Hungarian University of Agriculture and Life Sciences

EVALUATION OF DIFFERENT MICROENCAPSULATION TECHNIQUES FOR IMPROVING THE FUNCTIONAL EFFECTIVENESS OF PROBIOTIC-CONTAINING FOODS

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1 BACKGROUND AND OBJECTIVE OF THE RESEARCH

During the last decades there has been a considerable demand for various foods that can contribute to health and overall well-being. Growing evidence indicate that intake of foods fortified with probiotics, namely probiotic foods can potentially confer numerous benefits to human health, which in turn has established a big market of these foods worldwide. To date, different dairy products have served as traditional forms of probiotic consumption. Although, due to the high prevalence of people with lactose intolerance, milk protein allergy, hypercholesterolemia, calorie concerns and strict vegetarian dietary patterns in these days, more preference has been directed towards choosing non-dairy, particularly plant-based products. However, there are several technological challenges that need to be addressed when it comes to developing probiotic products, particularly of plant-based types. In specific, these probiotics have to confront a variety of stress factors during food processing (e.g. heat treatment), food storage (e.g. acidic conditions of some plant-based products) and subsequent gastrointestinal transit (e.g., gastric acid, bile salt), by which their health effects cannot be realised as effectively as desired. This, in turn, can question the claimed functionality of the probiotic products as well.

Microencapsulation of probiotics in a protective polymer matrix or with polymer coating is one of the recent potential approaches to protect the viability of probiotics under several harsh conditions, and also effectively deliver them to their therapeutic sites of action within the gastrointestinal tract, along with targeted release. Several delivery (capsule) systems for probiotics have been developed in previous studies to date. However, the main problem with them is their limited adaptability for food industrial and commercial applications, considering the scale-up difficulties of their microencapsulation processes, too large capsules for incorporating in food products, or insufficient probiotic protection against strong acidic and bile salt conditions, among others. Furthermore, the effect of these probiotic-loaded capsules in real food matrices, especially of plant-based types, has been insufficiently assessed so far. Last but not least, mucoadhesion aspect of these capsules – also necessary for more effective gastrointestinal delivery (and release) of encapsulated probiotics – has particularly not been reported in previous studies.

In light of above-mentioned concerns, the objective of my PhD work is to find the most promising encapsulation materials and techniques for development of microcapsule systems that can be utilised for effective protection and gastrointestinal delivery of probiotics, and at the same time, can be well-adapted for food industrial applications, thereby for developing novel probiotic non-dairy food products. In order to solve these research problems, the following tasks were set to accomplish:

- Formation of different probiotic-loaded capsule systems by applying different materials for microencapsulation process, including prebiotics such as resistant starch, lactulose and lactosucrose; hydrocolloid polymers such as alginate, gellan gum, xanthan gum, κ-carrageenan, locust bean gum, carboxymethyl cellulose and chitosan. In addition, polymer coating of bacteria-loaded alginate capsules with either chitosan or DEAE Sephadex was also aimed to perform.
- Encapsulation of a model probiotic strain with different chosen techniques, including the two most commonly reported techniques of extrusion and emulsification (external gelation-involving type), and the two less commonly studied ones of electrospraying and layer-by-layer self-assembly techniques.
- Evaluation and comparison of differently formed probiotic-loaded capsule systems – regarding encapsulation material and technique – for their efficacy in probiotic delivery, based on the following physical and physiological aspects:
    - Capsule size and size distribution
    - Encapsulation efficiency of viable probiotics
    - Viability of encapsulated probiotics under commonly applied *in vitro* gastric and/or intestinal conditions
        - Viability of encapsulated probiotics under *in vitro* digestion conditions based on a standardised Infogest protocol
    - Heat tolerance of encapsulated probiotics
    - Long-term storage stability and metabolic activity of encapsulated probiotics in different commercial plant-based beverages at different temperatures, in comparison to that of free cells.
    - Comparison of encapsulated probiotics with unencapsulated probiotics in terms of their survival rates under the above-mentioned stress conditions.
    - Mucoadhesion property of capsule systems.
- Comparison of encapsulation effect on the physiological activities (i.e., survival rate under stress conditions) of probiotic *Lactobacillus* and *Bifidobacterium* strains

2 MATERIALS AND METHODS

2.1 APPLIED PROBIOTIC MICROORGANISMS

In my study, the strains of ***Lactobacillus casei* 01**, ***Lactobacillus plantarum* NCDO 1752** and ***Bifidobacterium lactis* Bb-12** were applied as model probiotic bacteria for the encapsulation studies. The bacteria were obtained from Chr. Hansen in freeze-dried DVS (commercial Direct Vat Set) form, except the *Lactobacillus plantarum* strain, which was among the culture collection of UK National Collection of Dairy Organisms (Reading).

