KAUNAS UNIVERSITY OF TECHNOLOGY

SIGITA JEZNIENĖ

EVALUATION OF PROBIOTIC PROPERTIES OF *LACTOBACILLUS REUTERI, LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS HELVETICUS* ISOLATED FROM SOURDOUGH, THEIR ENCAPSULATION IN BIOPOLYMERIC SYSTEMS AND APPLICATION IN COSMETICS

Summary of Doctoral Dissertation
Technological Sciences, Chemical Engineering (T 005)

Kaunas, 2024

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

SIGITA JEZNIENĖ

***LACTOBACILLUS REUTERI*, *LACTOBACILLUS PLANTARUM* IR *LACTOBACILLUS HELVETICUS* BAKTERIJŲ, IŠSKIRTŲ IŠ RAUGŲ, PROBIOTINIŲ SAVYBIŲ VERTINIMAS, KAPSULIAVIMAS BIOPOLIMERINĖSE SISTEMOSE IR TAIKYMAS KOSMETIKOJE**

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ABBREVIATIONS

LAB	lactic acid bacteria
W/O/W	water-in-oil-in-water double emulsion

List of microorganism genera

B. cereus	*Bacillus cereus*
B. circulans	*Bacillus circulans*
C. freundii	*Citrobacter freundii*
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
K. pneumoniae	*Klebsiella pneumoniae*
L. helveticus	*Aactobacillus helveticus*
L. monocytogenes	*Listeria monocytogenes*
L. plantarum	*Lactobacillus plantarum*
L. reuteri	*Lactobacillus reuteri*
P. aeruginosa	*Pseudomonas aeruginosa*
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
S. typhimurium	*Salmonella typhimurium*

1. INTRODUCTION

Motivation for the research. The gradual increase in immune-related health problems, along with the rising healthcare costs, and the emergence of antibiotic-resistant pathogens have resulted in humanity's inclination toward natural healthcare remedies and the search for alternative medical treatments. Probiotic therapy is one of the alternative options gaining momentum in the field of health care. The use of probiotics is a method of infection control in the modern era. Protection from pathogenic microorganisms that is provided by probiotic bacteria is the basis of probiotic therapy.

Humans never cease to pursue aesthetics. Problems of the skin and oral cavity, such as skin hyperpigmentation, wrinkles, skin hydration, and bad breath, caused by improper care or external factors, force us to constantly look for ways to solve them. In addition to aesthetic imperfections, existing skin diseases such as dermatitis, acne, eczema, rosacea, etc. are also solved, or, at least, their symptoms are alleviated with the use of specially adapted dermatological cosmetics. As there are many causes of these skin problems, scientists are constantly developing safe and effective skincare products to address these issues. In recent years, experts and scientists have established that probiotics can be used as components of cosmetic products to solve the problems mentioned above. In addition, experimental studies have proven that probiotics do not have a toxic effect on human cells and can be widely applied in the field of skincare and dermatological cosmetics. Bacteriotherapy in the form of probiotic products inhibiting oral and skin pathogens is a promising and cost-effective concept, especially when used preventively.

In the cosmetics industry, it is challenging to develop topical formulations which would maintain the viability of real probiotics throughout the product's declared shelf life. The high amount of free water in cosmetic products allows inactivated microorganisms to rehydrate, multiply and eventually die, thereby necessitating anhydrous oil-based cosmetic formulations. However, the question is how easily microorganisms can detach from the oil applied to human skin and become metabolically active to provide the desired probiotic effect. Also, the majority of cosmetic formulations are not produced under aseptic conditions. Moreover, it is understood that the use of a cosmetic product is also not sterile, and therefore preservatives with bactericidal and/or bacteriostatic effects are used, which inevitably affects the viability of probiotics. One of the ways to maintain the viability of microorganisms in these systems is the encapsulation of probiotic bacteria. In the literature, the possibility of using probiotic bacteria in cosmetics is usually noted without specifying the exact and precise formulations of cosmetics or the principles of their composition. Furthermore, the selection of encapsulation technologies is very broad. Therefore, it is important to assess which microorganisms are encapsulated, and how specific strains will be affected by the

process parameters and the materials used. Additionally, there is a lack of research on the interaction of these bacteria with cosmetic products. A number of probiotic bacteria encapsulation methods have been described in the literature, but the viability of such encapsulated bacteria in cosmetics is still not clear. As a result, research on the encapsulation and practical applicability of probiotics in cosmetics is extremely relevant.

The aim of the thesis. This work is aimed to investigate the probiotic properties of different lactic acid bacteria (LAB) *Lactobacillus* species isolated from bread sourdough to encapsulate them in different biopolymeric systems and, after selecting the most suitable system, to evaluate the possibilities of practical use of the encapsulated bacteria in cosmetics.

The objectives of the research:

1. To determine the probiotic properties of *Lactobacillus reuteri* 182, *Lactobacillus plantarum* F1, and *Lactobacillus helveticus* 305 strains isolated from bread sourdough by various *in vitro* methods.
2. To study the physical and mechanical properties of double emulsions and to evaluate the possibilities of LAB encapsulation in them.
3. To study the physical and mechanical properties of alginate hydrogel capsules obtained by different methods and to evaluate the possibilities of LAB encapsulation in them.
4. After evaluating the various parameters of different encapsulation systems, to select the most suitable system for the encapsulation of live LAB cells.
5. To modify the selected encapsulation system by co-encapsulating prebiotics or coating the capsules with an additional layer of chitosan in order to increase the viability of LAB during long-term storage.
6. To investigate the application possibilities of lactic acid bacteria encapsulated in the selected system in cosmetic formulations.

The scientific novelty of the research. The probiotic properties and ability to colonize the human skin of the indigenous bacterial cultures isolated from bread sourdough, to which no commercial leaven has been added, have been investigated. For the first time, the individual compatibility of the encapsulation systems with specific potentially probiotic lactic acid bacteria, as well as the effect on bacteria dynamics, has been assessed. Having optimized the method chosen for an individual LAB culture, a safe and effective encapsulation system has been developed which would allow maintaining the viability of the bacteria and release the live probiotics at the target site after topical application to the host's skin.

Practical significance of the research. After having analyzed and compared several encapsulation systems and highlighted their advantages, we have adapted a system to each probiotic microorganism individually. We have developed an encapsulation system with high encapsulation efficiency and high

viability during long-term storage. This system has been successfully applied in the developed pilot probiotic cosmetic products.

Defended claims of the dissertation:

1. Bacteria isolated from bread sourdough and not belonging to commercial collections have probiotic properties and can colonize the human skin.
2. Non-spore-forming probiotic bacteria can be protected from the negative effects of preservatives and other ingredients of cosmetic compositions by encapsulating them, and thus probiotics can be used in model cosmetic products.

2. OBJECT AND METHODS

2.1. Research Scheme

The overall structure of the research is presented in Figure 2.1.

2.2. Research Objects and Methods

All objects and methods used in the research are outlined in Table 2.1.

Table 2.1. Research objects, analyzed properties and methods used

Objects	Properties	Methods
LAB strains: *L. reuteri* 182 *L. plantarum* F1 *L. helveticus* 305	Cell surface hydrophobicity and autoaggregation	Spectrophotometry (Krausova, Hyrslova and Hynstova, 2019)
	Biofilm formation	Crystal violet assay
	Antimicrobial activity	Agar well diffusion method
	Co-aggregation with pathogens	Spectrophotometry (Tuo, Yu, Ai, Wu, Guo and Chen, 2013)
	Inhibition of pathogenic biofilm formation	Counting of pathogen cells by the pour plate method (Woo, Ahn, 2013)
	Adhesion to HaCaT cells	Counting of LAB cells by the pour plate method; microscopy (Coman et al., 2019)
Encapsulation of LAB in W/O/W emulsions	Encapsulation efficiency and LAB viability during storage	Counting of LAB cells by the pour plate method
Encapsulation of LAB in cross-linked alginate capsules	Encapsulation efficiency and LAB viability during prolonged storage	Counting of LAB cells by the pour plate method
Modified selected encapsulation system	Morphology	SEM analysis; H&E staining; microscopic analysis
	Mechanical properties	Texture analysis: Young's modulus
	Encapsulation efficiency, release and viability of LAB during prolonged storage	Counting of LAB cells by the pour plate method
Cosmetic formulations: hand cream and toothpaste	Preservative challenge test	Counting of pathogen cells by the pour plate method
	Stability of cosmetic products	pH-metry and visual monitoring under accelerated conditions
	Viability of LAB during storage	Counting of LAB cells by the pour plate method
	Release of LAB cells at the time of consumption	Targeted surface swabbing; counting of LAB cells by the pour plate method

