 °C until they reached a constant weight.

2.8.1. Scanning electron microscopy (SEM)

The surface morphology and microstructure of microcapsules were examined by SEM using QUATTRO S-FEG - Thermofisher scientific at magnifications of 1000 and 2000. The gold-sputtered powder, was mounted on a SEM stub and examined.

2.9. Simulated gastric juice (SGJ) and simulated intestinal juice (SIJ) tolerance

According to (Rajam et al., 2015), the simulated gastric juice (SGJ) was generated with a few adjustments. Briefly, pepsin was added to sterile MRS broth (adjusted to pH 2.5) at a final concentration of 0.3 percent (v/v) after being filtered using a sterile membrane filter (0.45 µm). The SGF solution (9 mL) was then poured into the sterile test tubes. These various test tubes received separate additions of the samples of 10^8 of free cells (1 mL) and encapsulated cells (1 g), the tubes were vortexed for 1 min and then incubated at 37°C for 4 h. Similarly, the simulated intestinal juice (SIJ) was prepared by dissolving 0.3 percent (w/v) of bile salt in MRS broth. The provided solution was autoclaved and then inoculated as described above with free and encapsulated cells, separately and then incubated at 37°C for 4 h.

An aliquot (1 mL) from each treatment was obtained at 0 and 4h of incubation to calculate the viable cell counts as described above.

2.10. Milk fermentation

The selected strains' capacity for fermentation was tested in accordance with Sultana et al. (2000). In a water bath, 5% of skim milk (w/v) was pasteurized at 100°C for 5 min. The milk was maintained to cool until it reached 37°C and then was aseptically inoculated with the strains until it reached 10^8 CFU/mL and was then incubated at 37°C for 48 h. The free and the encapsulated cells were added into a saline solution, and each suspension was added to the milk. Non-fermented milk was used as control.

2.10.1. Kinetic of acidification

The rate of acidification was measured by periodically recording the pH value for each milk inoculated with free or encapsulated cells during 48 h.

2.10.2. Rheological parameters

An Anton Paar MCR 72 rheometer (Anton Paar, Graz, Austria), equipped with a Peltier plate-plate system (P-PTD 200/Air), was used to measure the viscosity of the samples both before and after fermentation. The samples were positioned between the two plates, the lower plate having a temperature control system set at 25 °C with a gap of 1 mm, and the upper plate having smooth parallel plate geometry of 50 mm diameter. To ensure thermal equilibrium before measurements, the sample surplus was taken out and permitted to rest for 10 min. With a linearly increasing shear rate from 5 to 300 1/s, each measurement was carried out twice.

2.11. Statistics

All the experiment were repeated three times, with the exception of spray dry process which was performed in duplicate. The results were expressed as mean ± standard deviation. For statistical analysis, ANOVA test was employed using GraphPad prism 8.0.2. One-way Anova was used to determine whether there are any statistically significant differences between the means of the repeated experiment, and two-way Anova was also used to compare the mean differences between the control and the other groups.

3. Results and discussion

3.1. Molecular identification

The isolate was identified as *Enterococcus mundtii* SRBG1 and the sequence was sent to GenBank and obtained the following accession number (ON204234).

3.2. Encapsulation yield and moisture content

Encapsulation yield after the spray-dry process is shown in Fig. 1A. Microcapsules yields ranged from 67 to 85 percent, which is consistent with typical B-290 spray-drier yields. The greater yield was recorded with microcapsules with inulin, maltodextrin and sodium alginate.

Similar results were found by Tan et al. (2022) when using *in situ* alginate crosslinking during spray-drying of lactobacilli probiotics. Otherwise, Kawakita et al. (2021) optimized the viability and yield of bacteria encapsulated by spray-drying and showed that when increasing the alginate concentration to 3% the encapsulation yield decreased to 31%, reporting that the encapsulation yield in alginate concentration dependent. While, Sohail et al. (2013), used 2% of alginate and/or 5% of maltodextrin and showed that the probiotic had similar viability reductions during drying processes with both or only one encapsulant.

