(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids
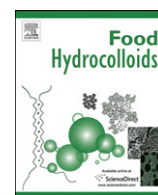
Anti-*Listeria monocytogenes* activity of enterocins microencapsulated by ionic gelation

Carolina Ibarguren [a], Carlos R.F. Grosso [d], María C. Apella [c], M. Carina Audisio [a,b,*]

[a] *Instituto de Investigaciones para la Industria Química – Consejo Nacional de Investigaciones Científicas y Técnicas (INIQUI-CONICET), Universidad Nacional de Salta, Buenos Aires 177, A4402FDC-Salta, Argentina*
[b] *Facultad de Ingeniería, Universidad Nacional de Salta, Buenos Aires 177, A4402FDC-Salta, Argentina*
[c] *Centro de Referencia para Lactobacilos – Consejo Nacional de Investigaciones Científicas y Técnicas (CERELA-CONICET), Chacabuco 145, T4000ILC-Tucumán, Argentina*
[d] *Faculdade de Engenharia de Alimentos, Departamento de Alimentos e Nutrição, Universidade Estadual de Campinas (FEA/UNICAMP), Caixa Postal 6121, CEP: 13083-862 – Campinas/SP São Paulo, Brazil*

ARTICLE INFO

ABSTRACT

The encapsulation of enterocins synthesized by *Enterococcus faecium* CRL1385 through ionic gelation with calcium ions was analyzed. Different enterocins samples were lyophilised and encapsulated using low-methoxyl pectin as the coating material. Lipids present in milk butter were also added to control the release of antimicrobial peptides from the capsules. The morphology of fresh and freeze-dried capsules was examined using light microscopy and scanning electron microscopy, respectively. Antimicrobial activity of encapsulated bacteriocins was assessed against *Listeria monocytogenes* 01/155 using the agar diffusion technique and direct contact in microplates. The capsules with higher lipid content showed a more spherical and uniform shape. Pathogen inhibition was observed for capsules prepared with different bacteriocin solutions both on solid (halo diameter = 8.5–13.5 mm) and in an aqueous medium (*ca.* 2 log orders decline in *L. monocytogenes* viability). The outcomes suggest that bacteriocin encapsulation through ionic gelation can be a potential alternative for the application of these antimicrobial peptides as biopreservatives in food.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The interest in application of natural metabolites like bacteriocins in the prevention of food spoilage and the extension of food shelf life has increased during the last decade. However, the interaction between these antimicrobial agents and food components as well as their uneven distribution in food products, reduces their efficiency against the pathogen when directly applied to the food. Therefore, it is necessary to develop an adequate distribution or delivery system to reduce these interactions and maximize the bioprotective potential of these compounds. Gálvez, Abriouel, López, and Omar (2007) emphasized the advantages of administration of immobilized enterocin preparations as food preservatives. The carrier would act as a reservoir and gradually diffuse the concentrated bacteriocin molecules throughout the food, thus assuring a gradual and continuous provision of the bacteriocins. Also, precise location of the molecules requires a relatively low bacteriocin dose as compared to the total food volume with the ensuing cost reduction. The immobilizing agent also protects the bacteriocins from interaction with food components or inactivation (Gálvez et al., 2007). There is information regarding the preservation of antimicrobial bacteriocin activity attached to a variety of surfaces such as silicates (Bower, McGuire, & Daeschel, 1995; Ibarguren, Audisio, Farfán Torres, & Apella, 2010; Wan, Gordon, Hickey, Mawson, & Coventry, 1996) and beads of different materials: alginates (Siragusa & Dickson, 1992), liposomes (da Silva Malheiros, Daroit, & Brandelli, 2010), niosomes (Kopermsub, Varissaporn, & Choochart, 2011) and phospholipid nanovesicles (Teixeira, dos Santos, Silveira, & Brandelli, 2008).

Microencapsulation is a process in which tiny solid, liquid or gaseous materials are coated to produce small capsules (beads) (Gouin, 2004). This technique is based on the embedding effect of a polymeric matrix, which creates a microenvironment in the capsule able to control the interactions between the internal part and the external one (Borgogna, Bellich, Zorzin, Lapasin, & Cesàro, 2010). Microencapsulation has unlimited applications in the food

industry, and in general food-grade polymers like alginate, chitosan, carboxymethyl cellulose (CMC), carrageenan, gelatine and pectin are used for coating (Anal & Singh, 2007). Encapsulation through ionic gelation is a simple, fast and low-cost technique that also allows the use of natural and inert polysaccharides as the coating (Mukai-Correa, Prata, Alvim, & Grosso, 2004; Willaert & Baron, 1996). This technology can be used to generate small beads which are desirable in food processing so that the addition of microencapsulated bioactive ingredients does not affect the sensory properties of food products (Champagne & Fustier, 2007).