2.2 APPLIED MATERIALS AND SOLUTIONS

2.2.1 Applied bacterial growth media

L. casei 01 and *L. plantarum* NCDO 1752 bacteria were grown in **MRS** (de Man Rogosa Sharpe) medium. For propagating *B. lactis* Bb12, **TPY** (Trypticase Phytone Yeast extract) medium was applied (De Man et al., 1960; Scardovi, 1981). For solidifying the growth media, **15 g/L of bacteriological agar** (Sigma Aldrich) was added to the liquid growth solutions. Before use, all these growth media – either with or without agar – were sterilised by autoclaving (at 121°C for 15 min).

2.2.2 Applied carrier materials (encapsulant agents) for the encapsulation of probiotics

For encapsulation of probiotics, I applied different types of carbohydrates, namely **sodium alginate** (alginic acid), **gellan gum, xanthan gum, κ-carrageenan, locust bean gum, chitosan, carboxymethyl cellulose** (Sigma Aldrich), **resistant starch** (National Starch Food Innovation, UK), **lactulose** (PanReac AppliChem, Germany), **lactosucrose LS40L**, lactosucrose LS55L (Ensuiko Sugar Refining Co., Japan), **DEAE Sephadex A50 (**Pharmacia Fine Chemicals, Sweden).

2.2.3 Applied food matrices

In the study of storage stability of probiotic cells, commercial organic **beetroot juice** (Steinberger) and **oat drink** (enerBio) were purchased and applied as model non-dairy food matrices. Some criteria were considered for the selection of non-dairy food products, such as the high degree of purity and no (or very minimal) antimicrobial content. Since both products were readily acquired in pasteurised form, no additional sterilisation step needed to be performed.

2.2.4 Other applied media or solutions

For preparing serial dilutions during the plating-based enumeration of bacteria, **0.85 % (w/v) saline solution** was used; for suspending and storing encapsulated/unencapsulated probiotic bacteria, either **0.1 % (w/v) peptone water** or **phosphate buffer saline (PBS)** were used; for dissolving gel capsules and thereby releasing encapsulated probiotics for enumeration and further examinations, **0.1**

M phosphate buffer solution was applied. The applied pH of this the latter solution necessarily varied between 6.8 and 7.5, depending on the specific polymer type of gel matrices to be dissolved. Before use, all of these solutions were sterilised in autoclave (at 121°C for 15 min).

2.3 APPLIED METHODOLOGIES

All the microbiological-related formulations and investigations mentioned in this present section were carefully carried out under aseptic conditions.

2.3.1 Bacterial culture preparation and its maintenance

The proliferation of *L. casei* 01 and *L. plantarum* NCDO 1752 was carried out by an incubation in MRS broth at **37°C** for **16-24 h**. In case of the growth of *B. lactis* Bb-12, anaerobic incubation at **37°C** for **24-72 h** in TPY liquid medium was applied. All types of the prepared culture were kept at 4°C in a refrigerator shortly before their further usage.

2.3.2 Determination of viable bacterial counts

Viable bacterial cell counts in CFU (Colony Forming Unit) per mL or g were enumerated with either by pour plating (Sanders, 2012) or surface drop plating technique (Miles et al., 1938). For determining viable bacterial cells encapsulated in gel capsules, these capsules were necessarily agitated in 0.1 M phosphate buffer until their complete gel disintegration (~ 15 - 60 min), right before the plating processes.

2.3.3 Applied techniques for encapsulation of probiotic bacteria

Prior to all the encapsulation processes, the previously grown bacterial culture suspension was harvested by centrifugation at 10 000 rpm, 4°C for 10 min, followed by double washing of the cell pellet for removing the growth medium residues and resuspension in 0.1% (w/v) peptone water (or PBS).