The moisture content of probiotic powder has a strong influence on product stability and can also have an impact on probiotic viability during storage, which is one of the quality parameters to consider for powders containing cells. The moisture content of encapsulated SRBG1 with different carriers is shown in Fig. 1B. The moisture content of probiotic powder is shown to increase in the presence of sodium alginate but still acceptable in food product (Yonekura et al., 2014). This

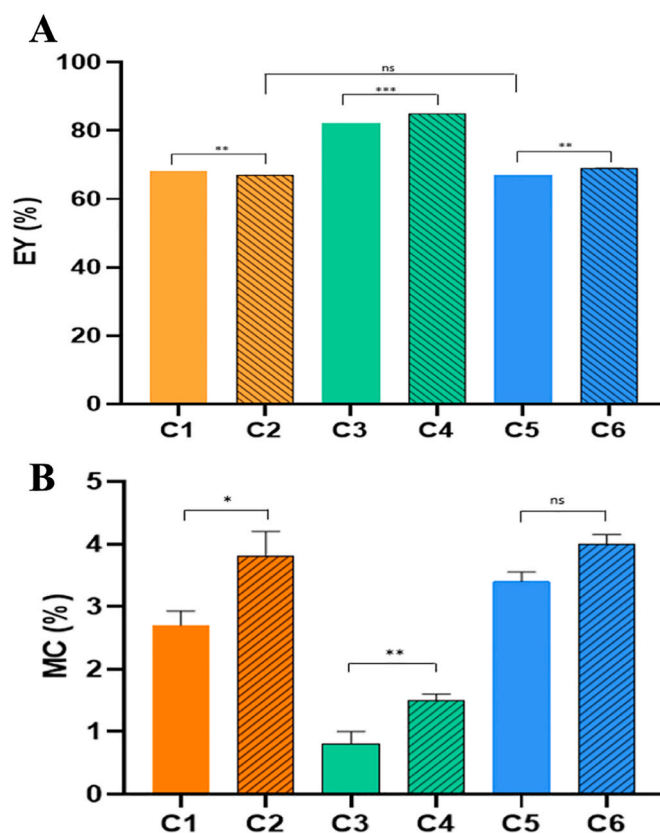


Fig. 1. Encapsulation yield (EY) and moisture content of encapsulated *Enterococcus mundtii* SRBG1 with different matrix (C1: Inulin, C2: Inulin + Sodium alginate, C3: Maltodextrin, C4: Maltodextrin + Sodium alginate, C5: Inulin + Maltodextrin, C6: Inulin + Maltodextrin + Sodium Alginate).

increment may be related to the water holding capacity of sodium alginate. Otherwise, the higher moisture content was observed for the combination of inulin, maltodextrin and sodium alginate with a percentage of 4%.

Khem et al. (2016) reported that several process variables, including inlet temperature, coating agent properties, and feed flow rate, may influence moisture content. They showed that when the feed was injected at the lowest flow rate and with the highest amount of inulin, the product had the lowest moisture content which is 4.2%.

3.3. SEM

The structure of the probiotic microcapsules was examined using scanning electron microscopy (Fig. 2). The probiotic microcapsules were spherical in shape. According to Chen et al. (2005), the concentration of sodium alginate affects the structure of the microcapsules; higher concentrations result in a smoother surface. In their study, probiotic microcapsules made with 3% alginate had a smooth surface and relatively

small pores, whereas optimum probiotic microcapsules made with 1% sodium alginate have a rough surface and significantly open and large pores. In our case, the microcapsules prepared with matrix containing inulin (with or without maltodextrin) have irregular (Smooth and rough) surfaces with small pores, when adding the sodium alginate, we observed that the pores become larger. Moreover, more regularly shaped particles were seen on the MD and on the combination of MD and SD, pointing to MD's beneficial influence on the development of microcapsules. Otherwise, Pandey and Mishra (2021) reported that compared to microcapsules made with prebiotics, inulin, and dextran, which adhere to stronger probiotic protection, those made with simply maltodextrin had some holes.

3.4. Viability of cells

The microorganism's survival rate during the encapsulation process is shown in Table 2. The composition of the matrix solutions had a significant effect on encapsulation efficiency. The microcapsules

