ENCAPSULATION OF BACTERIAL CULTURES LACTIPLANTIBACILLUS PLANTARUM IN ALGINATE MICROPARTICLE FORMULATIONS

Marko Vinceković[1], Nevijo Zdolec[2], Hana Zelić[1], Luna Maslov Bandić[1], Damir Iveković[3], Nataša Mikulec[1]

[1]University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[2]University of Zagreb, Faculty of Veterinary medicine, Heinzelova 55, 10000 Zagreb, Croatia
[3]University of Zagreb, Faculty of Food technology and biotechnology, Pierottijeva 6, Zagreb, Croatia

4th International Congress on Food Safety and Quality
ONE HEALTH
November 9th-12th, 2022 | Dubrovnik, Croatia

Abstract

Starter (bacterial) cultures are used in cheese production to acidify the milk and create desirable sensory properties. One of the most commonly used bacterial species in cheese production is Lactiplantibacillus Plantarum. The study aimed to prove that the encapsulation process of this bacterial species in alginate microparticles will ensure the appropriate number and activity of bacterial cells in the microparticles and thus enable the controlled release of components inside the microparticles during cheese production. The encapsulation process is carried out by the ionic gelation method. After the encapsulation process, a satisfactory number and activity of Lactiplantibacillus plantarum bacterial cells were achieved in alginate microparticle formulations. Physico-chemical characterization of alginate microparticles filled with the Lactiplantibacillus plantarum strain was successfully performed. Microscopic examination confirmed the presence of short, gram-positive rods of Lactiplantibacillus plantarum within alginate microparticles. The size of the bacterial cells and the size of the prepared microspheres were determined with an optical microscope. The dynamics of the release of Lactiplantibacillus plantarum bacterial cells from the microspheres were successfully measured. Lactobacillus plantarum strain is released immediately after encapsulation (4.82 ± 0.07 log CFU/ml). Most of it is released by the 5th day (7.65 ± 0.05 log CFU/ml). After the 20th day, the release of the Lactobacillus plantarum strain gradually decreases until the 40th day. Based on the analysis results, it can be concluded that Lactiplantibacillus plantarum bacteria are suitable for use in the technological process of cheese production.

Research aims

This study aimed to prepare and physico-cjemical characterized of optimal microsphere filled *with Lactiplantibacillus plantarum* bacteria cultures for the process of cheese production

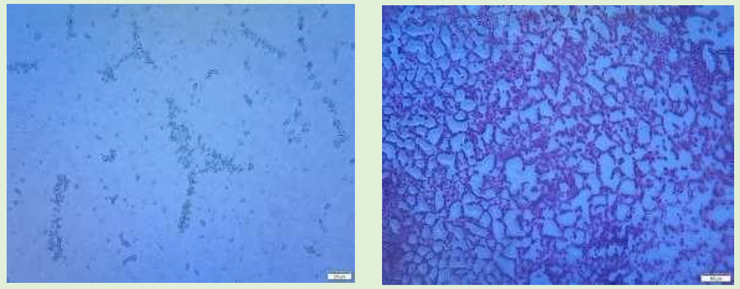


Figure 3. The appearance of *Lactiplantibacillus plantarum* cells under a light microscope: a) without staining, b) after gram staining

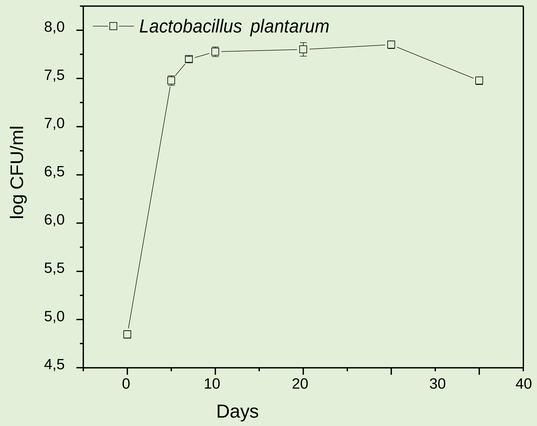


Figure 5. Release of Lactiplantibacillus plantarum cells from alginate microspheres. The number of the Lactiplantibacillus plantarum cell strain is shown as the mean value of log CFU/mL with the corresponding standard deviation

Materials & Methods

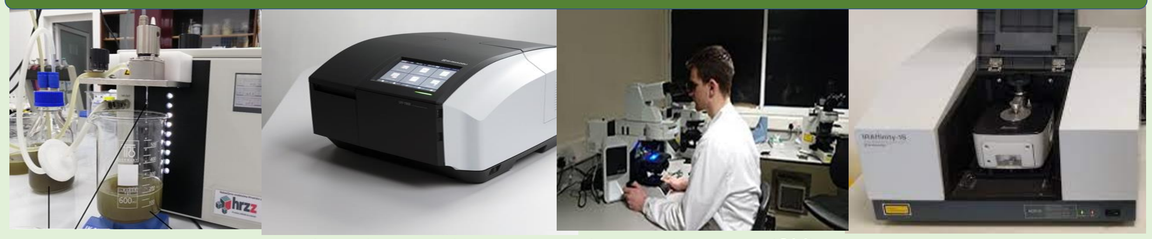
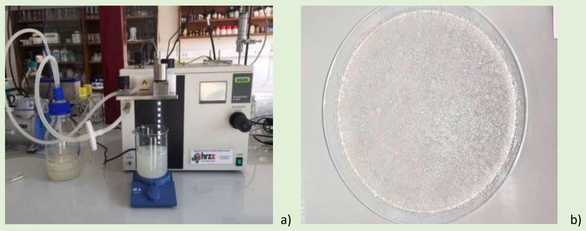


