Original article
Nisin-loaded alginate-high methoxy pectin microparticles: preparation and physicochemical characterisation

Ramin Khaksar,[1] Seyede Marzieh Hosseini,[1,2]* Hedayat Hosseini,[1]* Saeedeh Shojaee-Aliabadi,[1] Mohammad Amin Mohammadifar,[1] Amir Mohammad Mortazavian,[1] Kianoosh khosravi-Darani,[3] Nasrin Haji Seyed Javadi[3] & Rozita Komeily[3]

1 Department of Food Science and Technology, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, P.O. Box 19395-4741, Tehran, Iran
2 Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3 Research Department of Food Technology, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Science, P.O. Box 19395-4741, Tehran, Iran

Summary
The bacteriocin nisin has been extensively used as potential natural preservative in the food industry. However, antimicrobial activity of nisin due to its binding with food components and inactivation by enzymatic degradation is reduced when it is applied in food. Encapsulation of nisin is an efficient approach to overcome the problems related to the direct application of this antimicrobial peptide in foods. In this study, nisin was encapsulated in alginate-high methoxy pectin (HMP) microparticles, and its release studies were performed in water to determine the diffusion and the kinetic behaviour of the matrix. Results showed that the nisin content had a significant influence on encapsulation efficiency (EE), loading capacity (LC) and microparticles size. The values of EE, LC and particle mean diameter were about 47–54%, 16–21% and 57–131 μm, respectively. The nisin-loaded microparticles showed nearly spherical structure with fold on the surface, as displayed by scanning electron micrograph. Interaction between alginate and HMP was confirmed by the changes in the intensity and wave number of the stretching vibrations of the hydroxyl and carboxyl groups in alginate-HMP microparticles FTIR spectra. Furthermore, the addition of nisin resulted in a markedly increase in intensity of carboxylic peak at 1620 cm^{-1}, indicating the presence of nisin inside of the microparticles. The *in vitro* nisin release from these microparticles followed a sustained release profile consistent with a Fickian diffusion mechanism.

Introduction

Food safety and microbiological quality represent a major priority in the food industry. In recent years, there has been an increasing interest in the use of natural antimicrobials in the food products. Nisin is an antimicrobial peptide produced by certain strains of *Lactococcus lactis,* which acts as an effective agent in inhibiting the growth of many Gram-positive, food-borne pathogenic bacteria (Rodriguez, 1996). This bacteriocin is considered as 'generally recognised as safe' (GRAS) substance and has received particular attention because of its potential applications as a natural preservative (Register, 1988).

Several strategies have been proposed using nisin in combination with other antimicrobials and also use of liposomes, polymeric capsules or edible films to deliver bacteriocin and enhance its bioavailability in foods (Laridi *et al.*, 2003; Salmaso *et al.*, 2004; Jin *et al.*, 2009; Silva Malheiros *et al.*, 2010; Zohri *et al.*, 2010; Xiao & Zhong, 2011; Zou *et al.*, 2013). Encapsulation besides being able to reduce the interaction between nisin and food matrix components has additional potential to increase its stability and allows for a controlled release of used nisin (Laridi *et al.*, 2003; Xiao & Zhong, 2011).

Recently, using biopolymers in the design of delivery systems has received much attention due to their biocompatibility and biodegradability (Zamboni, 2005; De Salamanca *et al.*, 2006; Li *et al.*, 2011; Hosseini *et al.*, 2014). Alginate is a polyanionic polysaccharide consisting of various proportions of 1,4-linked b-mannuronic and a-guluronic acid residues which forms

*Correspondent: Fax: +98 21 22376480;
e-mails: hoseyni.fst@gmail.com; hedayat@sbmu.ac.ir

a gel through the cross-linking of the guluronic acid blocks by the divalent cations such as calcium ions, resulting in an 'egg-box' structure (Gombotz & Wee, 1998). This natural polymer is widely used in encapsulation applications because of its structural simplicity, low toxicity and immunogenicity and high availability (Su *et al.*, 2011; Goh *et al.*, 2012).

However, the cross-linked alginate microparticles are usually porous and exhibit poor mechanical stability (Chan *et al.*, 2011). As a result, most of the entrapped material is released from the microparticles quickly during application. Biopolymer blends is an alternative approach to improve desired functional properties of alginate such as stability, encapsulation efficiency and release profile. Alginate and pectin showed interesting synergistic properties, and mixture of these biopolymers leads to a microstructure very different from that of polymers alone (Islan *et al.*, 2012).