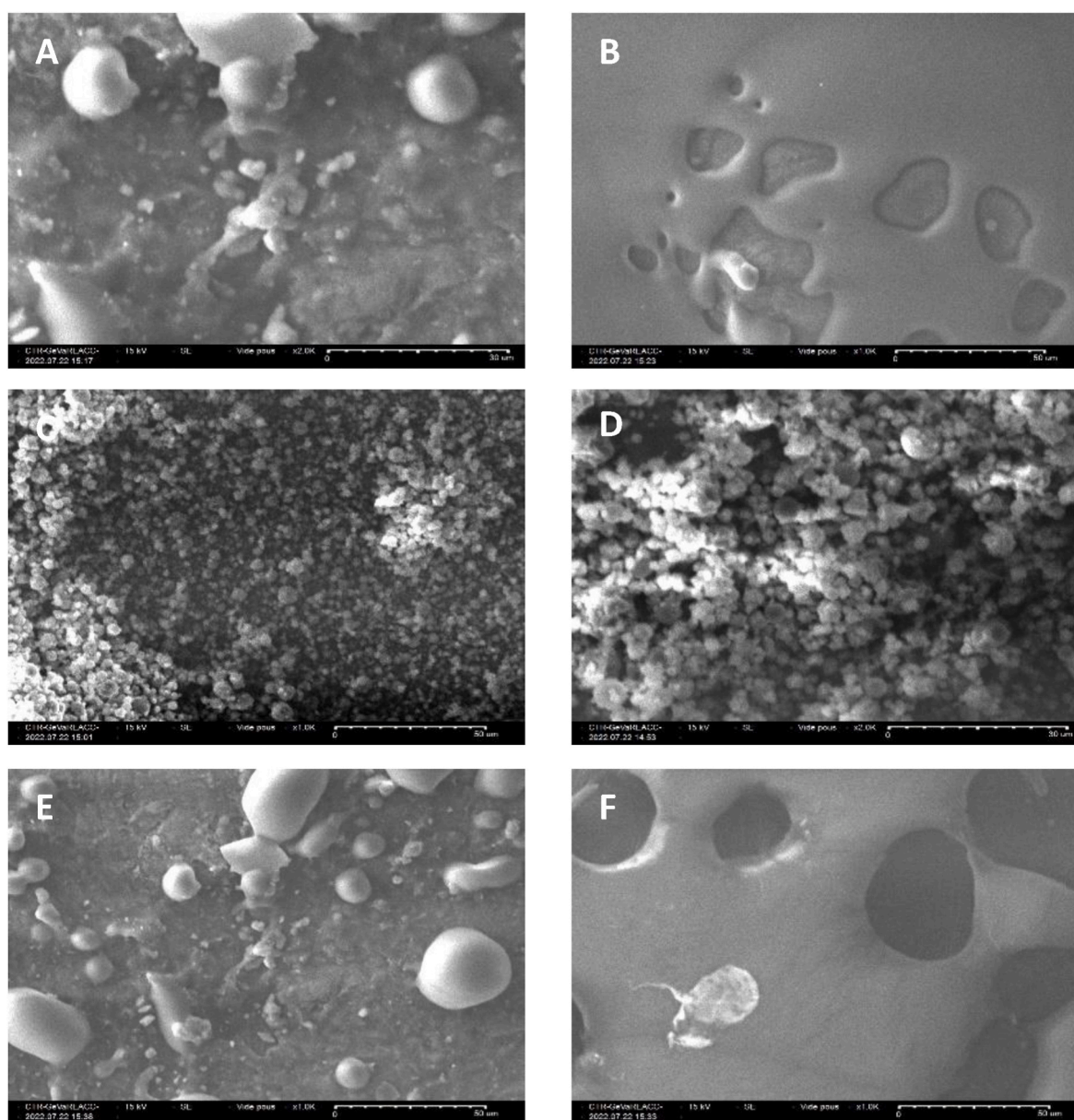


Fig. 2. Microstructure of encapsulated *Enterococcus mundti* SRBG1 in A: Inulin, B: Inulin + Sodium alginate, C: Maltodextrin, D: Maltodextrin + Sodium alginate, E: Inulin + Maltodextrin and F: Inulin + Maltodextrin + Sodium Alginate.

Table 2Viability of *Enterococcus mundtii* SRBG1 in different conditions after 4 h of incubation at 37°C (Log CFU/mL).

	Free cell	C1	C2	C3	C4	C5	C6
MRS broth	7.32 ± 0.01 ^a	6.65 ± 0.04 ^a	6.13 ± 0.00 ^a	7.16 ± 0.07 ^a	7.02 ± 0.01 ^a	7.22 ± 0.01 ^b	7.11 ± 0.01 ^a
Simulated gastric Juice(pH 2.5)	5.43 ± 0.01 ^a	6.39 ± 0.01 ^a	5.85 ± 0.02 ^a	6.40 ± 0.02 ^a	5.35 ± 0.06 ^b	7.12 ± 0.03 ^a	7.00 ± 0.01 ^a
Simulated intestinal Juice(Bile salts 0.3%)	4.29 ± 0.02 ^a	5.72 ± 0.01 ^a	4.65 ± 0.00 ^a	5.34 ± 0.07 ^a	4.32 ± 0.01 ^b	6.18 ± 0.02 ^a	5.43 ± 0.01 ^a