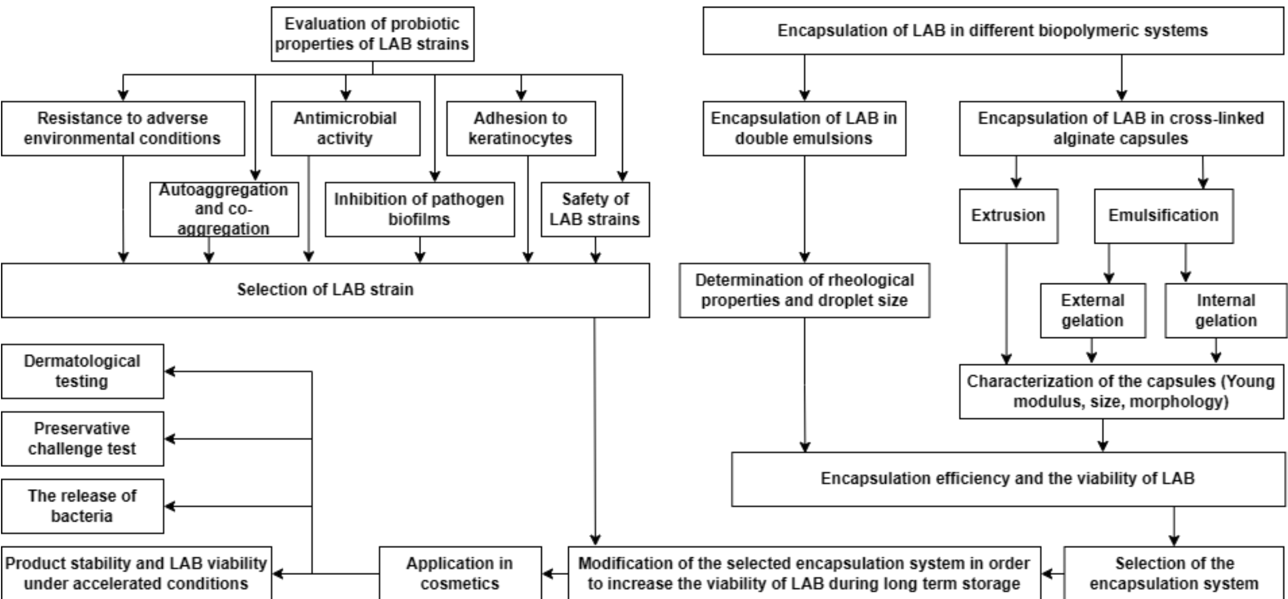

Figure 2.1. Research scheme

3. RESULTS AND DISCUSSION

3.1. *In vitro* Characterization of Probiotic Properties of LAB

3.1.1. Evaluation of LAB surface properties and cohesion

Cell surface hydrophobicity correlates with autoaggregation – the majority of higher hydrophobicity-holding strains have a higher autoaggregation ability (Collado, Meriluoto and Salminen, 2008). Such propensity is reflected well in our study. The correlation coefficient *r* between these two variables showed strong statistical dependency. Among the analyzed cultures, *L. plantarum* F1 was the least hydrophobic with the weakest autoaggregation. Meanwhile, the most hydrophobic *L. helveticus* 305 cells (11.15%) held also significantly ($p < 0.05$) the highest autoaggregation ability which was equal to 53.32%.

Another important characteristic of microorganisms is biofilm formation. In relation to pathogenic microorganisms, the formation of biofilms, which leads to their greater virulence, is undesirable. For probiotic bacteria, in contrast, it is considered a beneficial property which promotes colonization and a longer survival in the host (Terraf, Juarez, Nader-Macias and Silva, 2012). During the experiment with LAB strains, our study found that none of the tested cultures formed a biofilm on the polystyrene surface; the absorbance (570 nm) exceeded 0.1 in all the cases under investigation.

3.1.2. Antimicrobial activity of LAB and biological competition

The results of the antimicrobial activity of LAB against the selected bacteria that could be detected on the surface of human skin and mucosa and/or could cause skin or wound infections are presented in Table 3.1.

Table 3.1. Antimicrobial activity of LAB against pathogenic and opportunistic pathogenic microorganisms

Microorganism	**Inhibition zone, mm**		
	***L. reuteri* 182**	***L. plantarum* F1**	***L. helveticus* 305**
	Gram-positive		
B. cereus	11.3 ± 1.0^{aA}	17.8 ± 0.5^{bB}	18.0 ± 0^{cB}
B. circulans	13.0 ± 0.4^{bA}	19.4 ± 0.5^{cB}	19.3 ± 0.5^{dB}
C. freundii	19.1 ± 1.0^{cA}	19.3 ± 0.5^{cA}	21.3 ± 0.5^{eB}
E. faecalis	-	14.5 ± 0.6^{aA}	16.4 ± 0.5^{bB}
S. aureus	-	16.8 ± 0.5^{bB}	14.5 ± 0.6^{aA}
S. epidermidis	-	15.5 ± 0.6^{aA}	16.5 ± 0.6^{bB}
L. monocytogenes	19.5 ± 0.6^{cA}	19.6 ± 0.5^{cdA}	21.3 ± 0.5^{eB}
	Gram-negative		
E. coli	11.3 ± 0.5^{aA}	15.5 ± 0.6^{aC}	13.5 ± 0.6^{aB}
K. pneumoniae	11.1 ± 0.3^{aA}	15.0 ± 0^{aB}	16.0 ± 0.8^{bC}
P. aeruginosa	14.5 ± 0.6^{dA}	20.6 ± 0.5^{dC}	18.3 ± 0.5^{cB}
S. typhimurium	11.5 ± 0.6^{aA}	17.0 ± 0.8^{bB}	16.8 ± 0.5^{bB}

The results are presented as means with standard deviations; different lowercase letters in columns and different uppercase letters in rows indicate statistically significant ($p < 0.05$) differences

The experiment results show that *L. reuteri* 182 had the strongest antagonistic effect to *C. freundii* and *L. monocytogenes* as the diameter of the inhibition zone was 19.1 mm and 19.5 mm, respectively. Another tested LAB culture, *L. plantarum* F1, showed bactericidal activity against all the tested pathogenic cultures. Three gram-positive cultures – *B. circulans*, *C. freundii* and *L. monocytogenes* – and one gram-negative culture – *P. aeruginosa* – were the most sensitive to the antimicrobial activity of *L. plantarum* F1. As in the case of *L. reuteri* 182, significantly ($p < 0.05$) the strongest antimicrobial effect was exhibited by *L. helveticus* against *C. freundii* and *L. monocytogenes*. After performing the correlation analysis of the experimental data, no statistical relationship was observed between the variables. Therefore, it can be concluded that the antimicrobial activity of LAB is highly specific and does not depend on the composition of the cell wall of the target microorganism.

Another selected indirect method for the evaluation of the antimicrobial activity of LAB is the determination of their co-aggregation with pathogenic and opportunistic pathogenic microorganisms. The ability to co-aggregate is very important for the formation of biofilms, their inhibition and biological competition for binding sites. Also, probiotics with the property to co-aggregate with pathogens can produce antimicrobial substances very close to the pathogens, and thus can affect them more strongly (Keller, Hasslöf, Steckén-Blicks and Twetman, 2011).

The results exhibited in (Fig. 3.1) suggest that the highest co-aggregation of *L. reuteri* 182 occurred with *S. epidermidis*.

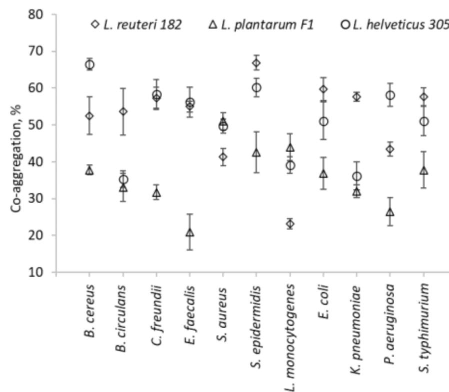

Figure 3.1. Co-aggregation ability of LAB strains with pathogens and opportunistic pathogens

Meanwhile, *L. helveticus* 305 co-aggregated most strongly with *B. cereus*. *L. plantarum* F1 had the weakest co-aggregation with almost all tested pathogens. It should be noted that the highest co-aggregation ability of *L. plantarum* F1 was

found with *L. monocytogenes*, while the co-aggregation of *L. reuteri* 182 with this culture was almost twice as low as with all the other cultures tested. Such results once again proved how uniquely microorganisms interact with each other.