To our knowledge, the effects of the reinforcement based on alginate and high methoxy pectin (HMP) on physicochemical properties of the encapsulated nisin were not reported by ionic external gelation technology. Therefore, this study aimed to develop nisin-loaded alginate-HMP microparticles by w/o emulsification and ionic gelation technique. The effect of nisin concentration on loading capacity (LC), encapsulation efficiency (EE) and mean particle size was investigated. Scanning electron microscopy of nisin-loaded alginate-HMP microparticles was performed to determine their morphologies, and the compatibility of nisin entrapped into microparticles was analysed by Fourier transform infrared (FTIR) spectroscopy. In addition, *in vitro* nisin release behaviours were studied.

Materials and methods

Chemicals

Nisin Z® (2.5% pure nisin) was supplied from Handary (SA, Brussels, Belgium). Sodium alginate with a molecular weight of 1.97×10^5 Da and mannuronate/guluronate ratio of 0.6 were used in this study (BDH, Poole, UK). High methoxy pectin (HMP) was obtained from Danicco Co., Copenhagen, Denmark. Brain heart infusion (BHI) was supplied from Merck Co. (Darmstadt, Germany). Sunflower oil with no added antioxidants was purchased from Nina (Tehran, Iran). Calcium chloride was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Preparation of alginate-HMP microparticles loaded with nisin

Microparticles were prepared using a w/o emulsion external cross-linking procedure (Moebus *et al.*,

2012) with a little modification. In this study, sunflower oil was used as organic phase. First, sodium alginate and HMP aqueous solutions were prepared separately using distilled water. These solutions were then mixed to obtain weight ratio of alginate to HMP of 1:2 and stirred for 10 min giving the biopolymers blend. Nisin was added to polymer solution to reach a 4:1, 2:1 and 1:1 nisin/alginate weight ratio (25%, 50% and 100% w/w, respectively) and stirred for another 30 min. Then, 30 mL sunflower oil (contained 1% v/v span 80 and 1% w/v Tween 80) and 20 mL polymeric solution were mixed using Ultra-Turrax (IKA T25-Digital Ultra-Turrax, Staufen, Germany) at a speed of 10 000 rpm for 3 min to get an w/o emulsion. Microparticles were formed by adding 8 g of calcium chloride solution (25% w/w) dropwise into 50 mL of w/o emulsion with continuous stirring and were allowed to cross-link with calcium ions for 20 min. The microparticles were recovered by centrifugation at 831 *g* for 5 min, followed by vacuum filtration and several washes with distilled water containing 0.1% v/v Tween 80. Finally, they were frozen at −80 °C for 2 h and lyophilised with a freeze drier for 14 h (ALPHA 2-4; Christ, Harz, Germany).

Nisin encapsulation efficiency and loading capacity

The content of nisin loaded in microparticles was determined by agar diffusion method. Accurately weighed amounts of microparticles from each formulation were dissolved under vigorous stirring in phosphate-buffered saline at 37 °C until they were completely dissolved. Nisin extraction was accomplished by adding 0.02 M hydrochloric acid followed by centrifugation at 3326 *g* for 10 min. The supernatant was used to determined nisin concentration.

Each sample was measured in triplicate. EE and LC were determined using the following equations:

$$EE(\%) = \frac{\text{Total amount of loaded nisin}}{\text{Initial amount of nisin}} \times 100 \quad (1)$$

$$LC(\%) = \frac{\text{Total amount of loaded nisin}}{\text{Weight of microparticles after freeze drying}} \times 100 \quad (2)$$

Agar diffusion assay

The agar diffusion method was used to detect nisin activity against food pathogen *Listeria monocytogenes* ATCC 19117 as a test microorganism. This assay is based on the measurement of the inhibition zone produced by nisin-sensitive microorganisms. First, 100 μL of an overnight broth culture containing 10^7–10^8 CFU mL^{-1} of *L. monocytogenes* was inoculated on

the BHI plates, and then, three wells of 6 mm diameter were punched into the agar (depth of wells = 3 mm).

For the standard curve, standard nisin solutions (0.01–2.5 mg mL^{-1}) were prepared in sterile 0.02 N HCl. Twenty microlitres of nisin standard solutions was placed into prepared wells. The plates without inversion were incubated at 30 °C for 24 h. Inhibition zone diameter was then measured using a caliper to the nearest 0.02 mm (Jozala *et al.*, 2011). To generate nisin standard curve, the log nisin concentrations were plotted against inhibition zone diameters ($y = 8.626x - 20.38$ $R^2 = 0.971$).