To our knowledge, there is no information about the immobilization of bacteriocins by encapsulation using ionic gelation with pectin as a coating agent and butter as a retaining agent. This work has assessed the encapsulation of anti-*Listeria monocytogenes* enterocins, produced by *Enterococcus faecium* CRL1385 (Audisio, Oliver, & Apella, 2001), to deliver these bacteriocins as biopreservatives for food products, like dairy products or ham. Microcapsules were obtained through ionic gelation, the morphology was examined and the activity of the bacteriocins, inserted in the microcapsules, was determined against *L. monocytogenes* 01/155.

2. Materials and methods

2.1. Bacterial strains and culture conditions

E. faecium CRL1385 was used as the bacteriocin-producing strain (Audisio et al., 2001) and *L. monocytogenes* 01/155 (Audisio, M.C. INIQUI-CONICET) as the indicator strain. The strains were routinely propagated in BHI broth and incubated at 37 °C for 12 h between transfers.

2.2. Bacteriocin solutions

Cell-free supernatant (CFS) of a 6-h culture of *E. faecium* CRL1385 in BHI broth (37 °C) was centrifuged at 10,000 g for 20 min at 10 °C. The pH was adjusted to 6 with sterile 2 M NaOH and the CFS was filtered (0.45 µm). The resulting solution was referred to as "crude bacteriocin solution", i.e. without purification (BS, 800 AU/mL). A fraction of this BS was concentrated via precipitation with 75% $(NH_4)_2SO_4$. The resultant precipitate was resuspended in sterile distilled water of approximately 1/20th of the initial volume (BS_{PREC}, 6400 AU/mL). A fraction of this suspension was desalted on Sephadex G-10 (Amersham Pharmacia, Switzerland) with a non-jacketed Luer-Lock column of 0.7×10 cm with 4 mL bed volume (Sigma–Aldrich, Germany). Aliquots of 400 µL of the resuspended precipitate (BS_{PREC}) were loaded onto the column, which was previously equilibrated with 50 mM phosphate buffer (pH 7), and eluted with the same buffer at a flow rate of 0.4 mL/min. Fractions of 400 µL were then collected every minute for 30 min and those that presented antimicrobial activity were pooled and constitute the BS GF fraction (200 AU/mL). The three BS fractions (BS, BS_{PREC} and BS GF) were lyophilised prior to encapsulation in a CT 110 lyophiliser (Heto, Denmark). In all cases the bacteriocin titre was determined against *L. monocytogenes* 01/155, using the serial dilution method and the suspension titre was expressed in arbitrary units per millilitre (AU/mL) and calculated as follows: $1000/(V_s \times D)$, where V_s: bacteriocin volume tested (µL), and D: the highest dilution that still inhibited cell growth (Daba et al., 1991).

2.3. Microcapsules

Four microcapsules batches were prepared using the following formula: 2% (w/v) pectin; 2–4% (w/v) milk butter and 4–8% (w/v) of different fractions of lyophilised BS (BS, BS_{PREP} or BS GF) prepared according to Section 2.2. Low-methoxyl amidated pectin

from GENU® citrus peel (26–28% degree of methoxylation, 15–21% degree of amidation) was obtained from CPKelco (Limeira, SP, Brazil), containing $3.16 \pm 0.05\%$ w/w nitrogen as determined by the Kjeldahl method (AOAC, 2002). Unsalted milk butter (84% w/w lipids (saturated fat 48%, trans-fat 3%), 0% w/w protein, 0% carbohydrate, 0% fibre (Aviação, São Sebastião do Paraiso, MG, Brazil)) was obtained from the local market. $CaCl_2.2H_2O$ and NaH_2PO_4 were obtained from Merck (São Paulo, SP, Brazil).

