Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 12, Issue 1 2023, 18
https://doi.org/10.33263/LIANBS121.018

Development and Characterization of Innovative Polymeric Oil-core Nanocarriers for Nisin Delivery

Cristian Mauricio Barreto Pinilla [1], Henrique Ataide Isaia [1], Adriano Brandelli [1,*]

[1] Laboratorio de Bioquímica e Microbiologia Aplicada, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, Brazil; christian_mao@hotmail.com (C.M.B.P.); henriqueisaia@yahoo.com.br (H.A.I.); abrand@ufrgs.br (A.B.);
* Correspondence: abrand@ufrgs.br (A.B.);

Scopus Author ID 35577298900
Received: 1.11.2021; Accepted: 5.12.2021; Published: 9.01.2022

Abstract: Nisin is an antimicrobial peptide broadly used as a preservative in the food industry. In this study, oil-core polymeric nanocapsules encapsulating nisin were prepared by a nanoprecipitation technique using three different synthetic biodegradable polymers, namely poly(butylene adipate-co-terephthalate) (PBAT), Eudragit RS-100® (EUD), and poly(ε-caprolactone) (PCL), and their physical characteristics, thermal resistance and antimicrobial activity against *Listeria monocytogenes* were investigated. All nanocapsule formulations showed entrapment efficiency superior to 96%. EUD and PCL nanocapsules showed average diameters ranging from 145 to 303 nm, while PBAT-nisin nanocapsules showed larger size and PDI index (556.2 nm and 0.51, respectively), possibly due to changes in the organic phase equilibrium during preparation. The thermogravimetric analysis indicates lower decomposition temperatures in the PCL and EUD nanocapsules containing nisin compared with the unload nanocapsule and the contrary effect in the PBAT. Moreover, all nisin-containing polymeric nanocapsules exhibited antimicrobial activity against *L. monocytogenes* in both agar diffusion tests and determination of antimicrobial units (AU/mL) in liquid media. Oil-core polymeric nanocapsules have not been previously described as carriers for nisin, and the results suggested that PBAT, EUD, and PCL could be suitable polymers for nisin delivery systems.

Keywords: antimicrobial; polymeric nanocapsules; nanoprecipitation; nisin; food pathogen.

1. Introduction

Novel technologies play an important role in food manufacturing and preservation. Microbial contamination of foods may result in human illness and important economic losses, but there is growing concern about chemicals used as food preservatives. Thus, bioactive compounds from natural sources such as essential oils and antimicrobial peptides have obtained great attention in the food industry [1]. Amidst commercially available natural preservatives, antimicrobial peptides produced by bacteria, also called bacteriocins, are an interesting subgroup [2]. Nisin is a bacteriocin naturally produced by strains of *Lactococcus lactis* subsp. *lactis* and has been used as a food preservative since 1950. Nisin is also Generally Regarded as Safe (GRAS) and is permitted by the US Food and Drug Administration for use as a natural preservative in some foods [3]. However, nisin can be partially degraded by proteolytic activity during food processing and could lose activity by interaction with fat and proteins present in the food matrix [4,5].

Because the effectiveness of bacteriocins can be reduced in a food matrix, encapsulation has been suggested as an alternative to deliver nisin, providing protection against undesirable interactions, controlled release, and increased antimicrobial properties [6]. Some examples of nanocarriers for nisin are liposomes [7,8], alginate [9], chitosan-carrageenan [10], silica xerogels [11] and silver nanoparticles [12]. However, the use of synthetic biodegradable polymers for nisin nanoencapsulation has been poorly investigated, despite these polymers having desirable properties for protection and stabilization of peptides, proteins, and enzymes against degradation induced by pH, temperature, chemicals, or light during delivery or fabrication procedures [13].

The objective of this study was to prepare nisin oil-core nanocapsules using Eudragit® RS-100, poly(ε-caprolactone), and poly(butylene adipate-co-terephthalate) using the nanoprecipitation technique. The developed nanocapsules were characterized in size, zeta potential, and entrapment efficiency. In addition, their thermal stability and antimicrobial activity against *Listeria monocytogenes* were evaluated.