Results are presented as the mean of three values ± standard deviation. C1: Inulin, C2: Inulin + Sodium alginate, C3: Maltodextrin, C4: Maltodextrin + Sodium alginate, C5: Inulin + Maltodextrin, C6: Inulin + Maltodextrin + Sodium Alginate. The same letter in the same row means that there is a significant difference and different letter means that there is no significant difference.

produced with the combination of inulin and sodium alginate had the greatest reduction in cell viability, corresponding to approximately 15% of the cell population of the initial cells, and in results had the lowest encapsulation efficiency. In contrast, the reduction in viable cell counts observed after spray drying of SRBG1 produced with the combination of maltodextrin and inulin and with only maltodextrin, resulting in higher encapsulation efficiencies of 85.07 and 75.31 percent, respectively. These findings indicate that the presence of maltodextrin had a positive effect on *Enterococcus* survival during the microencapsulation process, most likely due to its role as a thermoprotector of cells undergoing drying. preventing cell membrane disruption.

Once the probiotic food has been taken, probiotics are subject to gastrointestinal stress. For probiotics to have any positive effects, they must be able to survive in this environment. The possible protective effect of encapsulation on survival of *E. mundtii* SRBG1 in SGJ and SIJ is presented in Table 2. As it can be seen in Table 2, in comparison to free cells, SRBG1 was better protected by the microencapsulation of various matrix combinations when exposed to SGJ and SIJ. This finding suggests that microencapsulation is a viable alternative to boost probiotic tolerance in these environments. The viability of microparticles prepared with the combination of inulin and maltodextrin to SGJ conditions was 7.12 ± 0.03, which was greater than free SRBG1 that lost 1.89 CFU/mL under the same conditions. Better protection was shown in the absence of sodium alginate, this could be due to the larger pores present in sodium alginate capsules, as Bhagwat et al. (2020) have reported that the bile salts are known to affect the viability of entrapped cells by the action of detergent-like property that might cause emulsification of the entrapped and surface ingredient, thereby releasing the enterococci cells into bile salt solution.

Bhagwat et al. (2020) reported the relative levels of defense against the acidic media at pH 2.0 and 2.5 that the spray-dried microcapsules provide to the encapsulated *Enterococcus* cells by maltodextrin. They showed that at pH 2.0, the viability percentage decreased over the course of 4 h, going from 9.23 log CFU/mL to 7.56 log CFU/mL. That implies that cell viability at pH 2 decreased by a net 1.67 log CFU/mL. The count was 8.49 log CFU/mL at pH 2.5, with a net loss of only 0.74 log CFU/mL. On the spray-dried microcapsules, gastric juice's antibacterial effect was noticeably less pronounced.

Regarding the survival rate in acidic conditions, microcapsules prepared with only inulin showed the greater viability of 86.01% when compared to the free cells followed with microcapsules prepared with the combination of inulin and maltodextrin with a viability percentage of 85.59%. These results suggest that inulin had a better effect on the protection of the *Enterococci* under the acidic conditions. According to Fritzen-Freire et al. (2013), inulin may be the best material to transfer encapsulated probiotics to the colon because it is stable to the pH and ionic strength range found in the human gastrointestinal tract.

Darjani et al. (2016) reported that after 120 min, *L. casei* 431 microencapsulated with a combination of alginate and inulin in a saline solution with pH 1.5 showed a 69.8% of reduction. Additionally, Mantzouridou et al. (2012) claim that since the human gastrointestinal tract enzymes do not hydrolyze inulin, they have a positive impact on health by acting as dietary fiber.

3.5. Kinetic of milk acidification

After adding the encapsulated and non-encapsulated SRBG1 to skimmed milk, the acidification kinetics were determined over the duration of 48 h. Fig. 3 shows the results. Compared to free cells cultured under similar conditions, the acidification rate for the encapsulated cultures was lower. The encapsulated cells take longer to achieve the identical pH change end point than do the free cells, who reach it faster. As an example, free cells reached pH 4.67 after 12 h of fermentation but encapsulated cells took more than 24 h to reach the same pH level. The encapsulation of the probiotics could reduce the release of lactic acid into milk, thus reducing the acidification process. This might be as a result of the nutrients' and metabolites' gradual release across the encapsulant shell of the beads. Previous observations revealed a comparable result (Alvarado-Reveles et al., 2019; Pradeep Prasanna and Charalampopoulos, 2019). In our study, the milk fermented with microcapsules prepared with sodium alginate reach pH 4 after 24 h of fermentation, which is lower than the time took by the milk fermented with the encapsulated bacteria with inulin and/or maltodextrin, but still greater than the non-encapsulated cells.