The adequate quantities of pectin and butter necessary to prepare the different batches detailed above were mixed under heat to obtain a homogeneous solution and afterwards, the corresponding quantity of lyophilised BS, resuspended in distilled water, was added. The final volume of the filling solution was 100 mL. This mixture was homogenized (10,000 rpm, 1 min) in an Ultra-Turrax T50 (IKA, Germany) and pulverized in a double fluid atomizer (diameter = 1.5 mm) (SD-05, Lab Plant, Huddersfield, UK). The bulk of the emulsion was atomized in a $CaCl_2$ solution (300 mL, 2% w/v) maintained under magnetic stirring (320 rpm) using the following conditions: a peristaltic pump flow of 7.5 mL min^{-1} (Cole Parmer Instrument Co., Vernon Hills, IL, USA), an air flow of 0.15 kg cm^{-2} and a distance of 12 cm between the nozzle and the surface of the solution. After 30 min of curing at 25 °C in $CaCl_2$, microcapsules were sifted (25 µm), washed with abundant distilled water (400 mL) and finally lyophilised. The lyophilised capsules were kept frozen at −18 °C until analysis.

2.4. Microscopic analysis

2.4.1. Optical microscopy

The morphology of wet microcapsules of each CFS batch was examined with a NIKON Eclipse E800 optical microscope (Nikon, Japan) with 10× and 20× objectives. The images were captured with Image Pro Plus 4.0 software. Average capsule diameter of 100 beads of each batch was measured using the same software. Freeze-drying microcapsules swelling in contact with water were also observed by optical microscopy and the average diameter of the resultant capsules was determined following the same procedure.

2.4.2. Scanning electron microscopy

Lyophilised microcapsules were fixed on double-coated copper tape, placed on aluminium supports and coated with a fine gold layer in a Baltec SCD 050 sputter coater (Bal-Tec, USA) at 40 mA/ 150 s. After coating the samples were examined under a Joel JMS-T300 SEM (Joel Inc., Japan).

2.5. Inhibitory activity of encapsulated bacteriocins

Inhibitory activity of encapsulated bacteriocins was assayed using two methods: agar diffusion and direct contact in microplates. Both assays were carried out in triplicate.

2.5.1. Agar diffusion

A modified agar diffusion technique was used to determine the activity of encapsulated bacteriocins against *L. monocytogenes* 01/ 155 (Audisio, Terzolo, & Apella, 2005) with the agar wells being loaded with lyophilised microcapsules.

2.5.2. Direct contact in microplates

A modified version of the direct contact in microplate technique was used (Ibarguren, Raya, Apella, & Audisio, 2010). Wells of 6-well microplates (BD Falcon™) were loaded with *ca.* 50 mg of lyophilised microcapsules. Five mL of a suspension of *L. monocytogenes* 01/155 (*ca.* 10^4 cfu/mL) in peptone water were added to the wells and the viability was determined after 0, 0.5, 1, 2, 4 and 24 h of incubation through cell counts on BHI agar plates. The plates were

then incubated at 25 °C for 24 h. The same proportions of the pathogen suspension in peptone water or free BS (400 AU/mL) were used for both microcapsule assaying and controls. Quantification of bacteriocins released into the reaction medium, and measured through the titre using the serial dilution method (Daba et al., 1991), was determined after the same incubation intervals taking an aliquot of supernatant from the corresponding reaction system.

The same procedure was followed using the microcapsules from batch 2 (BS 4% w/v lipids), and comparing the effect of suspending the capsules in contact with the pathogen cells, using peptone water or phosphate buffer 50 mM (pH 7), as solvents.

2.6. Statistical analysis

GraphPad Prism 5.0 software (2009, GraphPad Software Inc., USA) was used for the statistical analysis of the results. $P < 0.05$ was considered significant. All assays were carried out in triplicate.

3. Results and discussion

3.1. Microcapsules

Insertion of *E. faecium* CRL1385 enterocins to a delivery system (pectin microcapsules) was assayed. The enterocins were partially purified (BS_{PREC} and BS GF) or directly present in the culture medium without purification (BS). Four batches of microcapsules with inserted bacteriocins were obtained. Their description is given in Table 1.

Milk butter was added to the capsules as a source of food-grade lipids (as the other ingredients used) so these lipids act as release stabilizers for the bacteriocins incorporated. Ionic gelation is an adequate method for encapsulation of biologically active compounds and the polysaccharides used are harmless, but the capsules thus prepared release the fill at a high rate, especially in the case of water-soluble and low-molecular-weight compounds. This is the case of enterocins, which are low-molecular-weight peptides (3–8 kDa). The cross-linked matrix formed by the gelified pectin in the presence of calcium ions is not sufficient to retain them, so a strategy is needed to improve the release characteristics. Langdon, Levine, and Jones (1985) and Mukai-Correa et al. (2004) found a higher retention of small compounds in the production of pectin microcapsules developed for fish larvae feeding, after the addition of a lipid mixture.