2. Materials and Methods

2.1. Materials.

The polymers used were poly(butylene adipate-co-terephthalate (PBAT; Ecoflex F Blend C1200, BASF Corporation, Florham Park, NJ, USA), Eudragit RS-100® (EUD; Evonik Healthcare, Dossenheim, Germany) and poly(ε-caprolactone) (PCL; M*n* 80,000; Sigma-Aldrich, St. Louis, MO, USA). Sorbitan monostearate (Span 60®) and caprylic/capric triglycerides (MCT) were from Delaware (Porto Alegre, Brazil); polysorbate 80 (Tween 80®) was from Synth (Diadema, Brazil). Nisin (Nisaplin®) was obtained from Danisco (Vargem Grande, Brazil), and other chemical reagents were analytic grade and acquired from regular suppliers.

2.2. Bacterial strains and media.

Listeria monocytogenes ATCC 7644 was used as a bacterial indicator in the antimicrobial activity assays. The strain was maintained on Brain Heart Infusion (BHI; Kasvi, São José dos Pinhais, Brazil) agar plates at 4°C, and subcultured periodically. Before each experiment, *L. monocytogenes* ATCC 7644 was grown in BHI broth at 37°C for 24 h in a rotary shaker (125 rpm).

2.3. Preparation of lipid-core nanocapsules loaded with nisin.

The nanocapsules of PCL, EUD, and PBAT with and without nisin were prepared by the solvent displacement method described previously [14], with minor modifications. For the PCL nanocapsules, PCL (1% w/v), Span 60® (0.78% w/v) and 1 mL nisin (5 mg/mL), previously mixed with the MCT (3.3% w/v), were dissolved in acetone under magnetic stirring at 40ºC for 60 min until total dissolution to form the organic phase. The same procedure was used for PBAT nanocapsules, changing the pure acetone by a mixture (1:1 v/v) acetone-methanol. For the EUD nanocapsules, the magnetic stirring was at room temperature for 30 min without the addition of Span 60®. Then, the organic phase was subjected to an ultrasonic bath (USC 700, Unique, Santo Amaro, Brazil) for 10 min (PCL and PBAT only) and poured into 25 mL of aqueous phase composed by ultrapure water and polysorbate 80 (0.78% w/v) at

a slow rate. Finally, the organic solvent was removed by rotary evaporation, and the final volume was adjusted to 10 mL to attempt 0.5 mg/mL nisin concentration. Polymeric nanocapsules prepared with ultrapure water instead of nisin were used as controls.

2.4. Characterization of nanocapsules.

The average diameter and polydispersity index (PDI) of the nanocapsules were measured by dynamic light scattering (DLS) using a Zetasizer® S90 (Malvern Instruments, Herrenberg, Germany) equipment. The zeta potential *(ζ)* was determined by electrophoretic mobility in a Zetasizernano-ZS ZEN 3600 (Malvern Instruments, Herrenberg, Germany). All samples were diluted in ultrapure water (1:500 v/v) before analysis. The entrapment efficiency was determined by quantifying free nisin (non-encapsulated), as described elsewhere [15]. Briefly, the nanocapsules loaded with nisin were placed in 10-kDa centrifugal filters (Ultracel YM-10; Millipore, Billerica, MA, USA) and centrifuged at 10,000 *g* for 30 min and 4°C. The concentration of nisin in the filtrate was determined by HPLC analysis as described in the same work [15]. The EE values were calculated separately for nisin according to the following equation:

$$EE = \frac{\text{Nisin (used in preparation} - \text{in the filtrate)}}{\text{Nisin used in the preparation}}\ x\ 100.$$

2.5. Thermogravimetric analysis (TGA).

The TGA analysis was used to determine the thermal stability of the nanocapsules, monitoring the weight change during heating at a constant rate. TGA was performed using a thermogravimetric analyzer TGA Pyris 1 (Perkin Elmer, Shelton, CT, USA). Lyophilized samples of each formulation were heated from 25°C to 600°C at the 20°C/min rate under a nitrogen atmosphere (flow rate 40 mL/min).