The analysis of the results of the inhibition of pathogenic biofilm formation showed that statistically significant ($p < 0.05$) inhibition was achieved in all cases (Fig. 3.2). The strength of the inhibition varied depending on the LAB strain and the target pathogen, as well as on the method used. The complete inhibition of *S. aureus* biofilm was achieved when co-cultured with *L. helveticus*. The inhibitory effect of *L. reuteri* 182 strain on *S. aureus* biofilm formation was similar to *L. plantarum* F1. Viable cells of *L. monocytogenes* were not detected when co-cultured with *L. reuteri* 182 and *L. helveticus* 305 strains, and complete inhibition of *L. monocytogenes* was observed in both tested methods with *L. helveticus* 305. The biofilm formed by *E. coli* was the most resistant to LAB cultures as complete inhibition of biofilm was not achieved in any of the cases. In the presence of *L. plantarum* F1, the number of *E. coli* biofilm cells was reduced by 6.67 lg in a competition assay. The ability of two other tested LAB strains to displace and competitively inhibit the biofilm formation by *E. coli* was weaker. Although the biofilm formed by *P. aeruginosa* was the strongest of all the pathogens tested, the co-cultivation with *L. plantarum* F1 and *L. helveticus* 305 completely inhibited pathogen biofilm formation.

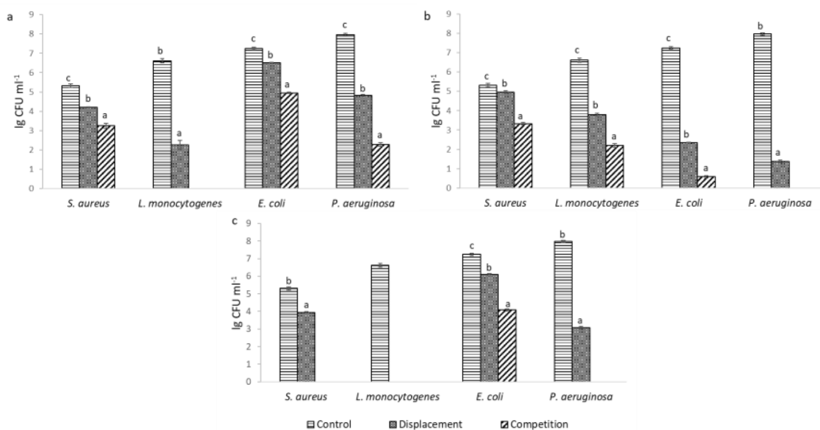

Figure 3.2. Inhibition of pathogenic biofilms: a – *L. reuteri* 182; b – *L. plantarum* F1; c – *L. helveticus* 305; results of the same pathogenic culture marked with different lowercase letters are statistically significantly ($p < 0.05$) different

3.1.3. Adherence of LAB to human skin cells

To find out whether LAB bacteria will be able to colonize the human skin, the immortalized human skin keratinocyte cell line (HaCaT) was chosen, and the

ability of PRB to adhere to said cells was measured. From the presented results (Fig. 3.3), it can be seen that two of the tested LAB strains exhibited very high adhesive capacity.

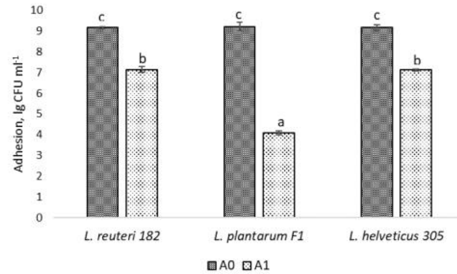

Figure 3.3. Adhesion of LAB cells to HaCaT: A0 – the initial amount of microorganisms used in the experiment; A1 – the number of LAB cells attached to keratinocytes; results marked with different lowercase letters are statistically significantly ($p < 0.05$) different

The adhesion capacity of *L. reuteri* 182 and *L. helveticus* 305 was similar and reached 77.94% and 77.63%, respectively. The ability of *L. plantarum* F1 to adhere to HaCaT cells was almost two times lower. Researchers Coman et al. (2019) analyzed the adhesion ability of the probiotic preparation SYNBIO®, consisting of *L. rhamnosus* IMC 501 and *L. paracasei* IMC 502, to human keratinocyte cells. The adhesion capacity was 19%. The conclusion presented by the authors mentions that such adhesion is sufficient for the successful use of probiotics in topical preparations.

The adhesion of LAB to HaCaT cells was verified by microscopic observations (Fig. 3.4).

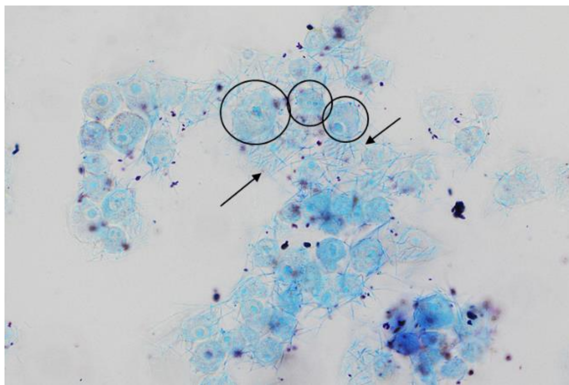

Figure 3.4. *L. helveticus* 305 bacteria attached to HaCaT cells (magnification 1000x); round keratinocyte cells are indicated by circles, and rod-shaped bacteria are indicated by arrows

3.2. Evaluation and Selection of Different Biopolymeric Encapsulation Systems

3.2.1. Encapsulation efficiency and viability of PRB during storage in double emulsions

Double emulsions were produced by alternating the components of the internal aqueous (v_1) and oil (a) phases. After determining the encapsulation efficiency, it was established that *L. helveticus* 305 is not suitable for this encapsulation method. The encapsulation efficiency of this strain was only 55.44%. Such low encapsulation efficiency could be due to the toxic effect of oxygen which was introduced into the system during the homogenization process. The encapsulation efficiency of *L. reuteri* 182 in double emulsions was also relatively low. The highest efficiency was achieved by the encapsulation of *L. plantarum* F1 cells (73.63%). The bacterial cell size of *L. plantarum* F1 cells is known to be the smallest among the studied LAB strains. It shows that *L. reuteri* 182 cells were too large to fit in the v_1 phase droplets.

As illustrated in Figure 3.5, the viability of bacteria I–IV emulsions did not differ after 7 days of storage at an elevated temperature (37 ºC) and reached about 93–94%. Statistically significantly ($p < 0.05$) higher survival levels were achieved by adding sea buckthorn extract to the oil phase of the double emulsion, and mannitol (V) and trehalose (VI) to the internal aqueous phase v_1. During the continuation of the experiment of storage under accelerated conditions, more significant differences were observed.

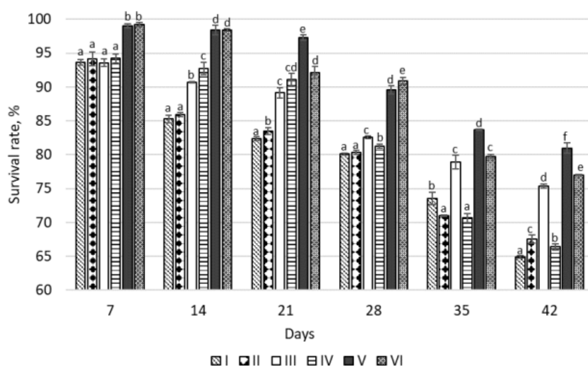

Figure 3.5. Influence of the composition of emulsions on the survival of *L. plantarum* F1 during storage at 37 ºC; lowercase letters indicate statistically significant ($p < 0.05$) differences when comparing emulsion types

In emulsions I and II, the survival rate of *L. plantarum* F1 cells was the lowest throughout the experiment. In contrast, the presence of mannitol provided the greatest protective effect on cells (emulsion V). After 42 days of storage under

accelerated conditions, the survival rate of bacteria in this emulsion was 80.99%. The enhanced viability of probiotic cells entrapped with prebiotics in the double emulsion inner phase was reported by other researchers (Frakolaki, Katsouli, Giannou and Tzia, 2020, Yee, Yee, Lin and Phing, 2019).

3.2.2. Encapsulation efficiency and viability of LAB in cross-linked alginate capsules

Extremely low encapsulation efficiency was observed when the capsules were formed by extrusion technique and calcium chloride was used as the cross-linking agent.