Unknown amount of nisin in this study was determined by loading twenty microlitres of samples into the wells, and the plates were incubated in the same conditions as the standard solutions. The average of six inhibition zone diameters was used to calculate nisin concentrations in each formulation.

Scanning electron microscopy

Scanning electron microscope (SEM; EM 3200; KYKY, Beijing, China) was used to study the morphology of the nisin-loaded alginate-HMP microparticles. The sample was fixed to the specimen holder with a double-sided adhesive tape and sputtered with gold. SEM images were taken at the required magnification at room temperature and examined using an acceleration voltage of 25 kV.

Fourier transform infrared analyses

Fourier transform infrared spectra of sodium alginate, HMP, alginate-HMP microparticles and nisin-loaded alginate-HMP microparticles were recorded in KBr pellet using an FTIR spectrophotometer (PerkinElmer Spectrum RX I, Waltham, MA, USA). Each sample was scanned 16 times in the range of 4000–450 cm^{-1} at a resolution of 4 cm^{-1}. Data were analysed by Spectrum v5.0.1 software (PerkinElmer, Waltham, MA, USA).

Particle size measurements
Mean size and size distribution were studied by laser diffraction using a Cilas 1090 particle-size analyzer (Orleans, France) equipped with a 5 mW He/Ne (635 nm) laser beam. Measurements were carried out on diluted aqueous microparticle dispersions in triplicate. Polydispersity was expressed by span factor. Span is a measure of the distribution width of particle size in dispersion, which was calculated using the following equation (Elversson *et al.*, 2003):

$$\text{Span} = \frac{(D_{0.9} - D_{0.1})}{D_{0.5}} \quad (3)$$

where $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$ are size of particles below which 10%, 50% and 90% of the samples lies, respectively.

In vitro release profile

The release of nisin from microparticles was studied in distilled water as release medium. Nisin-loaded microparticles (about 100 mg) were suspended in 25 mL distilled water under room temperature and static condition. At specific time intervals, 1 mL of the samples was withdrawn and replaced with fresh medium. The aliquots were centrifuged at 3326 g for 10 min, and supernatant was taken for the determination of nisin activity by agar diffusion test. The cumulative percentage of nisin released was plotted against time in hours. Three replicates of the experiments were performed.

To predict and correlate the release behaviour of the nisin from the polymeric matrix, it is necessary to fit it to an appropriate model. Thus, the release data were fitted to a well-known Korsmeyer and Peppas semi-empirical model (Ritger & Peppas, 1987):

$$\frac{m_t}{m_\infty} = k.t^n \quad (4)$$

where m_t/m_∞ is the fractional release of nisin at time t; k is a constant which indicates the characteristics of the macromolecular network system; n is the release exponent characterising the nisin release mechanism.

Values for n lower than 0.5 indicate a simple Fickian diffusion. Values higher than 0.5 are related to non-Fickian release: in other words, active agent release occurred through diffusion in the hydrated matrix and polymer relaxation (Paula *et al.*, 2011).

Statistical analysis of data

Data were subjected to statistical analysis using SPSS statistical software. version 16 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) followed by the Duncan's multiple range procedure was used to identify any significant differences between the samples. A value of $P < 0.05$ was considered statistically significant.

Results and discussions

Encapsulation efficiency and loading capacity

The percentage of EE and LC of microparticles are presented in Table 1. The amount of loaded nisin was determined by agar diffusion assay. The EE values of nisin were between 47.44 ± 1.40 and 53.83 ± 1.51%.

Microparticles	Nisin/alginate ratio (%w/w)	EE (%)	LC (%)
Alginate-HMP$_0$	0	0.00	0.00
Alginate-HMP$_1$	25	53.83 $\pm$ 1.51[a]	16.00 $\pm$ 0.56[a]
Alginate-HMP$_2$	50	49.90 $\pm$ 0.89[b]	18.83 $\pm$ 1.00[b]
Alginate-HMP$_3$	100	47.44 $\pm$ 1.40[c]	21.06 $\pm$ 1.36[c]

Values within each column with different letters are significantly different ($P < 0.05$).

*Data reported are average values $\pm$ standard deviations.

With increasing initial nisin concentration, EE tended to decrease, and thus the maximum EE value was obtained for the formulation prepared using the initial nisin to alginate weight ratio of 25% w/w (53%). This decrease in EE value with increasing nisin content might be due to the saturation of nisin into microparticles. This finding was in agreement with the results regarding the loading of nisin into solid lipid nanoparticles, which have been reported by Prombutara *et al.* (2012).