2.6. Antimicrobial activity of nisin-nanocapsules.

The bacterial susceptibility to polymeric nanocapsules containing nisin was evaluated against *L. monocytogenes* to visualize inhibitory zones by agar diffusion [15]. Aliquots of free and encapsulated nisin (0.5 mg/mL) were heated at 100ºC for 3 min to eliminate possible microbial contaminants in the formulations; after cooling, they were applied onto BHI (Kasvi, São José dos Pinhais, Brazil) agar plates previously seeded with a suspension of *L. monocytogenes* (10^7 CFU/mL) prepared in NaCl solution (0.85% w/v). After this, the plates were incubated at 37ºC for 24 h, and the inhibition zones diameters were measured. Furthermore, the antimicrobial activity units (AU) of NPs were determined by the serial two-fold dilution analysis as described elsewhere [16], where an aliquot of 10-µL each sample dilution was applied onto BHI agar plates previously seeded with *L. monocytogenes* (approximately 7 log CFU/mL). The AU/mL values were defined as the reciprocal equivalent of the dilution after the last serial dilution giving a zone of inhibition.

2.7. Data analysis.

The results were analyzed by one-way ANOVA and Tukey means comparison test at 5% significance level, using the Statistica® software (StatSoft. Inc., Tulsa, USA) version 10.

3. Results and Discussion

3.1. Production and characterization of nisin-nanocapsules.

Nisin was successfully loaded in three different polymeric nanocapsules using the nanoprecipitation method, also known as the solvent displacement technique [17]. This methodology can form lipid-core nanocapsules by diffusion the organic solvent (containing the polymer) into an aqueous phase, followed by solvent evaporation, resulting in nanocapsules formed by sorbitan monostearate (SM) and triglycerides in the core. The lipid core is surrounded by an appropriate polymer (PCL, EUD, or PBAT), which is covered by polysorbate 80, a food-grade non-ionic surfactant, which also promotes steric stabilization effect [18]. The *z*-average diameter of nanoparticles is presented in Table 1. The EUD nanocapsules presented a lower size value (145.6 nm), while the formulation of PBAT-nisin showed a higher size value (556.2 nm). The results of mean diameter for PCL and EUD were similar to those previously reported for the same polymers encapsulating the peptide P43 [14]. Significant differences were observed between the size values of PBAT formulations with and without nisin, indicating differences in the nisin-polymer interactions.

Furthermore, the PDI values were around 0.3 for EUD and PCL nanocapsules (Table 1), indicating a narrow size distribution of nanoparticles. In contrast, the PDI values for PBAT were 0.56 and 0.51 for bare and nisin-containing nanocapsules, respectively, suggesting a broad size distribution. The incorporation of high concentrations of nisin during the production of PBAT-nisin nanofibers resulted in bigger, less uniform nanofibers compared with the single PBAT nanofibers, as a result of a change in the ionic strength viscosity and conductivity of the solution [19]. The viscosity of the dispersed phase can be related to the size distribution of nanoparticles, where the mean size often increases by increasing the viscosity of the organic phase [20]. Thus, changes in the organic phase equilibrium could be responsible for the large size and PDI of the PBAT nanocapsules containing nisin.

Table 1. Physical parameters and entrapment efficiency (EE) of nisin-loaded and bare PCL, EUD, and PBAT polymeric nanocapsules.

Sample	Size (nm)	PDI	(z) (mV)	EE (%)
EUD	145.6 ± 2.3^{a}	0.22 ± 0.07^{a}	15.3 ± 7.7^{a}	
EUD-nisin	168.9 ± 14.9^{a}	0.17 ± 0.05^{a}	14.5 ± 1.2^{a}	97.9 ± 1.0^{a}
PCL	303.9 ± 5.9^{bc}	0.20 ± 0.04^{a}	-19.7 ± 7.5^{a}	
PCL-nisin	278.4 ± 3.9^{b}	0.29 ± 0.02^{b}	-24.1 ± 8.2^{a}	96.2 ± 3.1^{a}
PBAT	336.0 ± 7.1^{c}	0.56 ± 0.06^{c}	-16.7 ± 4.2^{a}	
PBAT-nisin	556.2 ± 45.0^{d}	0.51 ± 0.07^{c}	-17.7 ± 5.0^{a}	99.9 ± 0.1^{a}

^{a,b,c} Different letters in the same column indicate significant differences using the Tukey test (p <0.05). The data represent the mean ± standard deviation of three experimental repetitions.