The model probiotics were attempted to encapsulate with several techniques, by which the formation of the capsules was based on the principles of ionotropic gelation-involved **extrusion** (Krasaekoopt et al. , 2004)**, emulsification** (Sheu and Marshall, 1993)**, layer-by-layer deposition** (Diaspro et al., 2002) **and electrospraying** of encapsulation agents. *Layer-by-layer self-assembly-based encapsulation* involved **alternating electrostatic deposition of two oppositely charged carboxymethyl cellulose (-) (CMC) and chitosan (+) (CHI) polyelectrolytes** on the surface of the bacteria cell. *Electrospray-based encapsulation* was carried out using a commercially available **Spraybase® instrument** (Avectas Ltd., Ireland).

An additional *polymer coating* was also applied on the prepared capsules, based on the **electrostatic deposition of the applied charged polymer to a counter ionic capsule surface**. For coating alginate capsules with positive-charged chitosan and DEAE Sephadex A 50, the procedure outlined by **Krasaekoopt at el. (2004)** was applied with slight modifications.

2.3.4 Applied methods for physical characterisation of capsules

The physical observation of the resultant capsules was carried out by either **inverted microscopy** or **fluorescence microscopy**.

The dimension and size distribution of the resultant capsules were measured by using either a **caliper** or **laser light diffraction analysis** with **Metasizer 3000 instrument** (Malvern Instruments, Malvern, UK).

Texture analyses were carried out with applying **Brookfield LFRA 4500 Texture Analyzer**. Two types of compression cycle were preformed aiming to acquire two different texture profiles, namely about springiness and mechanical strength for each capsule type. To this end, a non-destructive test for the former and a destructive test for the latter texture profile were conducted on the same batch particle sample.

To examine mucoadhesive ability of the microcapsules on mucosal tissue, an ***in vitro* fluorescence flow-through retention (wash-off) test** was undertaken by modifying the traditional procedure applied by Cook et al. (2018), Kaldybekov et al. (2018) and Porfiryeva et al. (2019). In this study an *ex vivo* porcine gastric tissue was used as a model mucosal membrane. Retention on mucosal surface depends on the mucoadhesive strength of the microcapsules while continuously being washed off with simulated gastric fluid; this retention was monitored and investigated through the microscopic imaging of the fluorescently labelled microcapsules on the mucosal surface at regular time intervals. The main modification made in my study was that the retention was observed under a **1080P 1000X Zoom HD 8LED Digital USB Microscope Magnifier Endoscope Video Camera,** instead of the traditional fluorescence microscope. In this case, a Winzwon UV torch was used as an external light source to illuminate and detect the fluorescently-labelled microparticles. The AmCap ver. 9.0 software was used for recording the images of the samples. The mucoadhesion test was conducted with an own assembled experimental set-up. All these experiments were performed in triplicate for each formulation using an incubator at 37°C and under dark conditions.

2.3.5 Applied methods for physiological evaluation of encapsulated (and free) bacteria

The encapsulation efficiency was calculated using the formula below (Haghshenas et al., 2015):

$$\text{Encapsulation yield } (\%) = \frac{\log_{10}(\text{N})}{\log_{10}(\text{No})} \cdot 100$$

where N is the viable number of encapsulated cells released from the resultant capsules and No is a total number of viable free cells added initially to polymer solution and used up for their encapsulation.

The survival test in simulated gastric and intestinal fluids was carried out as described earlier by **Krasaekoopt et al. (2004),** with some modifications.

Simulated digestion study was also carried out according to the **static *in vitro* protocol** developed by an international consensus within the **COST Infogest network** (Minekus et al., 2014), which harmonises other several *in vitro* protocols reported for simulating human digestion and also better simulates the complexity of the enzyme activity and electrolyte content of the human digestion fluids.

Heat tolerance test were performed by heat treatments in 60°C and 85°C water bath. For performing storage stability analyses, storage experiments were carried out **in oat and beetroot drinks at 4°C and 20°C.**

2.3.6 Preparation of fluorescently labelled microcapsules

Fluorescently labelled alginate and resistant starch-alginate microcapsules were prepared by electrospraying polymer solutions containing **0.1% (w/v) sodium fluorescein**; in the case of the mucoadhesion study, **0.1% (w/v) fluorescein isothiocyanate (FITC)-dextran** was used. Chitosan polymer used as coating was labelled with **FITC** using the protocol described in a previous study (Cook et al., 2011). Chitosan-based particles used in the mucoadhesion study were prepared from chitosan solution labelled with **0.1 % (w/v) FITC**.