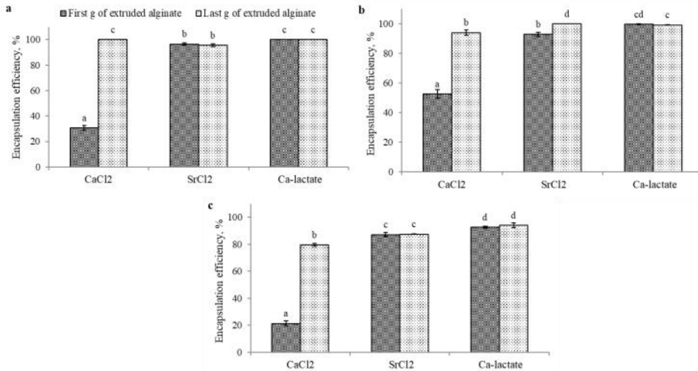

Figure. 3.6. Encapsulation efficiency of LAB in capsules obtained by extrusion method using different cross-linkers: a – *L. reuteri* 182; b – *L. plantarum* F1; c – *L. helveticus* 305

The encapsulation efficiency of *L. plantarum* F1 was 52.5% for the first 1 g (out of 10 g) of the extruded alginate. The encapsulation yield of *L. reuteri* 182 cells was 30.7% and only 21.2% for the *L. helveticus* 305, as shown in Fig. 3.6. In contrast, the viability of the cells in the lastly extruded alginate capsules was significantly ($p < 0.05$) bigger as it reached 93.9% for *L. plantarum* F1, 79.6% for *L. helveticus* 305, and showed the perfect encapsulation efficiency increase for *L. reuteri* 182 by scoring 100%. The obtained results are extremely important because $CaCl_2$ is probably the most widely used salt in encapsulation, but its toxicity to the encapsulated microorganisms has received very little attention in scientific research.

For the production of capsules by the extrusion method, the safest choice is to use calcium lactate salt. The overall encapsulation efficiency reached more than 92% with all the tested LAB strains in the capsules obtained with Ca-lactate.

The results of the emulsification-external gelation method experiment revealed that this method was best suited for *L. reuteri* 182 culture as the encapsulation efficiency of these bacteria was 100% regardless of the salt used.

Unlike in the case of extrusion, $CaCl_2$ salt did not have such a strong influence on the encapsulation efficiency of LAB cells in the capsules obtained by this method. This can be explained by the fact that the concentration of ions per drop of alginate-LAB in the emulsion is lower than when encapsulating by the extrusion method.

It appeared that the efficiency of the capsules obtained by the internal gelation method was lower than that of the external gelation and varied depending on the LAB strain.

The variation of LAB cell viability in macrocapsules during prolonged storage (365 days) at 4 °C is presented in Figure 3.7.

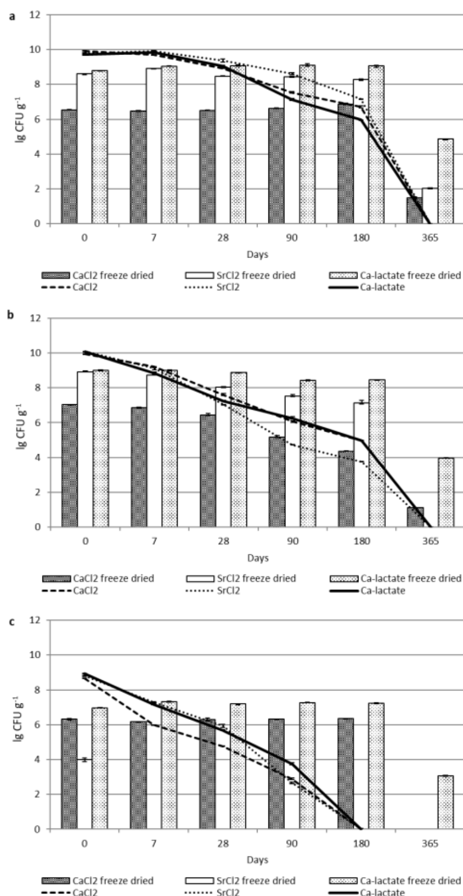

Figure 3.7. Viability of the encapsulated LAB in capsules obtained by extrusion method using different cross-linkers: a – *L. reuteri* 182; b – *L. plantarum* F1; c – *L. helveticus* 305

The viability of LAB cells in wet Ca-alginate and Sr-alginate capsules tended to decrease throughout the experiment, and no viable bacteria were detected after 365 days of storage. *L. reuteri* 182 and *L. plantarum* F1 were the most stable and demonstrated the highest viability in freeze-dried macrocapsules obtained by cross-linking alginate with calcium lactate salt. It is seen that the viability of *L. helveticus* 305 decreased more rapidly than the other tested LAB strains, and, after 180 days, no viable bacteria were present in the wet capsules. At the end of the experiment, viable cells of *L. helveticus* were found only in the freeze-dried capsules obtained by using calcium lactate. It is particularly important to mention that, after freeze drying, the viability of microorganisms decreased by 1–2 lg units, and, with calcium chloride, the viability decreased by ~3 lg units. The viability of microorganisms during freeze drying can be lost due to mechanical stress during the formation of ice crystals, high osmotic pressure, and water loss (Chávez, Ledeboer, 2007; Zayed, Roos, 2004). *L. reuteri* 182 and *L. plantarum* F1 demonstrated the highest viability in freeze-dried macrocapsules obtained with calcium lactate salt. It is notable that the viability of *L. helveticus* 305 decreased more rapidly than the other tested LAB strains, and, after 180 days, no viable bacteria were detected in the wet capsules. At the end of the experiment, viable cells of *L. helveticus* were found only in the freeze-dried capsules obtained by using calcium lactate. It is extremely important to emphasize that, after freeze drying, the viability of microorganisms decreased by 1–2 lg units, and, with calcium chloride, the viability decreased by ~3 lg units. The viability of microorganisms during freeze drying can be lost due to mechanical stress during the formation of ice crystals, high osmotic pressure, and water loss (Chávez, Ledeboer, 2007; Zayed, Roos, 2004).

The viability of LAB in microcapsules obtained by the emulsification-external gelation method is presented in Fig. 3.8. Having examined the viability of LAB in microcapsules obtained by the emulsification-external gelation method, it was determined that, at the end of the experiment, the amount of viable *L. reuteri* 182 bacteria in microcapsules obtained by using Ca-lactate reached as high as 6.71 lg CFU g^{-1}. Importantly, this value was the closest to the recommended amount of 10^7 CFU viable probiotic bacteria at the time of consumption. The viability of *L. plantarum* F1 varied similarly, only in this case the maximum amount of viable bacteria after 365 days was 4.35 lg CFU g^{-1}. Meanwhile, the viability of *L. helveticus* decreased the most among all the tested LAB strains. After 365 days, microcapsules made with calcium lactate were the only ones in which viable *L. helveticus* 305 bacteria were still detected. The viability of *L. reuteri* 182 in freeze-dried microcapsules obtained by the emulsification-internal gelation method was 3.17 lg CFU g^{-1} with $CaCO_3$ and 2.92 lg CFU g^{-1} with Ca-EDTA. *L. plantarum* F1 survived until the end of the experiment only in capsules obtained with calcium carbonate, and so did *L. helveticus* 305, yet the viability of the latter strain was relatively low and reached only 1.03 lg CFU g^{-1}.

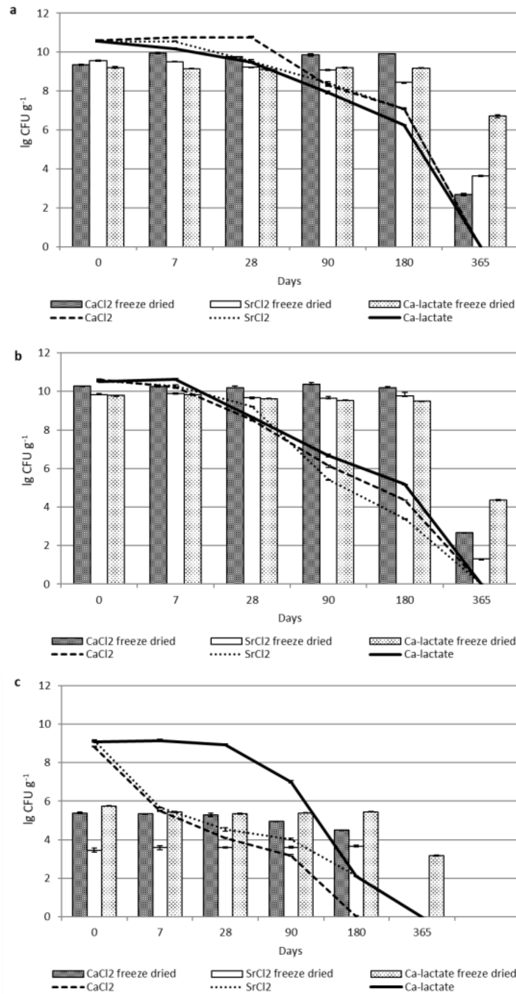

Figure 3.8. Viability of encapsulated LAB in capsules obtained by emulsification-external gelation using different cross-linkers: a – *L. reuteri* 182; b – *L. plantarum* F1; c – *L. helveticus* 305

Summarizing the obtained results, it can be stated that the most suitable source of divalent ions for the encapsulation of *L. reuteri* 182, *L. plantarum* F1 and *L. helveticus* 305 bacteria is Ca-lactate. Even though, after freeze drying, the viability of all LAB decreased by 1–3 lg units, to achieve stable viability during

prolonged storage, it is necessary to freeze-dry the obtained capsules. Stable viability of encapsulated microorganisms was also achieved by several other researchers, for example, after 42 days of storage, the number of viable *L. acidophilus* bacteria was 5.50 lg CFU g^{-1} in calcium alginate capsules (Mortazavian et al., 2008). Another study found that *E. faecium* MC13 remained viable in calcium alginate capsules coated with chitosan for 6 months (Kanmani et al., 2011). However, no studies of 365 days could be found in the scientific literature.