Regarding the zeta potential, the formulations with PCL and PBAT showed negative values (between -16.7 and -24.07 mV) due to the negative charge of carboxylic groups present at the polymer extremities. In the case of EUD nanocapsules, the positive electric charge can be explained by the presence of quaternary ammonium groups that compose the structure of Eudragit RS-100® [21]. The presence of nisin caused no significant changes to the zeta potential values of nanocapsules (Table 1). The zeta potential value results from the combination of the materials used, and a relatively high value is important to maintain the physicochemical stability of the colloidal suspension since large repulsive forces tend to avoid aggregation of adjacent nanoparticles [20]. The zeta potential values observed for EUD, PCL,

and PBAT nanoparticles suggest that they are in the instability range (between +30 and -30 mV), which can be attributed to the polysorbate 80 present in the nanoparticle wall. However, this non-ionic surfactant stabilizes the nanocapsules by a steric mechanism [22], preventing undesirable physical processes as aggregation and deposition.

The EE % of different nisin-loaded NCs, considering the ratio between the amount of encapsulated nisin and the total nisin measured in the suspension, was very high, with values greater than 96% (Table 1). These data indicate excellent encapsulation efficiency of nisin in the three polymers and higher values compared with some reports of nisin in lipid-based nanostructures [23].

3.2. Thermal properties of polymeric nanocapsules loaded with nisin.

The TGA methodology provides data on nanocapsules' decomposition pattern and thermal stability loaded with nisin. The weight loss profiles were determined for each nisin-containing nanocapsule and compared with control nanocapsules. As seen in Fig. 1, the thermal decomposition of all samples occurred by a one-step mechanism started at different temperatures depending on the nanocapsule composition. According to the respective profiles, the PCL and EUD nanocapsules containing nisin reduced thermal stability, anticipating the main decomposition event in around 50°C and 30°C, respectively. On the other hand, the PBAT nanocapsules containing nisin presented a delay in the initial decomposition step (10% of weight loss was attempted at 305°C for PBAT and 325°C for PBAT-nisin). The thermogram variations of the control PCL and EUD nanocapsules, as compared to those containing nisin, were probably due to the reduction of molecular interactions of polymer chains by the presence of nisin, which can affect the disposition of polymer chains and consequently change the thermal resistance [19,24]. Therefore, the PCL and EUD nanocapsules containing nisin were more susceptible to thermal degradation, while PBAT nanocapsules presented an increase of thermal stability with the incorporation of nisin.

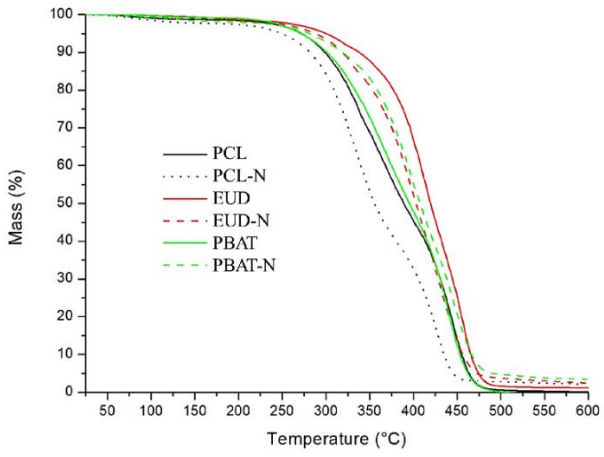
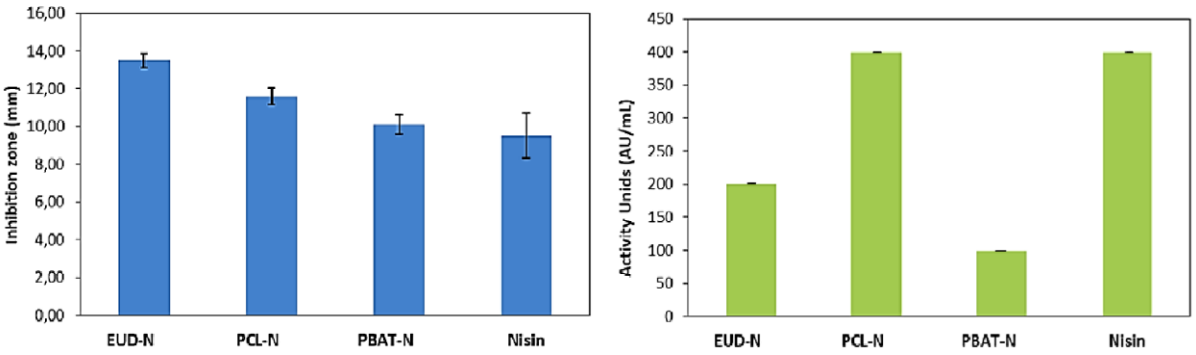