The capsules were also lyophilised as an additional strategy to extend their storage life. This dehydration method has advantages besides other drying techniques, because the process involves sublimation of water *in situ* without causing migration of the capsules feeling material to the periphery along with water during the evaporation progression (Talukander & Fassihi, 2004). Mukai-Correa et al. (2004) determined that the freeze-drying of microcapsules containing proteins and lipids added was efficient since the lipids addition kept the structure of the solid preventing

excessive breaks in the matrix, leading to a release behaviour similar to that of wet capsules.

The pectin emulsion including milk butter and bacteriocin used to prepare the microcapsules provided multinucleated particles with filling distribution throughout the extension of the matrix (Fig. 1A). In general, it was observed that microcapsules with a higher lipid content had a more spherical shape (Fig. 1Aii). The established working conditions (capillar size, spray height, air pressure, etc.) rendered capsules with an average diameter of 158 ± 80 μm. The lyophilisation process caused the loss of the spherical shape of the capsules, but maintained their physical integrity (Fig. 1B). Small spheres apparently made of lipid emerged encrusted on the solid microparticles and contrary to typical freeze-dried materials which show porosity, the obtained matrix although not smooth, appeared to be continuous without cracks or holes (Fig. 1Biii).

Swelling of the lyophilised capsules was observed immediately after contact with water due to the absorption of free or bulk water that fills the void regions of the polymer network and/or the centre of larger pores and macropores (Hoffman, 2002). Freeze-dried microcapsules instantly recovered their original round shape, appeared to be intact and almost reached the same size they had prior lyophilisation with an average diameter of 138 ± 70 μm.

3.2. Antimicrobial activity of encapsulated bacteriocins

Antimicrobial activity of encapsulated bacteriocins of the four batches, corresponding to the different BS types used as the bacteriocin source, was evaluated (Fig. 2). Bacteriocins in crude CFS (BS), without any previous concentration step, produced inhibition halos as well as those in BS concentrated through precipitation with ammonium sulphate (BS_{PREC}) (halo diameter = 8.5–12.5 mm and 13.5 mm, respectively), after their insertion in microcapsules. Inhibition with the BS GF fraction, obtained after desalting of the BS_{PREC} fraction by gel filtration, was too low to be detected after the encapsulation process, even though this fraction was purer. This could be expected, given the fact that the bacteriocin undergoes a diluting effect during the purification process since no concentration procedure was applied once recovered after gel filtration.

It is important to highlight that the bacteriocins kept their activity after encapsulation and a double lyophilisation process, as BS was lyophilised before and after encapsulation. Besides, the capsules kept their anti-*Listeria* activity after one year storage at −18 °C as observed with the agar diffusion technique. The results are similar to those observed in Fig. 2. These findings are encouraging since the antimicrobial activity endurance during the microcapsules processing and its' maintenance during a potential shelf life time, is essential for a probable future application of the microcapsules here obtained as food preservatives.

Analysis of antimicrobial activity against *L. monocytogenes* 01/155 of each microcapsules batch was done using a slightly-modified microplate technique. Since the pectin, butter and BS amount in each capsule batch are not the same, direct comparisons are not precise, but the result is indicative of the bacteriocin encapsulated response. As can be observed in Fig. 3 *L. monocytogenes* 01/155 cells inhibition was different depending on the BS contained in the capsules. Free BS immediately inhibited the pathogen cells with a decrease of almost 3 orders in cell viability and the inhibition was maintained until 24 h of contact. It is interesting to note that while the diffusion technique in agar did not reveal much difference in terms of inhibition by the microcapsules prepared with BS and BS_{PREC}, the direct contact microplate technique intimated a greater antibacterial effect of the capsules obtained from concentrated BS revealing a different antimicrobial diffusion profile in solid and liquid medium. The capsules of batch 3

Table 1
Pectin microcapsules composition with inserted BS (% w/v).