3.6. Apparent viscosity

Fig. 4 reports the apparent viscosity of the fermented milk with free and encapsulated SRBG1 with different matrix. As observed, in case of every fermented and non-fermented milk samples the viscosity decreased considerably, reaching final values between 85.7 ± 2.79 and 275.05 ± 4.21 mPa s. According to the viscosity results, every tested sample presented a shear-thinning (pseudo-plastic) behavior. The fast decrease in the viscosity of the samples, demonstrates that at the beginning when the sample is at rest the small particles are in a tangled mass and with the increase of shear rate they align. These results are in agreement with similar studies, regarding different food products

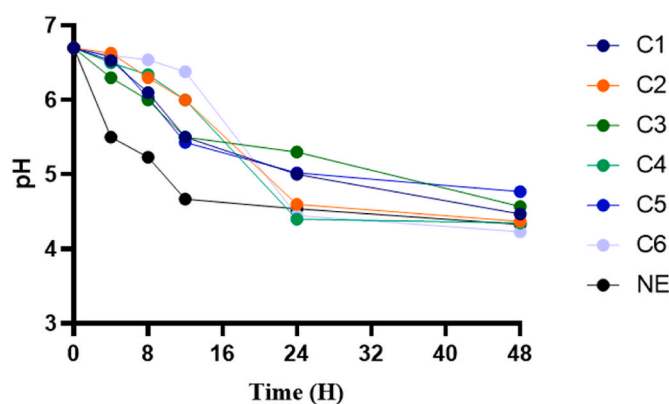


Fig. 3. Kinetic of milk acidification with the free (NE) and encapsulated *Enterococcus mundtii* SRBG1 (C1: Inulin, C2: Inulin + Sodium alginate, C3: Maltodextrin, C4: Maltodextrin + Sodium alginate, C5: Inulin + Maltodextrin, C6: Inulin + Maltodextrin + Sodium Alginate).

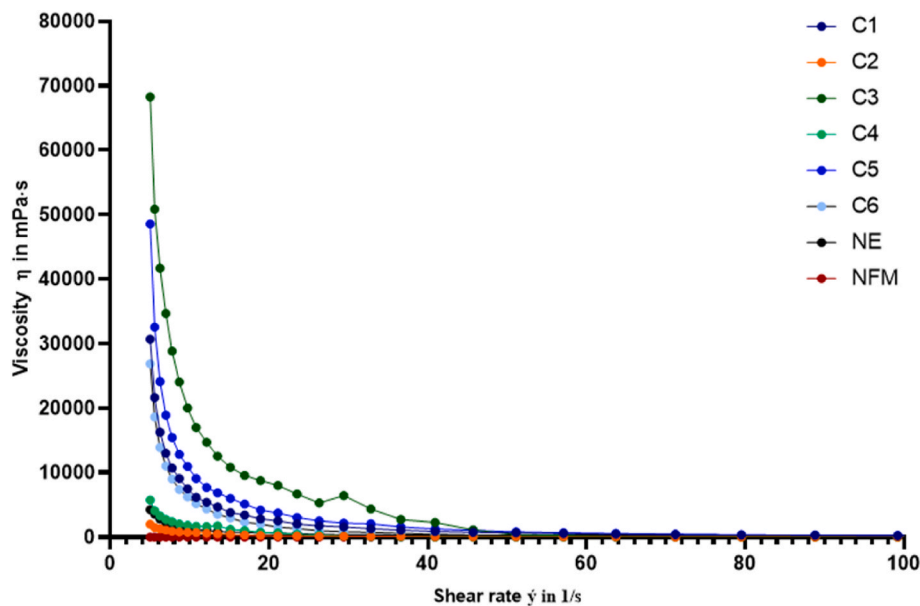


Fig. 4. Apparent viscosity of fermented milk with free *Enterococcus mundtii* SRBG1 (NE) and different matrix (C1: Inulin, C2: Inulin + Sodium alginate, C3: Maltodextrin, C4: Maltodextrin + Sodium alginate, C5: Inulin + Maltodextrin, C6: Inulin + Maltodextrin + Sodium Alginate) and Non-fermented milk (NFM).

(Muresan et al., 2014; Szabo et al., 2020). The apparent viscosity of milk fermented with encapsulated SRBG1 with a matrix non including sodium alginate was considerably ($P < 0.05$) higher (30666.4, 48543.2 and 68266 mPa s for inulin, maltodextrin + inulin and maltodextrin, respectively) than that of milk fermented with encapsulated SRBG1 with a matrix including sodium alginate and with the non-encapsulated one. These changes in viscosity may be related to higher degree of acidity provided by the free cells and the encapsulated one with sodium alginate or may be due to the high degree of polymerization of inulin and maltodextrin adsorbed on the surface of cells and forming viscous layer.