Taking into account that the sufficient amount of viable probiotic bacteria in the final consumer product is an essential criterion for the effectiveness of the product, the research findings on the monitoring of the encapsulation efficiency of LAB and the viability during storage provided extremely significant information for the use of probiotics in further products in the full range of areas, including food, pharmaceuticals, and cosmetics.

3.3. Enhancing the Viability of Encapsulated LAB in a Selected Biopolymer System and Modifying Capsule Properties

Based on the obtained results presented in section 3.2, LAB was encapsulated by the emulsification-external gelation method for further research. To additionally protect cells from negative impact during freeze-drying and TO further increase the viability during prolonged storage, several modifications of biopolymeric systems were performed, as illustrated in Table 3.2.

Table 3.2. Modification of calcium alginate capsules

Type	Modification
A	Control Ca-alginate capsules
A-T	Co-encapsulation with 4% of trehalose
A-T-P	Co-encapsulation with 4% of trehalose and 2% of inulin
A-Ch	Coating of capsules with chitosan

Since some properties that are extremely significant in further use cannot be determined in microcapsules, LAB was additionally encapsulated in macrocapsules obtained by the extrusion method which were assumed to perform analogous modifications.

3.3.1. Morphological and mechanical properties of modified Ca-alginate capsules

SEM images showed that trehalose had no significant effect on the surface morphology of the macrocapsules (A-T) in comparison with control (A) (Fig. 3.9). With the addition of inulin (A-T-P), the height and the thickness of surface irregularities decreased. Such results were in accordance with Balanč et al. (2016). Further analysis of SEM images revealed that freeze-dried A-Ch capsules remained spherical, with a smoother surface and without sharp edges. This means

that the external coating of the capsules with chitosan helped to protect the calcium alginate hydrogel matrix from collapse.

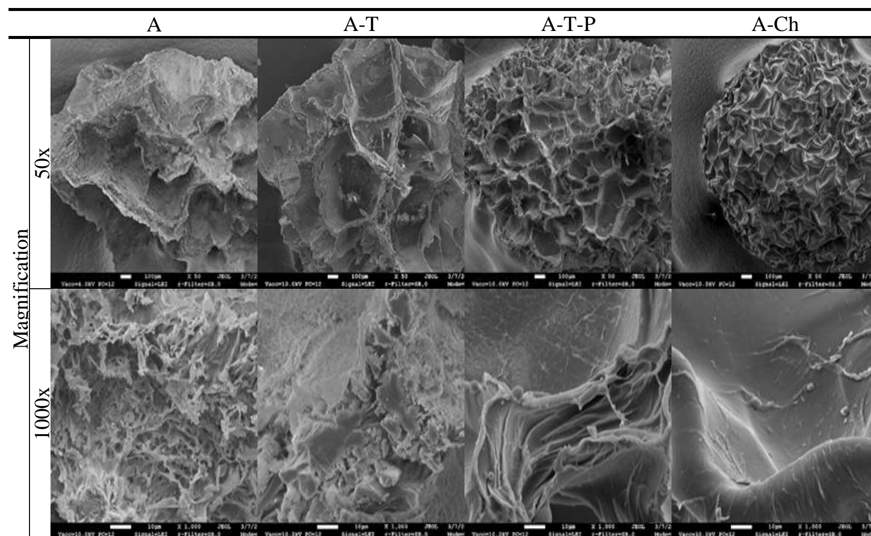

Figure 3.9. Scanning electron microscopy images of the various modifications of calcium alginate beads

After analyzing the microphotographs of the sections of the capsules stained with hematoxylin-eosin dye, rod-shaped LAB cells were stained darker than the matrix itself. No differences were observed between A, A-T and A-T-P capsules. However, after staining the A-Ch capsules, a darker-stained outer chitosan layer surrounding the capsules was observed.

Young's modulus of A-T macrocapsules remained similar to that of A capsules despite the addition of trehalose to the encapsulation matrix. Young's modulus of A-T-P capsules significantly ($p < 0.05$) decreased from 507 kPa to 471 kPa, as compared to the control sample A. Since trehalose had no significant effect on Young's modulus, it can be claimed that the addition of inulin reduced the stiffness of the hydrogel, which also affected the overall hardness. Such a result could be due to the fact that the degree of crosslinking of alginate decreased when inulin fibers were inserted between sodium alginate biomacromolecules. The reduced stiffness of calcium alginate capsules after the addition of inulin to the encapsulation matrix was also recorded by researchers, see Balanč et al. (2016). The synergistic interaction of polymers Ca-alginate and chitosan increased the value of Young's modulus of A-Ch hydrogel from 507±11 kPa to 658±17 kPa in comparison with the control (A) capsules. The ionic bonding between chitosan and calcium alginate functional groups created a rigid surface coating and strengthened the hydrogel. The positive effect of chitosan on the mechanical

properties of calcium alginate capsules was also stated in the publications of other authors (Obradović et al., 2015; Lin, Chang, and Peng, 2018).

This part of the experiment revealed that modifications of the capsules had a significant influence on their properties. Therefore, before making even minimal changes in the composition of the capsules, it is necessary to check their influence on the physical, mechanical and morphological properties.

3.3.2. Encapsulation efficiency of LAB, release and viability in modified Ca-alginate capsules

Examination of the encapsulation efficiency in modified calcium alginate capsules showed that the highest encapsulation efficiency was achieved in control capsules (A) and capsules with trehalose (A-T). However, in capsules A, the viability of LAB after freeze-drying was the lowest. It can be concluded that trehalose acted as a cryoprotectant. Co-encapsulation of trehalose and inulin with LAB significantly ($p < 0.05$) decreased the encapsulation efficiency. Similar results have been reported by other authors (Gandomi, Abbaszadeh, Misaghi, Bokaie and Noori, 2016; Kim et al., 2017; Chan, Pui, 2020). The reduction in encapsulation efficiency can be explained by prebiotics occupying part of the internal space of the capsules (Ng, Lai, Nyam and Pui, 2019).

According to other authors' studies, the chitosan coating did not significantly affect the encapsulation efficiency (Cook, Tzortzis, Charalampopoulos and Khutoryansky, 2011; Darjani, Nezhad, Kadkhodaee and Milani, 2016). However, our study showed that encapsulation efficiency decreased significantly ($p < 0.05$) after coating the capsules with a layer of chitosan, and its value was only 43.95%. The results demonstrated that chitosan exhibited antibacterial activity against LAB. According to Li and Zhuang (2020), positively charged protonated amino groups in the chitosan structure interact with negatively charged molecules on the surface of bacterial cells. This causes irreversible membrane permeabilization and leakage of intracellular substances (Li, Zhuang, 2020). Most likely, the cells located on the surface of the capsules were affected by chitosan, and, for this reason, the encapsulation efficiency decreased.

Based on the data of the conducted research (Figure 3.10), it can be stated that the modifications of calcium alginate microcapsules had a significant ($p < 0.05$) influence on the release rate of LAB cells. Moreover, the amount of the released bacteria varied depending on the LAB strain. Irrespective of the modification, *L. reuteri* 182 bacteria were mostly released, when comparing the strains.