3 RESULTS AND DISCUSSION

In this study, I applied and tested four different encapsulation techniques and many different carrier materials for encapsulating probiotic bacteria. In order to assess the suitability of the resultant capsules as probiotic delivery systems, several physical and physiological evaluations were carried out which are crucial factors when it comes to assure the optimal incorporation of probiotic-loaded capsules in food products, the consumption of probiotics in highest possible live number (min. 6-7 log CFU/g or mL for realisation of probiotic effects (Yao et al., 2020)), and the effective controlled gastrointestinal delivery of probiotics after encountering several environmental stress conditions. Accordingly, probiotic-loaded capsules were characterised by morphology, size, size distribution, textural attributes and mucoadhesive strength. Furthermore, the loading capacity of the capsules with viable probiotic cells (encapsulation efficiency) was also determined after each encapsulation process and different types of bacterial survival tests were also performed by exposure to simulated gastrointestinal fluids, and to high-temperature conditions. Finally, this study also aimed to analyse the storage stability of the encapsulated probiotics by long-term storage experiments in food matrices.

3.1 EVALUATION OF EXTRUSION-FORMED PROBIOTIC-LOADED CAPSULES

In my encapsulation study, extrusion technique-based encapsulation was performed with model probiotic *Lactobacillus casei* 01, during which I successfully encapsulated these bacteria into 9 different gel capsules, namely calcium alginate [2 % (w/v)], capsules consist of blends of alginate and prebiotic component like resistant starch, lactulose, lactosucrose LS40L and lactosucrose LS55L [2 % - 2 % (w/v)]; and non-alginate capsules like gellan gum-xanthan gum and κ-carrageenan - locust bean gum capsules. Furthermore, I also successfully prepared additional alginate capsules with outer chitosan and DEAE Sephadex-based polymer coatings (separately).

As a result of the extrusion-based gel capsule formulation, regular sphere (bead) shape could be formed in case of alginate-, resistant starch-alginate and every coated alginate-based gel formulation, whereas the shapes of lactulose-alginate, lactosucrose LS40L-alginate, lactosucrose LS55L-alginate, κ-carrageenan-locust bean gum and gellan gum-xanthan gum capsules were rather irregular. After evaluating each type of gel capsules, results showed that the physical (size, shape, texture) and physiological characteristics (encapsulation efficiency, bacterial protection ability) of the capsules produced varied with the type of applied encapsulating materials. For utilising as probiotic delivery systems, I revealed that those alginate capsules that blended with prebiotics had the most ideal characteristic since these simultaneously had the firmest gel structures, provided the highest

encapsulation yields (ranged between 77 % – 79 %) and the most effective protection of probiotic viability, especially the resistant starch-blended alginate ones, under strong acidic (pH = 2, either without or with pepsin activity) and bile salt conditions of simulated gastrointestinal fluids. In fact, I also reported a highly effective protection of probiotic viability with resistant starch-alginate capsules when examined with a more sophisticated, standardised static *in vitro* digestion protocol of COST Infogest network (Minekus et al., 2014); in this case, probiotic viability was only lost by roughly 1 log CFU/g after the sequential phases of simulated oral (2 min) (with amylase activity), gastric (120 min) (with pepsin activity, pH = 3) and intestinal (with bile salt content and pancreatin activity) (120 min) conditions. By further evaluating these resistant starch-blended alginate capsules, I observed that this encapsulation increased the long-term storage stability of the probiotics in acidic plant-based beverages like pre-fermented oat- and beetroot drinks, to the extent of maintaining the probiotic viability above the suggested minimal viable cell counts (Yao et al., 2020) even without the refrigerated storage and even for 3-4 months. Although, the positive effect of encapsulation was only clearly seen when the storage was performed for at least 3 months as the unencapsulated cells could also survive comparably well in the first 2 months' period. The effect of different storage temperature on the bacteria stability varied with the type of plant-based matrices; more specifically, the storage stability improvement in oat drink was only observed at ambient (20 °C) temperature, whereas the stability in beetroot drink was clearly improved under both ambient (20 °C) and refrigerated (4°C) storage conditions. According to the heat-treatment experiment performed in this work, the resistant starch-alginate encapsulation appeared to not considerably improve the thermal stability of (free) *L. casei* 01 at 60°C, and it failed to do so at a higher, 85°C temperature.