Our findings supported those previously published by Ajlouni et al. (2021). They have reported that when acidity is elevated, milk proteins are denatured and broken down, resulting in enhanced water flow and decreased viscosity. Because it gives a desired mouthfeel and reduces syneresis, viscosity is a crucial component of yogurt quality. While, Kailasapathy (2006), reported that divalent cations like calcium preferentially attach to the alginate polymer and hence increase viscosity or create gels.

4. Conclusion

Functional foods that contain probiotics are currently drawing interest on the market. In this study, *Enterococcus mundtii* SRBG1 isolated from Bat guano was spray dried and encapsulated with six combinations of different encapsulant (Inulin, Inulin + Sodium Alginate, Maltodextrin, Maltodextrin + Sodium Alginate, Inulin + Maltodextrin and Inulin + Maltodextrin + Sodium Alginate) to create a probiotic powder that may be utilized as a functional food ingredient. The efficiency and moisture of encapsulation were matrix dependent. The SEM results showed a spherical microcapsule in shape, and demonstrated the effect of adding sodium alginate on cell surface walls which become larger. In the gastrointestinal conditions, it was discovered that the viability of probiotic cells in capsules was higher than that of free cells. This demonstrated the effectiveness of combining inulin and maltodextrin to encapsulate substances in harsh environments, and that the addition of prebiotics in sodium alginate microcapsules improved the protection of the active microorganisms. Additionally, the fermentation of milk with the free and the encapsulated SRBG1 was also studied and showed that in the absence of sodium alginate the viscosity of milk was lower than with the other protectors.

Extensive studies are needed to prove the probiotic effect of the strain. Furthermore, a sensory evaluation must be conducted to clearly understand how the capsules alter the probiotic's milk's sensory qualities, such as color, texture, acidity, and flavor.

Funding

This research was supported by ERANET2/2018 (ProBone) and publication by (PFE)-37/2018–2020 and project number: PN-III-P4-IDPCE-2020-2126.

CRediT authorship contribution statement

Souraya Sakoui: Data curation, Conceptualization, Methodology, Writing – original draft. **Reda Derdak:** Writing – review & editing. **Oana Lelia Pop:** Funding acquisition, Visualization, Writing – review & editing. **Dan Cristian Vodnar:** Formal analysis, Supervision. **Boutaina Addoum:** Writing – review & editing. **Bernadette-Emöke Teleky:** Writing – review & editing. **Simon Elemer:** Writing – review & editing. **Abdelhakim Elmakssoudi:** Writing – review & editing. **Ramona Suharoschi:** Funding acquisition, Visualization. **Abdelaziz Soukri:** Supervision. **Bouchra El Khalfi:** Supervision, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

- Ajlouni, S., Ranadheera, C.S., Chua, E.L., 2021. Encapsulation increases the in vitro bioaccessibility of probiotics in yoghurt. *Int. J. Dairy Technol.* 74, 118–127. <https://doi.org/10.1111/1471-0307.12746>.
- Alvarado-Reveles, O., Fernández-Michel, S., Jiménez-Flores, R., Cueto-Wong, C., Vázquez-Moreno, L., Montfort, G.R.C., 2019. Survival and goat milk acidifying activity of lactobacillus rhamnosus GG encapsulated with agave fructans in a