Higher loading capacity for encapsulated products can be considered as a positive characteristic. Therefore, the effects of initial nisin contents on LC were studied. The results showed that the % LC values increased from 16.00 $\pm$ 0.56% to 21.06 $\pm$ 1.36% by increasing initial nisin to alginate weight ratio from 25 to 100% w/w (Table 1). The increase in LC as a function of initial active agent content has been reported previously (Hosseini *et al.*, 2013). The obtained result can be helpful in calculating the amount of microparticles needed to reach a determined nisin level in a food matrix.

Particle size analyses

The particle size parameters of microparticles prepared using different formulation are shown in Table 2. As can be seen, the unloaded microparticles (alginate-HMP$_0$) had a mean diameter of 57.57 $\pm$ 1.98 μm and a span factor of 1.59. In nisin-loaded microparticles,

the size and size distribution were affected significantly by the nisin content ($P < 0.05$). Mean diameter of nisin-loaded microparticles increased from 80.75 $\pm$ 2.37 to 131.28 $\pm$ 4.02 μm by increasing initial nisin to alginate weight ratio from 25 to 100% w/w. The larger particle size with higher nisin content was accompanied by an increase in the polydispersity obtained from the calculation of span factor. This increase was probably because of encapsulation of nisin in alginate-HMP microparticles. The increasing size as a function of initial nisin content was in agreement with previous studies related to the loading of nisin into poly-l-lactide nanoparticles and solid lipid nanoparticles, respectively (Salmaso *et al.*, 2004; Prombutara *et al.*, 2012).

Scanning electron microscopy

The morphology and surface of microparticles were analysed by scanning electron microphotography of freeze-dried microparticles (Fig. 1). Alginate-HMP microparticles containing nisin showed similarly acceptable spherical structure with fold on the surface. Similar folded surface was observed with alginate/HM pectin blends microspheres loaded with Ciprofloxacin,
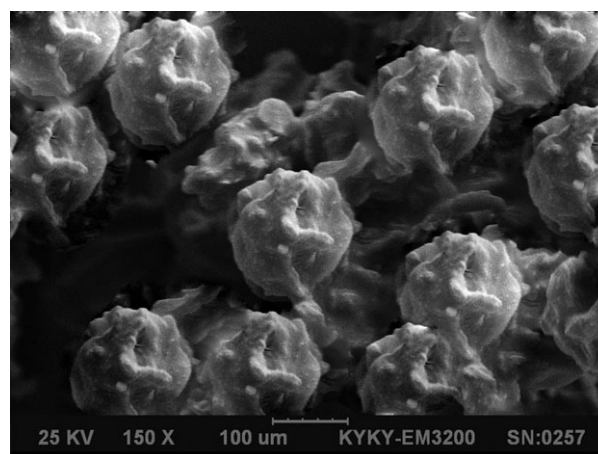
Figure 1 SEM micrograph nisin-loaded alginate-HMP microparticles.

| Microparticle | Particle size parameters | | | | |
	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	Mean diameter (μm)	Span
Alginate-HMP$_0$	13.03 $\pm$ 1.41[a]	55.50 $\pm$ 3.11[a]	101.73 $\pm$ 3.11[a]	57.57 $\pm$ 1.98[a]	1.59 $\pm$ 0.00[a]
Alginate-HMP$_1$	26.50 $\pm$ 2.12[b]	64.31 $\pm$ 1.42[b]	150.56 $\pm$ 2.82[b]	80.75 $\pm$ 2.37[b]	1.92 $\pm$ 0.02[b]
Alginate-HMP$_2$	30.51 $\pm$ 1.42[b]	70.55 $\pm$ 2.05[c]	194.97 $\pm$ 1.46[c]	105.28 $\pm$ 2.80[c]	2.33 $\pm$ 0.07[c]
Alginate-HMP$_3$	15.56 $\pm$ 1.50[a]	83.45 $\pm$ 1.41[d]	290.55 $\pm$ 2.19[d]	131.28 $\pm$ 4.02[d]	3.29 $\pm$ 0.05[d]

Values within each column with different letters are significantly different ($P < 0.05$).