Figure 1. TGA thermograms obtained for PCL, EUD, and PBAT nanocapsules and PCL, EUD, and PBAT nanocapsules led with nisin (PCL-N, EUD-N, and PBAT-N).

3.3. Antimicrobial properties against L. monocytogenes.

The antimicrobial activity of free and encapsulated nisin was evaluated. The results of antimicrobial tests using the agar diffusion method for each formulation of polymeric nanocapsule (PCL, EUD, and PBAT) containing nisin against *L. monocytogenes*, can be

observed in Fig. 2a. In the agar diffusion test, the EUD-nisin nanocapsules presented the higher inhibition zone with a diameter of 13.5 mm. In contrast, free nisin showed the lower inhibition zone (diameter 9.5 mm). The control nanocapsules did not display antimicrobial activity. Factors such as agar concentration, pH, cellular density, and temperature affect the nisin diffusivity in agar bioassays [25]. Thus, the greater inhibition zone values indicate that polymeric nanocapsules containing nisin have good diffusion in agar media and presented the sustained release of the antimicrobial peptide, despite the different composition and charge of the polymeric nanocapsules.



(**a**) (**b**)

Figure 2. Antimicrobial activity of free and encapsulated nisin in PCL, EUD, and PBAT polymeric nanocapsules. Inhibition zone (a) and antimicrobial units (b) tests. Values are the means ± standard deviations of three independent experiments.

In contrast to the inhibition zone tests in agar plates, the determination of antimicrobial units (AU) by the serial-dilution method is related to the effective concentration of antimicrobial agents available in the media. As observed in Fig. 2b, free nisin and the PCL-nisin presented 400 AU/mL, while lower values were obtained for EUD-nisin (200 AU/mL) and PBAT-nisin (100 AU/mL). These results indicate that 24 h incubation at 37°C is sufficient to reach a complete release of nisin from the PCL nanocapsules. The different values of AU for EUD and PBAT indicate a lower release behavior due to a lower polymer degradation profile or higher affinity with the nisin. These factors could be delaying the release of the nisin, resulting in the decrease of AU. However, a slower release rate may suit applications where long-term antimicrobial protection is required. Therefore, the use of different biodegradable polymers is an interesting strategy to design efficient nanostructures for nisin delivery with applications as direct preservatives or as additives for food packaging.

4. Conclusions

In this study, oil-core nanocapsules containing nisin were produced with high entrapment efficiency using PCL, PBAT, and Eudragit polymers. All parameters evaluated in the characterization suggest good stability, particularly for the formulations PCL-nisin and EUD-nisin. Thermal analyses indicate that nisin reduces the degradation temperature of the nanocapsules prepared with PCL and EUD polymers but increases the thermal stability in the case of PBAT. Concerning biological activity, all nanocapsules containing nisin were effective against *L. monocytogenes* with some differences in diffusivity (inhibition zone) and nisin delivery (AU/mL). Thus, the use of polymeric nanocapsules as carriers of nisin represents an innovative alternative as delivery systems of nisin. However, additional studies should be

conducted to determine the different delivery rates of nisin from each formulation and to explore applications of these nanocapsules as delivery systems in the food sector.