- buttermilk protein matrix. *Probiotics Antimicrob. Proteins* 11, 1340–1347. <https://doi.org/10.1007/S12602-018-9475-Y/TABLES/1>.
- Anekella, K., Orsat, V., 2013. Optimization of microencapsulation of probiotics in raspberry juice by spray drying. *LWT—Food Sci. Technol.* 50, 17–24. <https://doi.org/10.1016/J.LWT.2012.08.003>.
- Arepally, D., Goswami, T.K., 2019. Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. *LWT—Food Sci. Technol.* 99, 583–593. <https://doi.org/10.1016/J.LWT.2018.10.022>.
- Barajas-Álvarez, P., González-Ávila, M., Espinosa-Andrews, H., 2022. Microencapsulation of *Lactobacillus rhamnosus* HN001 by spray drying and its evaluation under gastrointestinal and storage conditions. *LWT—Food Sci. Technol.* 153, 112485. <https://doi.org/10.1016/J.LWT.2021.112485>.
- Bhagwat, A., Bhushette, P., Annature, U.S., 2020. Spray drying studies of probiotic *Enterococcus* strains encapsulated with whey protein and maltodextrin. *Beni-Suef Univ. J. Basic Appl. Sci.* 9, 1–8. <https://doi.org/10.1186/S43088-020-00061-Z/FIGURES/2>.
- Campos, C.A., Rodríguez, Ó., Calo-Mata, P., Prado, M., Barros-Velázquez, J., 2006. Preliminary characterization of bacteriocins from *Lactococcus lactis*, *Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (*Psetta maxima*). *Food Res. Int.* 39 (3), 356–364. <https://doi.org/10.1016/j.foodres.2005.08.008>.
- Chen, K.N., Chen, M.J., Liu, J.E.R., Lin, C.W., Chiu, H.Y.I., 2005. Optimization of incorporated prebiotics as coating materials for probiotic microencapsulation. *J. Food Sci.* 70, M260. <https://doi.org/10.1111/J.1365-2621.2005.TB09981.X>. M266.
- Darjani, P., Hosseini Nezhad, M., Kadkhodae, R., Milani, E., 2016. Influence of prebiotic and coating materials on morphology and survival of a probiotic strain of *Lactobacillus casei* exposed to simulated gastrointestinal conditions. *LWT—Food Sci. Technol.* 73, 162–167. <https://doi.org/10.1016/J.LWT.2016.05.032>.
- Fernandes, R.V.D.B., Borges, S.V., Botrel, D.A., 2014. Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. *Carbohydr. Polym.* 101, 524–532. <https://doi.org/10.1016/J.CARBPOL.2013.09.083>.
- Fritzen-Freire, C.B., Prudêncio, E.S., Pinto, S.S., Muñoz, I.B., Amboni, R.D.M.C., 2013. Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastrointestinal conditions and heat treatments. *LWT—Food Sci. Technol.* 50, 39–44. <https://doi.org/10.1016/J.LWT.2012.07.037>.
- Kailasapathy, K., 2006. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT—Food Sci. Technol.* 39, 1221–1227. <https://doi.org/10.1016/J.LWT.2005.07.013>.
- Kawakita, R., Leveau, J.H.J., Jeoh, T., 2021. Optimizing viability and yield and improving stability of Gram-negative, non-spore forming plant-beneficial bacteria encapsulated by spray-drying. *Bioproc. Biosyst. Eng.* 44, 2289–2301. <https://doi.org/10.1007/S00449-021-02604-9/FIGURES/7>.
- Khem, S., Small, D.M., May, B.K., 2016. The behaviour of whey protein isolate in protecting *Lactobacillus plantarum*. *Food Chem.* 190, 717–723. <https://doi.org/10.1016/J.FOODCHEM.2015.06.020>.
- Mantzouridou, F., Spanou, A., Kiosseoglou, V., 2012. An inulin-based dressing emulsion as a potential probiotic food carrier. *Food Res. Int.* 46 (1), 260–269.
- Muresan, V., Danthine, S., Racolta, E., Muste, S., Blecker, C., 2014. The influence of particle size distribution on sunflower tahini rheology and structure. *J. Food Process. Eng.* 37 (4), 411–426. <https://doi.org/10.1111/jfpe.12097>.
- Okuro, P.K., Thomazini, M., Balieiro, J.C.C., Liberal, R.D.C.O., Fávoro-Trindade, C.S., 2013. Co-encapsulation of *Lactobacillus acidophilus* with inulin or polydextrose in solid lipid microparticles provides protection and improves stability. *Food Res. Int.* 53, 96–103. <https://doi.org/10.1016/J.FOODRES.2013.03.042>.
- Pandey, P., Mishra, H.N., 2021. Co-microencapsulation of γ -aminobutyric acid (GABA) and probiotic bacteria in thermostable and biocompatible exopolysaccharides matrix. *LWT—Food Sci. Technol.* 136, 110293. <https://doi.org/10.1016/J.LWT.2020.110293>.
- Pingitore, E.V., Todorov, S.D., Sesma, F., de Melo Franco, B.D.G., 2012. Application of bacteriocinogenic *Enterococcus mundtii* CRL35 and *Enterococcus faecium* ST88Ch in the control of *Listeria monocytogenes* in fresh Minas cheese. *Food Microbiol.* 32 (1), 38–47. <https://doi.org/10.1016/j.fm.2012.04.005>.