By investigating another probiotic bacteria of different genus, extrusion-based encapsulation could also successfully be applied to *Bifidobacterium lactis* Bb-12, in which case higher level of viability protections were generally observed under the same challenges of *in vitro* gastrointestinal and especially high temperature (60°C) stresses. This result indicates that the effect of encapsulation can vary in this regard.

3.2 EVALUATION OF EMULSIFICATION-FORMED PROBIOTIC-LOADED CAPSULES

Considering that extrusion technique resulted unfavourably large capsule size (~ 2 – 5 mm) for food incorporation, I carried out further probiotic encapsulation studies with emulsification/external gelation. As a result of it, as opposed to extrusion, smaller sized capsules (roughly in the range of 0.8

- 10 mm) can be partially formed by emulsification/external gelation method, which would be generally more favourable for food incorporation. However, it has been revealed that encapsulation by this technique – into the same resistant starch-alginate gel matrix – tends to provide generally worse protection of probiotics from the challenges of strong acid and the high temperature than when encapsulation is done by extrusion technique. Although, in practical aspect, the long-term storage stability of probiotics in low pH of plant-based matrices seems to be rather irrelevant as to which of the two encapsulation procedures is applied.

Subsequently, I also applied and investigated this same emulsification/external gelation technique for the encapsulation of *B. lactis* Bb-12 strain, where I revealed again that the same encapsulation approach could provide higher degree of viability protection to the bifidobacteria against various environmental stress factors than that to the lactobacilli.

3.3 EVALUATION OF ELECTROSPRAY-FORMED PROBIOTIC-LOADED CAPSULES

For the possibility of preparing particles in fine grain size and in mass amount, electrospray technique was also applied and examined for *L. plantarum* NCDO 1752 encapsulation. By electrospraying of the same cell-containing resistant starch-alginate solution, it was possible to encapsulate probiotics into capsules with both uniformly fine size – in the range of 30-600 μm – and spherical shape, unlike with the extrusion or the emulsification techniques. It further appears that, encapsulation into these fine sized capsules can still result in high encapsulation yield and high protection of probiotics against strong acid challenge during the simulated gastric treatment. Furthermore, I also found that although blending with resistant starch had the great protective effect, the mucoadhesive property of alginate capsules – which would be important for assuring the extended residence time of encapsulated probiotics within the gastrointestinal tract for sufficiently releasing them from the capsules and exerting their health effects – tended to be weakened by the effect of the non-ionic nature of starch, although not by a great extent. This property, on the other hand, could be considerably improved if capsules were supplied with cationic chitosan coating, through which strong ionic interaction could potentially be established with anionic mucin.

3.4 EVALUATION OF ENCAPSULATED PROBIOTICS FORMED BY LAYER-BY-LAYER SELF-ASSEMBLY OF POLYELECTROLYTES

Within the encapsulation study, I also encapsulated *L. casei* 01 by electrostatic layer-by-layer (alternating) self-assembly of two oppositely charged polyelectrolytes like carboxymethyl cellulose

(–) and chitosan (+). This encapsulation approach differed from the three previous ones in that probiotic cells are individually coated with nano-sized multilayered polymer film, rather than entrapping multiple cells within a polymer (gel) matrix. In my work, total 6 alternate polyelectrolyte layers (carboxymethyl cellulose/chitosan) were shown to be successfully deposited on the bacteria surface. However, I observed that the physiological activity of the bacteria decreased as more and more polyelectrolyte layers were applied on the bacteria surface. As for the protective effect, 4 alternate carboxymethyl cellulose/chitosan layers improved the viability of *L. casei* 01 under strong acidic (pH = 2, with including pepsin) and bile salt conditions, but fell short when compared with extrusion-based encapsulation with resistant starch-blended alginate or other prebiotic-blended alginate (among others), or with electrospray-based encapsulation with resistant starch-alginate.