Funding

This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil), grant number 306936/2017-8.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Batiha, G.E.-S.; Hussein, D.E.; Algammal, A.M.; George, T.T.; Jeandet, P.; Al-Snafi, A.E.; Tiwari, A.; Pagnossa, J.P.; Lima, C.M.; Thorat, N.D., Zahoor, M.; El-Esawi, M.; Dey, A.; Alghamdi, S.; Hetta, H.F.; Cruz-Martins, N. Application of natural antimicrobials in food preservation: Recent views. *Food Control* **2021**, *126*, https://doi.org/10.1016/j.foodcont.2021.108066.
2. Soltani, S.; Hammami, R.; Cotter, P.D.; Rebuffat, S.; Ben Said, L.; Gaudreau. H.; Bédard, F.; Biron, E.; Drider, D.; Fliss, I. Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations. *FEMS Microbiol Rev* **2021**, *45*, https://doi.org/10.1093/femsre/fuaa039.
3. Ibarra-Sánchez, L.A.; El-Haddad, N.; Mahmoud, D.; Miller, M.J.; Karam, L. Invited review: Advances in nisin use for preservation of dairy products. *J Dairy Sci* **2020**, *103*, 2041-2052, https://doi.org/10.3168/jds.2019-17498.
4. Aasen, I.; Markussen, S.; Møretrø, T.; Katla, T.; Axelsson, L.; Naterstad, K. Interactions of the bacteriocins sakacin P and nisin with food constituents. *Int J Food Microbiol* **2003**, *87*, 35-43, https://doi.org/10.1016/s0168-1605(03)00047-3.
5. Brandelli, A.; Lopes, N.A.; Boelter, J.F. Food applications of nanostructured antimicrobials. In: *Food Preservation.* Grumezescu, A.M. Ed.; Academic Press: London, UK, **2017**; pp. 35-74, https://doi.org/10.1016/B978-0-12-804303-5.00002-X.
6. McClements, D. Encapsulation, protection, and delivery of bioactive proteins and peptides using nanoparticle and microparticle systems: A review. *Adv Colloid Interface Sci* **2018**, *253*, 1-22, https://doi.org/10.1016/j.cis.2018.02.002.
7. Pinilla, C.M.B.; Reque, P.M.; Brandelli, A. Effect of oleic acid, cholesterol, and octadecylamine on membrane stability of freeze-dried liposomes encapsulating natural antimicrobials. *Food Bioprocess Technol* **2020**, *13*, 599-610, https://doi.org/10.1007/s11947-020-02419-8.
8. Lopes N.A.; Mertins O.; Pinilla C.M.B.; Brandelli A. Nisin induces lamellar to cubic liquid-crystalline transition in pectin and polygalacturonic acid liposomes. *Food Hydrocoll* **2021**, *112*, https://doi.org/10.1016/j.foodhyd.2020.106320.
9. Maresca, D.; Prisco, A.; Storia, A.; Cirillo, T.; Esposito, F.; Mauriello, G. Microencapsulation of nisin in alginate beads by vibrating technology: Preliminary investigation. *LWT Food Sci Technol* **2016**, *66*, 436-443, https://doi.org/10.1016/j.lwt.2015.10.062.
10. Webber, J.L.; Namivandi-Zangeneh, R.; Drozdek, S.; Wilk, K.A.; Boyer, C.; Wong, E.H.H.; Bradshaw-Hajek, B.H.; Krasowska, M.; Beattie, D.A. Incorporation and antimicrobial activity of nisin Z within carrageenan/chitosan multilayers. *Sci Rep* **2021**, *11*, https://doi.org/10.1038/s41598-020-79702-3.
11. Brum, L.F.W.; dos Santos, C.; Zimnoch Santos, J.H.; Brandelli A. Structured silica materials as innovative delivery systems for the bacteriocin nisin. *Food Chem* **2022**, *366*, https://doi.org/10.1016/j.foodchem.2021.130599.
12. Zhao, X.; Kuipers, O.P. Synthesis of silver-nisin nanoparticles with low cytotoxicity as antimicrobials against biofilm-forming pathogens. Colloids Surf B **2021**, *206*, https://doi.org/10.1016/j.colsurfb.2021.111965.
13. Deng, S.; Gigliobianco, M.R.; Censi, R.; Martino, P.D. Polymeric nanocapsules as nanotechnological alternative for drug delivery system: Current status, challenges and opportunities. *Nanomaterials* **2020**, *10*, https://doi.org/10.3390/nano10050847.
14. Isaia, H.A.; Pinilla, C.M.B.; Brandelli, A. Evidence that protein corona reduces the release of antimicrobial peptides from polymeric nanocapsules in milk. *Food Res Int* **2021**, *140*, https://doi.org/10.1016/j.foodres.2020.110074.