Batch	BS fraction	Lyophilized SB	Pectin	Butter
1	BS	8 *(60)*	2 *(20)*	2 *(20)*
2	BS	8 *(60)*	2 *(13)*	4 *(27)*
3	BS_{PREC}	4 *(50)*	2 *(25)*	2 *(25)*
4	BS GF	4 *(50)*	2 *(25)*	2 *(25)*

Values in parenthesis correspond to *dry weight (% w/w)*. BS: Bacteriocin solution, PREC: precipitated with 75% ammonium sulphate, GF: Desalted through gel filtration.
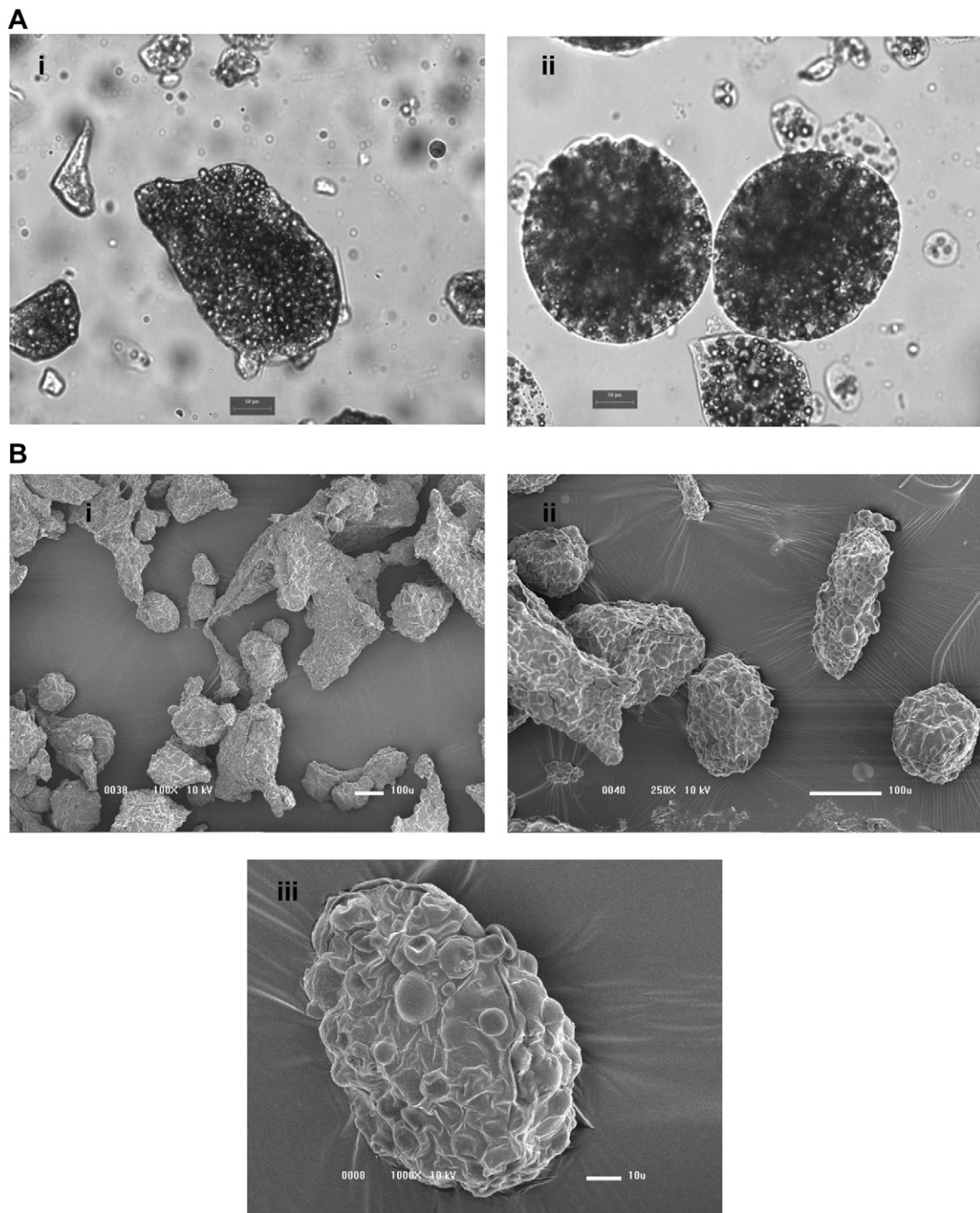
Fig. 1. Micrographs of (A) fresh capsules of BS with (i) 2% and (ii) 4% w/v lipids obtained by optical microscopy (10×, bars = 100 μm) and (B) lyophilized capsules of BS with (i) 2% (100×) and (ii, iii) 4% w/v lipids (250× and 1000×) obtained by Scanning electron microscopy (i and ii, bars = 100 μm; iii, bar = 10 μm).