4 CONCLUSIONS AND RECOMMENDATIONS

In my research work, I evaluated different ***encapsulation materials*** and ***encapsulation techniques*** for their suitability to develop effective (micro)capsules as protective and gastrointestinal (targeted) delivery systems for probiotics. To this end, various **physical** and **physiological evaluations** were performed on the resultant capsule formulations.

Overall, I demonstrated based on my results that the use of ***electrospray*** as encapsulation technique and prebiotics, especially ***resistant starch*** as encapsulants (excipients) can play a potential role in the development of effective and ideal micro delivery systems for probiotics that can maintain their viability above the minimal recommended level of 6 log CFU/g or mL (Yao et al., 2020) during their delivery and also can be put forward for plant-based food applications, thereby offering the possibility to develop novel probiotic non-dairy food products. It is also worth mentioning that electrospray-based encapsulation would be also easily adaptable for industrial-scale applications, and, without the necessary use of intensive heating, it can be also more cost-effective than the similar but more commonly reported encapsulation techniques such as spray drying. However, with a view to design a proper controlled gastrointestinal delivery of encapsulated probiotics, the weak mucoadhesive performance arising from the inclusion of non-ionic components like starch still needs to be overcome. For this purpose, one of the potential approaches may be to apply chitosan – or other highly mucoadhesive polymer – coating in combination with the prebiotic (resistant starch)-blending. Furthermore, there is still room for improvement in terms of probiotic protection at high temperature conditions in order to safely expose them to such common technological processes like pasteurisation or sterilisation (if needed for the specific applied probiotics and the technological processing of foods).

Finally, it is also recommended that further research be undertaken in the following aspects and areas (among others): (1) assessing the release nature of probiotics from these starch-contained microcapsules to the target site within gastrointestinal tract as the percentage of the probiotics delivered may be considerably less than that administered; (2) further assessing the protection ability of the microcapsules using *in vivo* approaches or at least a dynamic *in vitro* gastrointestinal model system (e.g., SHIME, TNO), considering that the real physiology of human gastrointestinal tract is highly complex and tends to vary greatly between subjects and several factors such as time since eating and age (Cook et al., 2012); (3) further studying the effect of lactosucrose LS55L and lactulose, or even other types of prebiotics as encapsulants on the physical characteristics and protection ability of microcapsules.

5 NEW SCIENTIFIC RESULTS

1.) I proved that the physical stability (regarding hardness and springiness) of the resultant alginate gel capsules was greatly increased by the addition of 2% (w/v) resistant starch into alginate, and I also demonstrated that the probiotic protection effect of alginate capsules against simulated gastric and intestinal conditions was also substantially improved with this firmer textural characteristic . Moreover, investigating with Infogest gastrointestinal model system, the protective effect of blend resistant starch-alginate capsules was highly satisfactory in a way that they could maintain the viability of probiotics above the recommended minimum level of 6 log CFU/mL for realising the therapeutic effects thereof.

2.) I found that with the encapsulation technology, especially with the resistant starch-contained capsules, the long-term storage viability of probiotic *L. casei* 01 could effectively maintained above the recommended minimum level of 6 log CFU/mL not only through refrigerated storage at 4°C, but even through 3 months of storage at 20°C in either oat or beetroot drinks. This result advocates a great promise of developing an effective probiotic plant-based products, considering that many of the commercial plant-based food products are rather stored and marketed at room temperature.

3.) By examining different encapsulation techniques, I proved that electrospray technique was the most promising approach to microencapsulate probiotics as applying this technique not only ensured high encapsulation efficiency (~ 87 %) and high viability protection (with an only loss of 3.68 log CFU/mL through 2h of simulated gastric treatment), but also enabled a production of the capsules in micron-size range (30 – 600 μm). Formation of microcapsules in this size range would enhance the adaptation of this type of microencapsulation into food industrial applications and would also allow a more cost-effective mass industrial production of the microcapsules.

4.) I found that same encapsulation (i.e., extrusion and emulsification) approaches provided better viability protection for *Bifidobacterium lactis* Bb-12 against the same strong acidic and bile salt effects of *in vitro* gastrointestinal conditions and particularly against the high-temperature (60°C) conditions, as compared to that for *Lactobacillus casei* 01. With this, I proved that the effectiveness of encapsulation can vary at a strain-specific level.