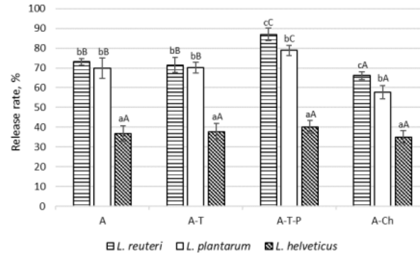

Figure 3.10. Effect of Ca-alginate microcapsule modifications on LAB release; different lowercase letters indicate statistically significant ($p < 0.05$) differences between strains; different uppercase letters indicate statistically significant ($p < 0.05$) differences between capsule modifications

The variation of encapsulated LAB viability during storage is presented in Fig. 3.11.

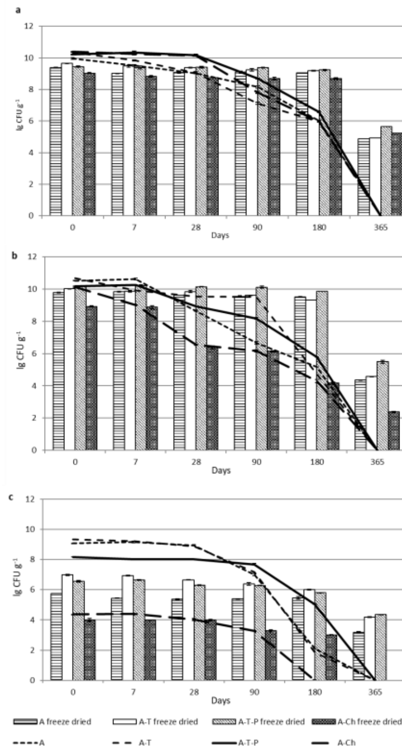

Figure 3.11. Viability of LAB in modified Ca-alginate capsules during prolonged storage: a – *L. reuteri* 182; b – *L. plantarum* F1; c – *L. helveticus* 305

It was determined that the highest viability of *L. reuteri* 182 after 1 year of storage was in freeze-dried A-T-P capsules and reached 6.97 lg CFU g^{-1}. In conclusion, trehalose and inulin not only showed a protective effect during freeze-drying but also increased the viability of LAB during storage. This finding is in agreement with the results previously reported by Nami, Lormezhad, Kiani, Abdullah and Haghshen (2020) and Ng, Lai, Nyam and Pui (2019). The prebiotics used in the experiment acted not only as cryoprotectants but also could have a positive effect on the recovery of LAB cells by acting as nutrients. The lowest number of viable LAB cells was detected in A-Ch capsules.

3.4. Application of Selected Biopolymer System with Encapsulated LAB in Cosmetics

The analysis of modified calcium alginate capsules led to the conclusion that it is most appropriate to co-encapsulate inulin and trehalose with LAB. Also, based on the analysis of LAB's probiotic properties and viability in capsules, further practical application studies with *L. reuteri* 182 strain were conducted.

In order to investigate the practical application of encapsulated LAB in cosmetics, a hand cream and a toothpaste were created.

3.4.1. Safety assessment of cosmetic composition

Preservative challenge test. The effectiveness of the preservative must be checked to prevent the reproduction of pathogenic microorganisms which would pose a risk to human health. Pathogenic microorganisms can enter cosmetic products with contaminated raw materials, during production or normal use. The preservative challenge test showed that the preservative system used in the hand cream and toothpaste was effective. After evaluating the reduction in the amount of microorganisms, the products met the requirements of criterion A.

Dermatological testing. Having carried out the patch tests on the chosen population, it was determined that the products should not cause any irritating effect.

3.4.2. Stability of cosmetic formulations, viability and release of encapsulated LAB

The stability of cosmetic products. Waterman et al. (2007) described a biopharmaceutical stability program under accelerated conditions. The method is based on the Arrhenius equation which determines the dependence of the reaction rate on temperature. The testing of cosmetic products under accelerated conditions is based on this method and allows for relatively accurate prediction of the product's shelf life, thus shortening the product development-testing-marketing period (International Federation of Societies of Cosmetic Chemists [IFSCC], 1992).

In summary, the results of the stability test of cosmetic products showed that the hand cream remained stable for 1 month under accelerated conditions, while no signs of product instability were observed when testing the toothpaste. Based on the results of the pH and organoleptic properties evaluation of the storage at an elevated temperature, the estimated preliminary shelf life for the hand cream is up to 6 months, and at least 6 months for the toothpaste.

Viability of LAB. As expected, the viability of non-encapsulated, planktonic *L. reuteri* 182 bacterial cells in the hand cream was almost halved after 24 hours and completely lost after 48 hours due to the preservative in the product (Figure 3.12). Meanwhile, after encapsulating the cells, the viability of *L. reuteri* 182 bacteria was preserved. When stored at room temperature, even 5.94 lg CFU g^{-1} bacteria were detected after 35 days. When tested under accelerated conditions, the number of viable bacteria was slightly lower and reached 3.67 lg CFU g^{-1} at the end of the experiment.

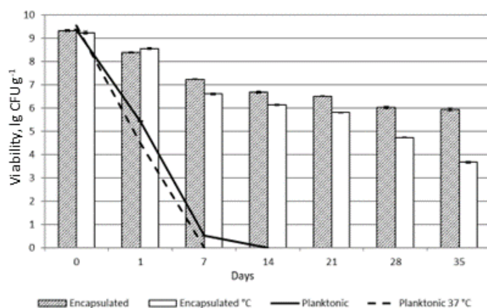

Figure 3.12. Viability of *L. reuteri* 182 in hand cream during storage

Although the effect of toothpaste on the viability of planktonic *L. reuteri* 182 bacteria was similar to that of hand cream, this cosmetic formulation had a stronger effect on encapsulated bacteria (Figure 3.13).

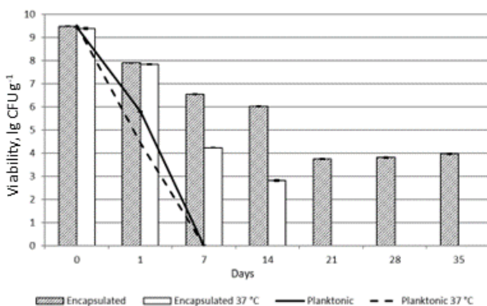

Figure 3.13. Viability of *L. reuteri* 182 in toothpaste during storage

The results demonstrated that, after 7 days of storage under accelerated conditions, the viability decreased drastically, and, after 21 days, all LAB cells had perished. When the sample was stored at 20 ºC temperature, at the end of the experiment, the number of viable *L. reuteri* 182 was 3.97 lg CFU g^{-1}. The increased storage temperature of the product accelerated the reactions, and we can thus make the assumption that the capsules swelled faster. Therefore, the preservative molecules could diffuse through the hydrogel pores more quickly.

In conclusion, to achieve the therapeutic effect of the product, the necessary number of viable *L. reuter*i bacteria in the toothpaste would be maintained for approximately 1 month. The probiotic effectiveness of the bacteria in the hand cream would potentially last longer, as this product had a lower effect on the viability of probiotics.

Release of LAB. It is equally important that the probiotics not only remain viable but also that the required amount of them would be released from the capsules during consumption. When using toothpaste and applying hand cream, the force used is not very high; so, it is important to predict whether such a shear force will be sufficient to fragment the capsules and release the required amount of bacteria (Garti, McClements, 2012). As it can be seen from the data presented in Figure 3.14, the release of bacteria from the capsules in the toothpaste was high and reached 89.02%.

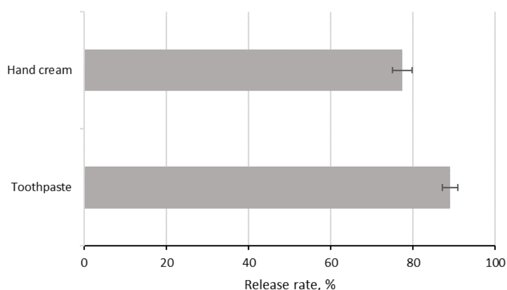

Figure 3.14. Release of encapsulated *L. reuteri* 182 from cosmetic products during consumption

The release from the capsules in the hand cream was slightly lower and reached 77.36%. Therefore, it is recommended to extend the application time of the hand cream or increase the amount of encapsulated LAB cells added to the cream.