(BS$_{PREC}$) showed the highest inhibitory effect, with an initial decrease in viability of 1 order decreasing to 2 orders after 24 h of contact, compared to the control of cell growth in peptone water. The content of lipid (butter) of the capsules made with BS, determined different behaviours. Initially the inhibition profile was similar for both batches (1 and 2), but after 24 h the capsules with a higher lipid content (batch 2, 4% w/v butter) produced an order decrease on the viability of the cells of *L. monocytogenes* 01/155, whereas the lot 1 capsules (low lipid content) after 24 h of contact reached an inhibition level similar to that of the capsules made

with BS GF (batch 4). The latter capsules were those which carried out the least antimicrobial effect, as already observed by the agar diffusion technique. These results suggest that batches 2 and 3 were the best in terms of inhibition. The greater inhibition of batch 2 (BS, 4% w/v lipid) can be attributed to the retention effect that the higher amount of lipid added in its formulation exerts and the subsequent gradual release of the bacteriocins from the capsules. The increased activity of capsules from batch 3 (BS$_{PREC}$) is to be expected as they were prepared with a BS with higher antimicrobial activity.
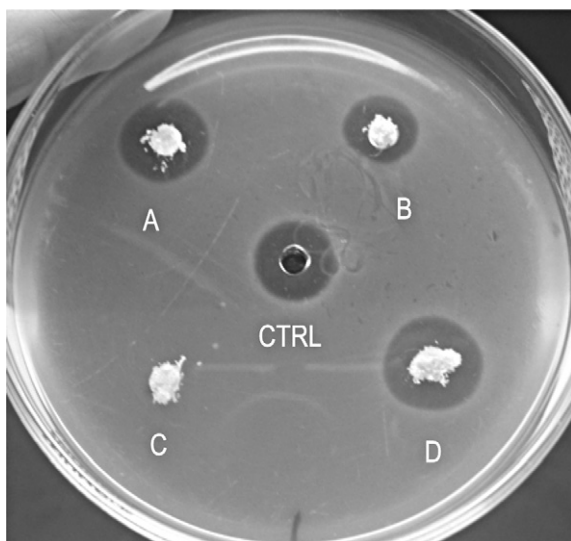
Fig. 2. Inhibition halos of pectin microcapsules with different fractions of BS synthesized by *E. faecium* CRL1385 against *L. monocytogenes* 01/155, with 4% and 2% (w/v) lipids (A and B respectively), BS GF (C), BS$_{PREC}$ (D) and BS not encapsulated (CTRL).

Fig. 4. *L. monocytogenes* 01/155 viability in peptone water (▲) and phosphate buffer (□) in contact with microcapsules with SB incorporated, according to the direct microplate contact bioassay. Dotted lines (●): growth control of *L. monocytogenes* 01/155 in peptone water.

Environmental conditions such as medium pH, ionic strength and presence of sequestering ions, clearly affect the release process of the filling material and in this particular case, the activity of inserted bacteriocins. Therefore, anti-*Listeria* activity of microcapsules prepared with BS encapsulated bacteriocins plus 4% w/w butter was assayed against a pathogen suspension in water peptone and in a phosphate buffer (Fig. 4). This batch was chosen based on the results from the previous assays and because the microcapsules presented a more uniform and spherical morphology. Phosphate ions sequester calcium ions from the matrix of the calcium–pectin gel, disintegrating the beads and releasing the fill. As expected, inhibition was slightly higher with cells suspended in 50 mM phosphate buffer (pH7). The beads suspended in water peptone maintained their structure, but the BS incorporated was
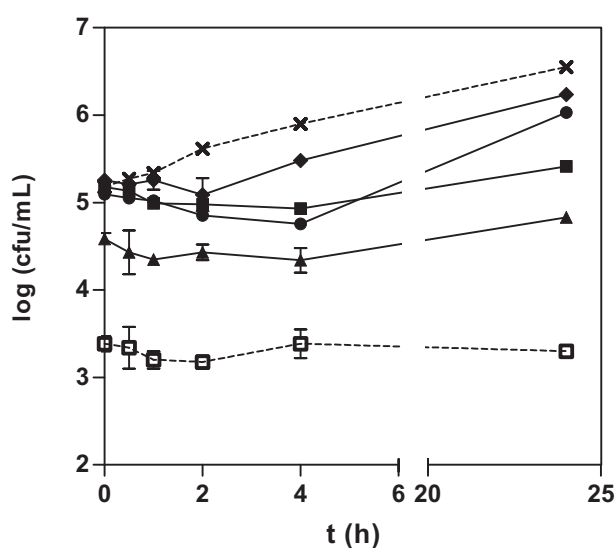
nonetheless released into the reaction medium and inhibited *Listeria* cells. After 24 h of contact, inhibition of the pathogen was similar for bacteriocin beads suspended in water peptone and in phosphate buffer. This result is important since it shows that the capsules could be active in several food environments.