*Data reported are average values $\pm$ standard deviations.

which have been reported by Islan *et al.* (2012). These authors represented that by increasing HMP on the blend matrix, microspheres with no symmetry and folded surface were obtained. As can be seen, nisin-loaded alginate-HMP microparticles showed nonporous surface. The most probable explanation for this structure is reinforcement of alginate gel with the addition of HMP. This combination is significant because the alginate forms a porous matrix and HMP may contribute to decreasing the pore size of this polymeric system.

FTIR analyses

To indentify functional groups and determine the interaction between biopolymers and nisin, the FTIR analyses were used. The FTIR spectra of sodium alginate, HMP, alginate-HMP microparticles and nisin-loaded alginate-HMP microparticles are shown in Fig. 2. Sodium alginate and HMP spectra showed similar characteristic peaks which can be attributed to their comparable structure.

Fourier transform infrared spectra of sodium alginate showed the characteristic absorption bands at 3421, 2934, 1658, 1469 and 1034 cm^{-1}, which were due to the stretching of O–H, C–H, COO^{-} (asymmetric), COO^{-} (symmetric) and C–O–C, respectively. This spectra pattern of sodium alginate has been reported previously (Hosseini *et al.*, 2013). HMP showed two prominent peaks at 1746 cm^{-1}, assigned as the ester carbonyl (C = O) groups, and at 1627 cm^{-1}, attributed to carboxyl ion stretching band (COO^{-}). Stronger intensity of ester carbonyl peak at 1746 compared to free carboxyl band at 1627 indicated an increase in the
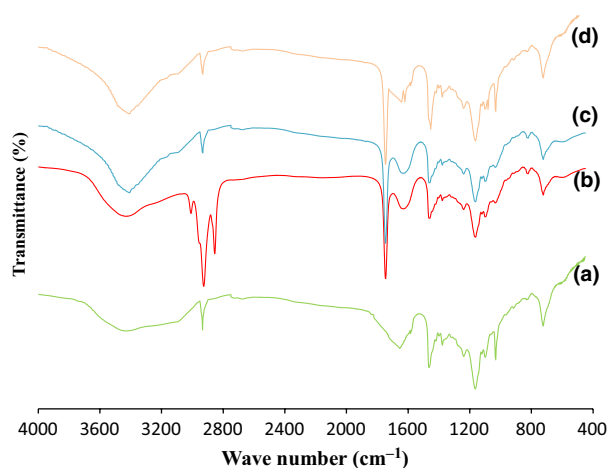
degree of esterification. With increase in the degree of esterification, the absorbance band of the ester carbonyl groups increased, but intensity of the free carboxyl stretching band showed a decrease (Singthong *et al.*, 2005).

Alginate-HMP microparticles showed visible alterations in the spectrum. The peak of hydroxyl group increased in intensity and shifted to lower wave numbers. At the same time, the peak of COO^{-} shifted from 1654 and 1464 cm^{-1} to 1641 and 1453 cm^{-1}, respectively. These changes were attributed to the increase in the hydrogen bonding between the alginate and HMP. Furthermore, the decrease in intensity observed for the C-H stretching vibrations (around 2930) could primarily be explained by the movement inhibition of these groups in the blend matrix (Sartori, 1997). In nisin-loaded microparticles, the peaks around 1640 cm^{-1} were sharper and shifted to lower wave numbers. This change was considered to the presence of nisin inside of the microparticles. This finding is consistent with the results regarding the loading of nisin into chitosan/alginate nanoparticles, which have been reported by Zohri *et al.* (2010).
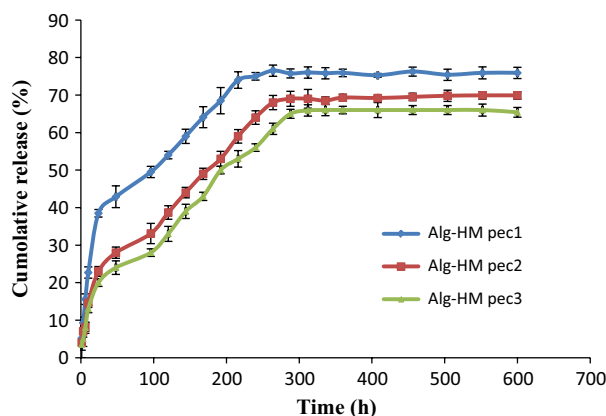
Figure 3 *In vitro* release profile of nisin from alginate-HMP microparticles prepared using different formulations. Error bars represent the SD ($n = 3$).