4. CONCLUSIONS

1. The results derived from the investigation of the probiotic properties of *lactic acid bacteria* (LAB) isolated from bread sourdough demonstrated that the metabolites of the tested LAB cultures exhibited antimicrobial activity against pathogens. It was also found that LAB was able to co-aggregate with the tested pathogenic microorganisms. Moreover, LAB inhibited the formation of pathogenic *S. aureus*, *L. monocytogenes*, *E. coli*, and *P. aeruginosa* biofilms, and, in some cases, complete inhibition occurred. The potency of the aforementioned probiotic properties was highly specific and varied depending on the LAB strain and the target pathogenic microorganism. Study results further showed that *L. reuteri* 182 and *L. helveticus* 305 demonstrated a very high adhesive ability to HaCaT cells – 77.94±1.84% and 77.63±2.46%, respectively.
2. It was determined that double emulsions with prebiotics and sea buckthorn extract had a protective effect on encapsulated LAB cells during storage at 20 °C and 37 °C temperatures. However, the encapsulation efficiency in such emulsions was not as high as expected and depended on the LAB strain: *L. reuteri* 182 scored 64.46±0.43%, whereas the numbers for *L. plantarum* F1 were 73.63±0.14%, and *L. helveticus* 305 scored 55.44±0.01%.
3. Strontium-alginate capsules proved to have the best mechanical properties, but $SrCl_2$ salt above 5% concentration was toxic to the tested LAB strains, as well as $CaCl_2$. Meanwhile, Ca-EDTA salt at more than 0.1% concentration inhibited the growth of LAB or had a bactericidal effect, depending on the concentration and the strain tested. Thus, after evaluating the toxicity of divalent ions and their salts used for alginate gelation, our findings showed that calcium lactate and calcium carbonate are the most suitable salts for alginate gelation in this regard.
4. After evaluating the effectiveness of PRB encapsulation in biopolymeric systems and the viability of LAB, it can be concluded that the most suitable system for LAB encapsulation is freeze-dried calcium alginate capsules, obtained by the emulsification-external gelation method using calcium lactate as a cross-linking agent. In this system, the encapsulation efficiency of *L. reuteri* 182 was 100%, and the amount of live bacteria was the highest when comparing biopolymer systems and reached as much as 6.71±0.07 lg CFU g^{-1} after 365 days of storage at 4 °C temperature.
5. After carrying out the modifications of the selected encapsulation system, it was established that:
    - 5.1. Co-encapsulation of prebiotics together with LAB showed that 4% trehalose and 2% inulin acted as cryoprotectants and increased the survival of *L. reuteri* 182 and *L. plantarum* F1 during lyophilization by ~5%, and *L. helveticus* 305 by as much as 17.8%. Also, co-

encapsulation of LAB with these materials increased the viability of LAB during long-term storage as, after 365 days of storage, the viability of *L. reuteri* 182 was 6.97±0.09 lg CFU g^{-1}.

5.2. Although coating the capsules with chitosan increased the Young's modulus from 507±11 kPa to 658±17 kPa, the encapsulation efficiency decreased twice, specifically, from 94.80±1.68% (in the control capsules not coated with chitosan) to 43.95±1.40%, and viable bacteria were no longer detected in capsules after 365 days of storage.

6. Having studied the effect of the substances commonly used in cosmetic compositions on LAB and selected those with the least effect, two cosmetic products were created – a toothpaste and a hand cream. Although the study confirmed the efficacy of the preservative against non-encapsulated *L. reuteri* 182 and pathogenic microorganisms *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*, and *A. niger*, the encapsulated *L. reuteri* 182 bacteria remained viable during the product stability study. After the overall evaluation of the viability of the encapsulated LAB cells and the stability of the cosmetic products when stored at 20 °C and under accelerated conditions at 37 °C, it can be concluded that the shelf life of the hand cream would be 6 months. The shelf life of the toothpaste would possibly be shorter. In the presently mentioned product, the amount of viable *L. reuteri* 182 bacteria needed to achieve a therapeutic effect would remain for about 1 month.

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LIST OF SCIENTIFIC PUBLICATIONS ON THE THEME OF THE DISSERTATION

In publications indexed in the *Web of Science* with Impact Factor (JCR SCIE)

1. Šipailienė, Aušra; **Petraitytė, Sigita**. Encapsulation of probiotics: proper selection of the probiotic strain and the influence of encapsulation technology and materials on the viability of encapsulated microorganisms // *Probiotics and Antimicrobial Proteins*. New York, NY: Springer. ISSN 1867-1306. eISSN 1867-1314. 2018, vol. 10, iss. 1, p. 1–10. DOI: 10.1007/s12602-017-9347-x. [Science Citation Index Expanded (Web of Science); Scopus; MEDLINE] [IF: 2.962; AIF: 3.927; IF/AIF: 0.754; Q2 (2018, InCites JCR SCIE)] [FOR: T 005] [Input: 0.500]
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REZIUMĖ

Temos aktualumas. Laipsniškas imuninės kilmės sveikatos problemų daugėjimas, išlaidų sveikatos priežiūrai didėjimas bei antibiotikams atsparių patogenų atsiradimas sąlygojo nulėmė žmonijos polinkį rinktisnatūralioms natūralias sveikatos priežiūros priemonėms priemones bei ir ieškoti alternatyvios medicinos gydymo būdų ieškojimą. Vienas iš alternatyviųjų gydymo būdų, įgyjančių vis didesnį pagreitį sveikatos apsaugos srityje, –- probiotikų terapija. Probiotikų naudojimas - – tai infekcijų kontroliavimo metodas moderniojoje eroje. Šių gerųjų bakterijų suteikiama apsauga nuo patogeninių mikroorganizmų yra probiotikų terapijos pagrindas.

Žmonės niekada nesiliauja siekę estetikos. Odos ir burnos ertmės problemos, tokios kaip odos hiperpigmentacija, raukšlės, odos dehidratacija, nemalonus burnos kvapas, kurias sukelia netinkama priežiūra ar išoriniai veiksniai, verčia žmones nuolatos ieškoti jų sprendimo būdų. Be estetinio netobulumo, egzistuojančios odos ligos, tokios kaip dermatitas, aknė, egzema, rožinė ir kt., taip pat yra spendžiamos ir / ar simptomai palengvinami naudojant specialiai pritaikytą dermatologinę kosmetiką. Yra daugybė šių odos problemų priežasčių, todėl mokslininkai nuolat kuria saugius ir efektyvius odos priežiūros produktus. Pastaraisiais metais ekspertai ir mokslininkai nustatė, kad probiotikai gali būti naudojami kaip kosmetikos produktų komponentai anksčiau minėtoms problemoms spręsti. Be to, atlikus įvairius eksperimentinius tyrimus įrodyta, jog probiotikai neturi toksinio poveikio žmogaus ląstelėms ir gali būti plačiai taikomi odos priežiūros ir dermatologinės kosmetikos srityje. Bakterioterapija probiotinių produktų pavidalu, slopinanti burnos ir odos patogenus, yra perspektyvi ir ekonomiškai efektyvi koncepcija, ypač kai ji naudojama profilaktiškai.

Kosmetikos pramonėje sukurti kompozicijas, kurios išlaikytų tikrųjų probiotikų gyvybingumą nuo gamybos iki vartotojo bei visą deklaruojamą produkto tinkamumo vartoti laiką, yra sudėtinga. Didelis laisvo vandens kiekis, esantis kosmetikos produktuose, leidžia anabiozės būsenos mikroorganizmams rehidratuotis ir daugintis arba žūti, todėl reikalingos bevandenės, aliejaus pagrindu pagamintos kosmetinės kompozicijos. Be to, kyla klausimas, kaip lengvai mikroorganizmai gali išsiskirti iš aliejaus, patekusio ant žmogaus odos, ir tapti metaboliškai aktyvūs, kad suteiktų norimą probiotinį poveikį. Taip pat didžioji dalis kremų ir kitų kosmetinių kompozicijų nėra gaminami steriliai, todėl naudojami konservantai, turintys bakteriocidinį ir / arba bakteriostatinį poveikį, kurie neišvengiamai paveiktų ir probiotikų gyvybingumą. Vienas iš būdų, leidžiančių išlaikyti gyvybingus mikroorganizmus šiose sistemose, yra probiotinių bakterijų kapsuliavimas. Literatūroje dažniausiai tik pažymima probiotinių bakterijų naudojimo kosmetikos priemonėse galimybė, nenurodant konkrečių kosmetikos priemonių receptūrų ar jų sudarymo principų. Kapsuliavimo technologijų pasirinkimas labai platus, todėl svarbu įvertinti, kokie

mikroorganizmai yra kapsuliuojami ir kaip konkrečias jų rūšis paveiks proceso parametrai bei naudojamos medžiagos. Negana to, pasigendama šių bakterijų sąveikos su kosmetikos produktais tyrimų. Literatūroje aprašyta nemažai probiotinių bakterijų kapsuliavimo metodų, tačiau tokių kapsuliuotų bakterijų gyvybingumas kosmetikos priemonėse nėra aiškus, todėl probiotikų kapsuliavimo ir praktinio pritaikomumo tyrimai yra itin aktualūs.