- Pinto, S.S., Verruck, S., Vieira, C.R.W., Prudêncio, E.S., Amante, E.R., Amboni, R.D.M.C., 2015. Influence of microencapsulation with sweet whey and prebiotics on the survival of *Bifidobacterium*-BB-12 under simulated gastrointestinal conditions and heat treatments. *LWT—Food Sci. Technol.* 64, 1004–1009. <https://doi.org/10.1016/J.LWT.2015.07.020>.
- Pradeep Prasanna, P.H., Charalampopoulos, D., 2019. Encapsulation in an alginate–goats' milk–inulin matrix improves survival of probiotic *Bifidobacterium* in simulated gastrointestinal conditions and goats' milk yoghurt. *Int. J. Dairy Technol.* 72, 132–141. <https://doi.org/10.1111/1471-0307.12568>.
- Rajam, R., Kumar, S.B., Prabhasankar, P., Anandharamakrishnan, C., 2015. Microencapsulation of *Lactobacillus plantarum* MTCC 5422 in fructooligosaccharide and whey protein wall systems and its impact on noodle quality. *J. Food Sci. Technol.* 52, 4029–4041. <https://doi.org/10.1007/S13197-014-1506-4/FIGURES/8>.
- Sakoui, S., Derdak, R., Addoum, B., Pop, O.L., Vodnar, D.C., Suharoschi, R., Soukri, A., El Khalfi, B., 2022. The first study of probiotic properties and biological activities of lactic acid bacteria isolated from Bat guano from Er-rachidia, Morocco. *LWT—Food Sci. Technol.* 159, 113224. <https://doi.org/10.1016/J.LWT.2022.113224>.
- Schell, D., Beermann, C., 2014. Fluidized bed microencapsulation of *Lactobacillus reuteri* with sweet whey and shellac for improved acid resistance and in-vitro gastrointestinal survival. *Food Res. Int.* 62, 308–314. <https://doi.org/10.1016/J.FOODRES.2014.03.016>.
- Sharma, R., Rashidinejad, A., Jafari, S.M., 2022. Application of spray dried encapsulated probiotics in functional food formulations. *Food Bioprocess Technol.* 2022 1–202220. <https://doi.org/10.1007/S11947-022-02803-6>.
- Silva, K.C.G., Cezarino, E.C., Michelon, M., Sato, A.C.K., 2018. Symbiotic microencapsulation to enhance *Lactobacillus acidophilus* survival. *LWT—Food Sci. Technol.* 89, 503–509. <https://doi.org/10.1016/J.LWT.2017.11.026>.
- Sohail, A., Turner, M.S., Coombes, A., Bhandari, B., 2013. The viability of *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* NCFM following double encapsulation in alginate and maltodextrin. *Food Bioprocess Technol.* 6, 2763–2769. <https://doi.org/10.1007/S11947-012-0938-Y/FIGURES/6>.
- Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., Kailasapathy, K., 2000. Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int. J. Food Microbiol.* 62, 47–55. [https://doi.org/10.1016/S0168-1605\(00\)00380-9](https://doi.org/10.1016/S0168-1605(00)00380-9).
- Szabo, K., Teleky, B.E., Mitrea, L., Călinoiu, L.F., Martău, G.A., Simon, E., et al., 2020. Active packaging—poly (Vinyl alcohol) films enriched with tomato by-products extract. *Coatings* 10 (2), 141. <https://doi.org/10.3390/coatings10020141>.
- Tan, L.L., Mahotra, M., Chan, S.Y., Loo, S.C.J., 2022. In situ alginate crosslinking during spray-drying of lactobacilli probiotics promotes gastrointestinal-targeted delivery. *Carbohydr. Polym.* 286, 119279. <https://doi.org/10.1016/J.CARBPOL.2022.119279>.
- Van Zyl, W.F., Deane, S.M., Dicks, L.M.T., 2016. *Enterococcus mundtii* ST4SA and *Lactobacillus plantarum* 423 excludes *Listeria monocytogenes* from the GIT, as shown by bioluminescent studies in mice. *Benef. Microbes* 7 (2), 227–235. <https://doi.org/10.3920/BM2015.0082>.
- Xavier dos Santos, D., Casazza, A.A., Aliakbarian, B., Bedani, R., Saad, S.M.I., Perego, P., 2019. Improved probiotic survival to in vitro gastrointestinal stress in a mouse containing *Lactobacillus acidophilus* La-5 microencapsulated with inulin by spray drying. *LWT—Food Sci. Technol.* 99, 404–410. <https://doi.org/10.1016/J.LWT.2018.10.010>.
- Yonekura, L., Sun, H., Soukoulis, C., Fisk, I., 2014. Microencapsulation of *Lactobacillus acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: technological characterization, storage stability and survival after in vitro digestion. *J. Funct. Foods* 6, 205–214. <https://doi.org/10.1016/J.JFF.2013.10.008>.