Quantification of bacteriocins released into the reaction medium after the same incubation intervals showed dissimilar behaviour compared to that shown by *L. monocytogenes* 01/155 survival. Bacteriocin released from capsules from batch 1 and 2 (BS, 2% w/w and 4% w/w lipids, respectively) was similar (*ca.* 200 AU/mL during the 24 h of the assay), while capsules with BS$_{PREC}$ (batch 3) inserted released an amount of bacteriocin similar to that detected in free BS system (*ca.* 400 AU/mL) during the first 4 h of contact and decreased after 24 h to *ca.* 200 AU/mL. No bacteriocin activity was detected in the supernatant recovered from the batch 4 reaction system (BS GF) by this method although a decrease in the viability of *L. monocytogenes* 01/155 in contact with the capsules was detected. These results suggest once more that the bacteriocin diffusion is favoured in liquid medium, since beside the low antimicrobial activity titres detected by the agar diffusion technique, the viability curves demonstrate that the bacteriocins are released "active" from the resuspended capsules and they inhibited the pathogen growth.

4. Conclusions

Microencapsulation of *E. faecium* CRL1385 bacteriocins through ionic gelation with pectin as the coating and the addition of milk butter as microencapsulation coadjutant would be an effective delivery system for antimicrobial agents as food biopreservatives.

Bacteriocins insertion provided bioactive beads that kept anti-*L. monocytogenes* activity on solid and in a liquid medium, even after lyophilisation and storage at −18 °C for one year. Lipid addition to the capsules helped their integrity maintenance during freeze-drying and seemed to favour antimicrobial compounds gradual liberation; but more studies concerning the bacteriocin–matrix interactions and its effect on the subsequent peptide release from the capsule should be done. These preliminary results are encouraging, and this alternative enterocin delivery system is advantageous, as it uses food-grade, low-cost materials and a simple,

Fig. 3. *L. monocytogenes* 01/155 viability in peptone water in contact with microcapsules with SB incorporated: (●) BS 2% w/w lipid, (■) BS 4% w/w lipid, (▲) BSPREC, (◆) BS GF according to the direct microplate contact bioassay. Dotted lines: growth control of *L. monocytogenes* 01/155 (x) in peptone water and (□) in free BS (400 AU/mL).
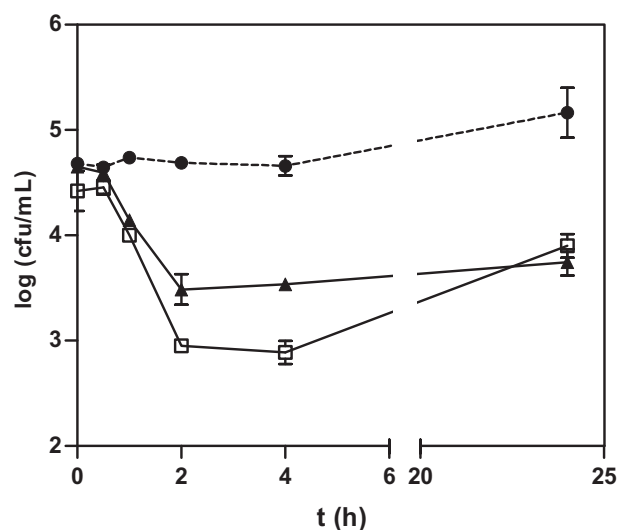
efficient and economical technique. The microcapsules can be effectively applied to high water content foodstuffs and can act both at food surface and core.