15. Pinilla, C.M.B.; Brandelli, A. Antimicrobial activity of nanoliposomes co-encapsulating nisin and garlic extract against Gram-positive and Gram-negative bacteria in milk. *Innov Food Sci Emerg Technol* **2016**, *36*, 287-293, https://doi.org/10.1016/j.ifset.2016.07.017.
16. Motta, A.S.; Brandelli, A. Characterization of an antibacterial peptide produced by *Brevibacterium linens*. *J Appl Microbiol* **2002**, *92*, 63-70, https://doi.org/10.1046/j.1365-2672.2002.01490.x.
17. Venturini, C.G.; Jäger, E.; Oliveira, C.P.; Bernardi, A.; Battastini, A.; Guterres, S.; Pohlmann, A. Formulation of lipid core nanocapsules. *Colloids Surf A* **2011**, *375*, 200-208, https://doi.org/10.1016/j.colsurfa.2010.12.011.
18. Martínez-Muñoz, O.I., Ospina-Giraldo, L.F., Mora-Huertas, C.E. Nanoprecipitation: Applications for entrapping active molecules of interest in pharmaceutics. In: *Nano- and Microencapsulation - Techniques and Applications*. Abu-Thabit, N. Ed.; IntechOpen: London, UK, **2020**; https://doi.org/10.5772/intechopen.93338.
19. Zehetmeyer, G.; Meira, S.M.M.; Scheibel, J.M.; Silva, C.B.; Rodembusch, F.; Brandelli, A.; Soares, R.M.D. Biodegradable and antimicrobial films based on poly(butylene adipate-co-terephthalate) electrospun fibers. *Polym Bull* **2017**, *74*, 3243-3268, https://doi.org/10.1007/s00289-016-1896-8.
20. Rodrigues, D.F.; Couto, R.O.; Sinisterra, R.D.; Jensen, C.E.M. Novel Eudragit®-based polymeric nanoparticles for sustained release of simvastatin. *Braz J Phar Res* **2020**, *56*, https://doi.org/10.1590/s2175-97902019000418363.
21. dos Santos, J.; da Silva, G.S.; Velho, M.C.; Beck, R.C.R. Eudragit®: A versatile family of polymers for hot melt extrusion and 3D printing processes in pharmaceutics. *Pharmaceutics* **2021**, *13*, https://doi.org/10.3390/pharmaceutics13091424.
22. Mora-Huertas, C.E.; Fessi, H.; Elaissari, A. Polymer-based nanocapsules for drug delivery. *Int J Pharm* **2010**, *385*, 113-142, https://doi.org/10.1016/j.ijpharm.2009.10.018.
23. Pinilla, C.M.B.; Lopes, N.A.; Brandelli, A. Lipid-based nanostructures for the delivery of natural antimicrobials. *Molecules* **2021**, *26*, https://doi.org/10.3390/molecules26123587.
24. Bastarrachea, L.; Dhawan, S.; Sablani, S.S.; Mah, J.-H.; Kang, D.-H.; Zhang, J.; Tang, J. Biodegradable poly(butylene adipate-*co*-terephthalate) films incorporated with nisin: Characterization and effectiveness against *Listeria innocua*. *J Food Sci* **2010**, *75*, E215-E224, https://doi.org/10.1111/j.1750-3841.2010.01591.x.
25. Lalpuria, M.; Karwa, V.; Anantheswaran, R.; Floros, J. Modified agar diffusion bioassay for better quantification of Nisaplin®. *J Appl Microbiol* **2013**, *114*, 663-671, https://doi.org/10.1111/jam.12078.