5.) With a portable microscope and an UV torch, I constructed and tested an experimental set-up that can be used as an effective alternative approach to performing a fluorescence imaging-based retention test for mucoadhesion analysis. With this approach of experiment, there are a number of technical and economic advantages offered to researchers over the traditional

fluorescent microscopy approach (e.g., the possibility for real-time monitoring and imaging the formulations in micro-scale resolution, the possibility for video recording the whole *in vitro* wash-off process, the user-friendliness and increased affordability of the experiment).

6.) By analysing the retention rate of different alginate-based gel capsules on *ex vivo* porcine gastric mucosa, I observed that the mucoadhesive property of the alginate capsules was only slightly weakened (by about 5.8 % of retention rate) when blending with resistant starch was applied. In this case, a retention rate of around 60 % on *ex vivo* porcine gastric mucosa could be still observed after 50 min of washing with simulated gastric fluid.

6 SCIENTIFIC PUBLICATIONS ON THE TOPIC OF THE DOCTORAL DISSERTATION

PEER-REVIEWED JOURNAL ARTICLES

In English

TA, L.P., BUJNA, E., KUN, S., CHARALAMPOPOULOS, D., KHUTORYANSKIY, V. V. (2021): Electrosprayed mucoadhesive alginate-chitosan microcapsules for gastrointestinal delivery of probiotics. *International Journal of Pharmaceutics,* 597, 120342. ***(IF = 5.875)***

TA, L. P., BUJNA, E., ANTAL, O., LADÁNYI, M., JUHÁSZ, R., SZÉCSI, A., KUN, S., SUDHEER, S., GUPTA, V.K., NGUYEN, Q.D. (2021): Effects of various polysaccharides (alginate, carrageenan, gums, chitosan) and their combination with prebiotic saccharides (resistant starch, lactosucrose, lactulose) on the encapsulation of probiotic bacteria *Lactobacillus casei* 01 strain. *International Journal of Biological Macromolecules,* 183, 1136–1144. ***(IF = 6.953)***

CONFERENCE ABSTRACT

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TA, L. P., CHARALAMPOPOULOS, D., KHUTORYANSKIY, V.V. (2019): Electrospray-based fabrication of polymer particles and their applicability for gastrointestinal delivery of probiotics. In: Abstract Book of the Conference on Innovations in Encapsulation, p. 24 (London, United Kingdom)

TA, L. P., BUJNA, E., KUN, SZ. (2018): Comparison study between external and internal gelation through emulsification technique regarding their suitability to develop micro delivery system for probiotics. In: Third International Conference on Food Science and Technology, ISBN:9789632697949 (Budapest, Hungary)

TA, L. P., BUJNA, E., KUN, SZ. (2018): Niosome as novel potential delivery system for probiotics. In: II. Book of Abstracts of the Young Researchers' International Conference on Chemistry and Chemical Engineering, Budapest, Hungary, ISBN:9789639970786, p.79 (Budapest, Hungary)

NGUYEN, Q. D., BUJNA, E., **TA, L. P.**, KUN, SZ., TRAN, A. M. T., DAM, M. S., & REZESSYNÉ-SZABÓ, J. (2017): Encapsulation of probiotics: recent developments and perspectives. In: Abstracts of the International Symposium of Food Security and Sustainable Development. ISBN: 9786049200656, p. 29 (Ho Si Minh City, Vietnam)

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TA, P. L., SÖRÖS, K., BUJNA, E., KUN, SZ. (2016): Optimization of medium composition for enhancing growth of *Bifidobacterium bifidum* b7.1 and *Lactobacillus casei* 01. In: *Acta Microbiologica et Immunologica Hungarica* 2017 supplement, 64 (1), ISSN: 12178950, p. 84 (Keszthely, Hungary)

In Hungarian

TA, P. L., BUJNA, E., KUN, SZ. (2017): Különböző összetételű gélgyöngyök alkalmazhatósága a *Lactobacillus casei* 01 életképességének megőrzésében. In: III. Big Food Konferencia absztraktfüzet, p. 15 (Budapest, Hungary)

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