Darbo tikslas. Šis darbas yra skirtas ištirti skirtingų *Lactobacillus* bakterijų rūšių, išskirtų iš duonos raugų, probiotines savybes, kapsuliuoti jas skirtingose biopolimerinėse sistemose ir, atrinkus tinkamiausią sistemą, įvertinti kapsuliuotų bakterijų praktinio panaudojimo kosmetikoje galimybes.

Darbo uždaviniai:

1. Įvairiais *in vitro* metodais nustatyti iš duonos raugų išskirtų *Lactobacillus reuteri* 182, *Lactobacillus plantarum* F1 ir *Lactobacillus helveticus* 305 bakterijų probiotines savybes.
2. Ištirti dvigubųjų emulsijų fizikines ir mechanines savybes bei įvertinti pieno rūgšties bakterijų kapsuliavimo jose galimybes.
3. Ištirti skirtingais metodais gautų alginato hidrogelio kapsulių fizikines ir mechanines savybes bei įvertinti pieno rūgšties bakterijų kapsuliavimo jose galimybes.
4. Įvertinus skirtingų kapsuliavimo sistemų parametrus atrinkti tinkamiausią sistemą gyvoms pieno rūgšties bakterijoms kapsuliuoti.
5. Modifikuoti atrinktą kapsuliavimo sistemą su pieno rūgšties bakterijoms ko-kapsuliuojant prebiotikus ar padengiant kapsules papildomu chitozano sluoksniu, siekiant padidinti bakterijų gyvybingumą ilgalaikio saugojimo metu.
6. Ištirti pieno rūgšties bakterijų, kapsuliuotų atrinktoje sistemoje, panaudojimo galimybes kosmetiniuose preparatuose.

Mokslinio darbo naujumas. Ištirtos vietinės kilmės bakterijų kultūrų, išskirtų iš savaiminių duonos raugų, probiotinės savybės bei jų geba kolonizuoti žmogaus odą. Pirmą kartą buvo įvertintas kapsuliavimo sistemų suderinamumas su konkrečiomis potencialiai probiotinėmis pieno rūgšties bakterijomis bei poveikio bakterijoms dinamika. Optimizavus individuliai pieno rūgšties bakterijų kultūrai parinktą metodą, sukurta saugi kapsuliavimo sistema, kuri leido veiksmingai atpalaiduoti gyvus probiotikus tikslinėje vietoje po vietinio panaudojimo ant šeimininko odos.

Praktinė darbo vertė. Išanalizavus ir tarpusavyje palyginus kelias kapsuliavimo sistemas, buvo nustatyti jų privalumai, kurie leido pritaikyti sistemą individualiai bakterijų rūšiai. Sukurta kapsuliavimo sistema, pasižyminti dideliu kapsuliavimo efektyvumu bei bakterijų gyvybingumu ilgalaikio saugojimo metu. Ši sistema buvo sėkmingai pritaikyta sukurtuose modeliniuose probiotiniuose kosmetiniuose gaminiuose.

Ginamieji disertacijos teiginiai:

1. Iš duonos raugų išskirtos ir komercinėms kolekcijoms nepriklausančios bakterijos pasižymi probiotinėmis savybėmis ir geba kolonizuoti žmogaus odą.
2. Nesporinės probiotinės bakterijos gali būti apsaugotos nuo neigiamo kosmetinių kompozicijų ingredientų poveikio jas kapsuliuojant ir panaudojamos modeliniuose kosmetiniuose gaminiuose.

IŠVADOS

1. Ištyrus iš maisto produktų išskirtų pieno rūgšties (PRB) bakterijų *L. reuteri* 182, *L. plantarum* F1 ir *L. helveticus* 305 probiotines savybes, nustatyta, kad tirtų PRB kultūrų metabolitai pasižymėjo antimikrobiniu poveikiu patogenams. Taip pat nustatyta, kad PRB gebėjo koagreguoti su patogeniniais mikroorganizmais ir pasižymėjo *S. aureus*, *L. monocytogenes*, *E. coli*, ir *P. aeruginosa* bioplėvelių susidarymo inhibicija. Minėtų probiotinių savybių stiprumas buvo labai specifiškas ir kito priklausomai nuo PRB kultūros ir patogeninio mikroorganizmo taikinio. Taip pat nustatyta, kad *L. reuteri* 182 ir *L. helveticus* 305 pasižymėjo labai didele adhezine geba prie HaCaT ląstelių, atitinkamai 77,94±1,84 % ir 77,63±2,46 %.
2. Nustatyta, kad dvigubosios emulsijos su prebiotikais ir šaltalankių ekstraktu pasižymėjo apsauginiu poveikiu kapsuliuotoms PRB bakterijoms laikymo +20 °C ir +37 °C temperatūrose metu. Tačiau kapsuliavimo efektyvumas tokiose emulsijose nebuvo didelis ir priklausė nuo PRB rūšies: *L. reuteri* 182 – 64,46±0,43 %, *L. plantarum* F1 – 73,63±0,14 % ir *L. helveticus* 305 – vos 55,44±0,01 %.
3. Nustatyta, kad geriausiomis mechaninėmis savybėmis pasižymėjo stroncio alginato kapsulės, tačiau 5 % koncentracijos $SrCl_2$ druska buvo toksiška tirtoms PRB rūšims, kaip ir $CaCl_2$, o daugiau nei 0,1 % koncentracijos Ca-EDTA druska veikė baktericidiškai arba slopino PRB augimą. Taigi, įvertinus alginato geliaciją sukeliančių dvivalenčių jonų ir jų druskų toksiškumą PRB, nustatyta, kad šiuo atžvilgiu tinkamiausios druskos alginato hidrogelio sudarymui yra kalcio laktatas ir kalcio karbonatas.
4. Įvertinus PRB kapsuliavimo biopolimerinėse sistemose efektyvumą ir PRB gyvybingumą nustatyta, kad tinkamiausia sistema PRB kapsuliuoti – liofilizuotos kalcio alginato kapsulės, gautos emulsavimo-išorinės geliacijos metodu, alginatui sutinklinti naudojant kalcio laktatą. Šioje sistemoje *L. reuteri* 182 kapsuliavimo efektyvumas buvo 100 %, o gyvų bakterijų kiekis po 365 parų buvo didžiausias lyginant biopolimerines sistemas ir siekė net 6,71±0,07 lg KSV g^{-1}.

5. Atlikus atrinktos kapsuliavimo sistemos modifikacijas nustatyta:
    5.1. Kartu su PRB ko-kapsuliavus prebiotikus nustatyta, kad 4 % trehalozė bei 2 % inulinas veikė kaip krioprotektoriai ir ~5 % padidino *L. reuteri* 182 ir *L. plantarum* F1 išgyvenamumą liofilizacijos metu, *o L. helveticus* 305 – net 17,8 %. Taip pat PRB kokapsuliavimas su šiomis medžiagomis padidino PRB gyvybingumą ilgalaikio saugojimo metu – po 365 parų laikymo gyvybingų *L. reuteri* 182 bakterijų kiekis buvo 6,97±0,09 lg KSV g^{-1}.
    5.2. Nors kapsulių padengimas chitozanu padidino Jungo modulį nuo 507±11 kPa iki 658±17 kPa, tačiau kapsuliavimo efektyvumas sumažėjo daugiau nei dvigubai – nuo 94,80±1,68 % (kontrolinės kapsulėse, nepadengtose chitozanu) iki 43,95±1,40 %, o gyvybingų bakterijų po 365 parų nebebuvo aptikta.
6. Ištyrus kosmetikos kompozicijose dažniausiai naudojamų komponentų poveikį PRB ir atrinkus mažiausią poveikį turėjusius, buvo sukurti du kosmetiniai gaminiai – dantų pasta ir rankų kremas. Nors konservanto poveikis nekapsuliuotoms *L. reuteri* 182 bakterijoms ir patogeniniams mikroorganizmams *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* ir *A. niger* buvo patvirtintas atlikus tyrimą, kapsuliuotos bakterijos išliko gyvybingos gaminių stabilumo tyrimo metu. Bendrai įvertinus kapsuliuotų PRB gyvybingumą bei kosmetinių gaminių stabilumą, galima daryti išvadą, jog rankų kremo tinkamumo naudoti trukmė būtų 6 mėnesiai. Dantų pastos tinkamumo naudoti trukmė trumpesnė – gyvybingų *L. reuteri* 182 bakterijų kiekis, reikalingas terapeutiniam probiotikų efektui suteikti, išliktų apie 1 mėnesį.

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