Acknowledgements

References

Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology, 18*, 240–251.

AOAC. (2002). *Official methods of analysis of AOAC international* (17th ed., Revision 1). Maryland: AOAC International.

Audisio, M. C., Oliver, G., & Apella, M. C. (2001). Effect of different complex carbon sources on the growth of *Enterococcus faecium* and on its bacteriocin synthesis. *International Journal of Food Microbiology, 63*, 235–241.

Audisio, M. C., Terzolo, H. R., & Apella, M. C. (2005). Bacteriocin from honeybee beebread *Enterococcus avium*, active against *Listeria monocytogenes*. *Applied & Environmental Microbiology, 71*, 3373–3375.

Borgogna, M., Bellich, B., Zorzin, L., Lapasin, R., & Cesàro, A. (2010). Food microencapsulation of bioactive compounds: rheological and thermal characterisation of non-conventional gelling system. *Food Chemistry, 122*, 416–423.

Bower, C. K., McGuire, J., & Daeschel, M. A. (1995). Suppression of *Listeria monocytogenes* colonization following adsorption of nisin onto silica surfaces. *Applied & Environmental Microbiology, 61*, 992–997.

Champagne, C. P., & Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. *Current Opinion in Biotechnology, 18*, 184–190.

Daba, H., Pandian, S., Gosselin, J. F., Simard, R. E., Huang, J., & Lacroix, C. (1991). Detection and activity of a bacteriocin produced by *Leuconostoc mesenteroides*. *Applied & Environmental Microbiology, 57*, 3450–3455.

Gálvez, A., Abriouel, H., López, R. L., & Omar, N. B. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology, 120*, 51–70.

Gouin, S. (2004). Microencapsulation: industrial appraisal of existing technologies and trends. *Trends in Food Science & Technology, 15*, 330–347.

Hoffman, A. S. (2002). Hydrogels for biomedical applications. *Advance Drug Delivery Reviews, 43*, 3–12.

Ibarguren, C., Audisio, M. C., Farfán Torres, M. E., & Apella, M. C. (2010). Silicates characterization as potential bacteriocin-carriers. *Innovative Food Science & Emerging Technologies, 11*, 197–202.

Ibarguren, C., Raya, R. R., Apella, M. C., & Audisio, M. C. (2010). *Enterococcus faecium* isolated from honey synthesized bacteriocin-like substances active against different *Listeria monocytogenes* strains. *Journal of Microbiology, 48*(1), 44–52.

Kopermsub, P., Varissaporn, M., & Choochart, W. (2011). Potential use of niosomes for encapsulation of nisin and EDTA and their antibacterial activity enhancement. *Food Research International, 44*, 605–612.

Langdon, C. J., Levine, D. M., & Jones, D. A. (1985). Microparticulate feeds for marine suspension-feeders. *Journal of Microencapsulation, 2*(1), 1–11.

Mukai-Correa, R., Prata, A. S., Alvim, I. D., & Grosso, C. R. F. (2004). Controlled release of protein from hydrocolloid gel microbeads before and after drying. *Current Drug Delivery, 1*, 265–273.

da Silva Malheiros, P., Daroit, D. J., & Brandelli, A. (2010). Food applications of liposome-encapsulated antimicrobial peptides. *Trends in Food Science & Technology, 21*(6), 284–292.

Siragusa, G. R., & Dickson, J. S. (1992). Inhibition of *Listeria monocytogenes* on beef tissue by application of organic acids immobilized in a calcium alginate cell. *Journal of Food Science, 57*, 293–296.

Talukder, R., & Fassihi, R. (2004). Gastroretentive delivery systems: hollow beads. *Drug Development and Industrial Pharmacy, 30*(4), 405–412.

Teixeira, M. L., dos Santos, J., Silveira, N. P., & Brandelli, A. (2008). Phospholipid nanovesicles containing a bacteriocin-like substance for control of *Listeria monocytogenes*. *Innovative Food Science & Emerging Technologies, 9*, 49–53.

Wan, J., Gordon, J., Hickey, M. W., Mawson, R. F., & Coventry, M. J. (1996). Adsorption of bacteriocins by ingestible silica compounds. *Journal of Applied Microbiology, 81*, 167–173.

Willaert, R. G., & Baron, G. V. (1996). Gel entrapment and micro-encapsulation: methods, applications and engineering principles. *Reviews in Chemical Engineering, 12*, 1–205.