Figure 1. Left to right, encapsulation process, UV(VIS, optical microscope (OM) and FTIT-ATR

Preparation of microspheres

The microspheres (MS) were prepared in one step by ionic gelation at room temperature Alginic acid sodium salt solution (1.8% (w/v)) with *Lactiplantibacillus* plantarum *bacteria* was dripped into the calcium chloride solution (1 M) with continuous magnetic stirring mixers was dripped through the encapsulator nozzle size of 150 µm (Büchi-B390 Encapsulator, Bütchi Labortechnik AG, Flawil, Switzerland). Encapsulation conditions (1800 Hz frequency, 0.4 bar pressure, 3 amplitude) were set up to obtain microspheres of optimal form and physicochemical properties. Formed microspheres were kept on a magnetic stirrer for an additional 30 min to promote gel strengthening. Microspheres were washed three times with deionized water to remove excess $CaCl_2$. Control microspheres were prepared using the same procedure.



a)

b)

Figure 2 a) Microencapsulation device, Encapsulator Büchi-B390, BÜCHI Labortechnik AG, Switzerland and b) microspheres applied to a Petri dish after filtering and washing)

Results

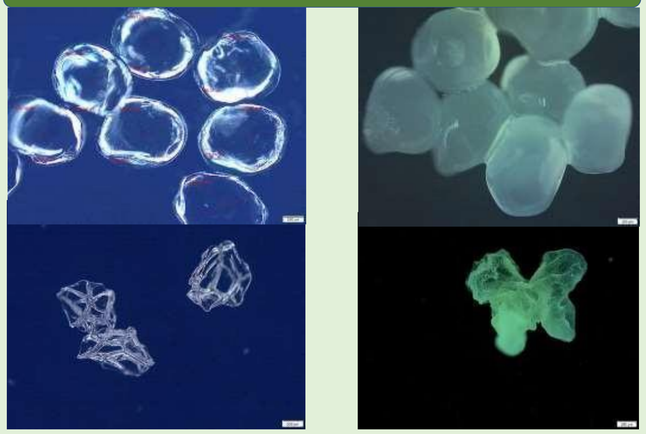


Figure 4. Left to right, optical microscope microphotographs of wet and dry microspheres (without and *with Lactiplantibacillus plantarum* cells

***In Vitro* Release Profiles of *Lactiplantibacillus plantarum* cells from Microspheres**

Release kinetics parameters from wet microspheres were monitored by dispersion of 1 g of microspheres with Lactiplantibacillus plantarum strain in 10 mL of liquid BHI medium. Sampling was performed in duplicate after 0, 5, 7, 10, 20, 30, and 40 days after microencapsulation (n = 14). For each sampling, a control was prepared where 4 g of empty microspheres were weighed in a Falkon tube with the addition of 10 mL of liquid BHI medium (n = 7) at room temperature. After incubation, individual colonies were counted at dilutions where their number ranged between 30- 300. The number of colonies forming units (colony forming units) was calculated according to the formula: CFU=(number of colonies grown/sample volume used) x reciprocal of the decimal dilution The obtained CFU was expressed as a logarithmic value of CFU/mL of supernatant or CFU/g of microspheres.

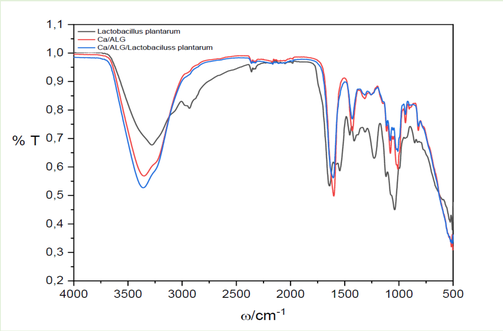


Figure 6. FTIR spectra of lyophilized cells of *Lactiplantibacillus plantarum* (black line), microsphere Ca/ALG (red line) and microsphere Ca/ALG/*Lactiplantibacillus plantarum* (blue line)

Conclusions

- Novel microspheres filled with Lactiplantibacillus plantarum cells were prepared and characterized
- Prepared microspheres loaded with Lactiplantibacillus plantarum cells are stable and easily applicable.
- From the results of the power-law equation, the release of Lactiplantibacillus plantarum cells was governed by the diffusion mechanism/anomalous transport.
- The main interactions in prepared microspheres are electrostatic.
- The prepared microspheres containing apitoxin were spherical, but due to the small sizes and the presence of water molecules, they tended to coalesce together. After drying to a constant mass the size of the microspheres decreased. It was observed that the initial spherical shape was lost and became irregular resulting in a relatively wrinkled surface.

Acknowledgments

The research was done as part of the project: "Potential of microencapsulation in cheese production", KK.01.1.1.04.0058.

The funding was ensured from the Operational Program Competitiveness and Cohesion 2014 – 2020. Investment in science and innovations - first call, KK.01.1.1.04.