Table 3 Kinetic parameters obtained from release curve (n and K) for different formulation of nisin-loaded alginate-HMP microparticles

Microparticles	Kinetic parameters		
	n	K (s^{-n})	R^2
Alginate-HMP$_1$	0.405	0.865	0.94
Alginate-HMP$_2$	0.468	0.6	0.98
Alginate-HMP$_3$	0.488	0.549	0.98

Figure 2 FTIR spectra of sodium alginate (a), HMP (b), alginate-HMP microparticles (c) and nisin-loaded alginate-HMP microparticles (d).

Release studies

Release studies of nisin from polymeric matrix are an important tool to examine the suitability of this matrix to use as carrier for controlled release of bacteriocin. Release pattern was obtained by plotting the amount of cumulative release against the time. As can be observed from Fig. 3, in all formulation, the *in vitro* release profile of nisin from alginate-HMP microparticles can be described as two stages: an initial rapid nisin release phase (burst effect) followed by a slower, gradual release phase, until the nisin content was constant. An obvious increase in the release rate of nisin at first stage corresponds to nisin being physically entrapped in the surface of microparticles. In addition, volume expansion of the biopolymers has an important role in the initial burst release of nisin from the polymeric matrix (Beirão-da-Costa *et al.*, 2013). On the other hand, delayed release in the second phase can be attributed to the diffusion of the nisin from the inside of microparticles, which needed more time to be extracted. Release properties of nisin were affected by initial nisin level. The amount of released nisin decreased with increasing initial nisin to alginate weight ratio from 25 to 100% w/w. Moreover, lower initial level of nisin gives higher fractional release. Microparticles formulated with 25% w/w nisin to alginate weight ratio released about 75% of the encapsulated bacteriocin after 240 h, followed by a sustained release profile. When initial nisin to alginate weight ratio was increased to 100% w/w, accumulative release decreased from 75 to 56% after 240 h. The most probable explanation for these results is the particle size effect. Generally, the release of nisin from microparticles with smaller size is faster due to greater surface to volume ratios of microparticles. A similar correlation between particles size and release rate of active agent has been reported in the previous studies (Chen & Subirade, 2007; Hosseini *et al.*, 2013).

To predict the release behaviour of the nisin from alginate-HMP microparticles, it is necessary to fit the release data to a suitable model. Hence, Korsmeyer and Peppas semi-empirical model was used for this purpose. Table 3 shows the values of *n* and K, obtained by applying this model, as well as correlation coefficients (R^2) for three formulations. As can be seen, the *n* values for all formulations ranged from 0.405 to 0.488, indicating that nisin release from the microparticles follows Fickian diffusion.

Conclusion

Blending of alginate and HMP followed by cross-linking with calcium chloride solution resulted in the formation of microparticles, which shows a promise to function as a carrier for controlled release of nisin.

The FTIR analysis of bacteriocin-loaded microparticles confirmed the presence of nisin in this matrix. SEM images showed that prepared microparticles were nearly spherical in shape with size range 57–131 µm. By increasing the initial nisin content in formulation, LC% is increased, while EE% is decreased. The results of the experiments of release kinetics showed microparticles followed a Fickian diffusion mechanism. In addition, the *in vitro* release studies indicated that the content of nisin in the microparticles influenced its release rate. Higher release rates were observed in microparticles with lower levels of nisin. Thus, these results improve the possibility of using nisin-loaded alginate-HMP microparticles as an antimicrobial agent in the food products.

References

Beirão-da-Costa, S., Duarte, C., Bourbon, A.I. *et al.* (2013). Inulin potential for encapsulation and controlled delivery of Oregano essential oil. *Food Hydrocolloids*, **33**, 199–206.

Chan, E.S., Wong, S.L., Lee, P.P. *et al.* (2011). Effects of starch filler on the physical properties of lyophilized calcium-alginate beads and the viability of encapsulated cells. *Carbohydrate Polymers*, **83**, 225–232.

Chen, L. & Subirade, M. (2007). Effect of preparation conditions on the nutrient release properties of alginate–whey protein granular microspheres. *European Journal of Pharmaceutics and Biopharmaceutics*, **65**, 354–362.

De Salamanca, A.E., Diebold, Y., Calonge, M. *et al.* (2006). Chitosan nanoparticles as a potential drug delivery system for the ocular surface: toxicity, uptake mechanism and in vivo tolerance. *Investigative Ophthalmology and Visual Science*, **47**, 1416–1425.

Elversson, J., Millqvist-Fureby, A., Alderborn, G. & Elofsson, U. (2003). Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying. *Journal of Pharmaceutical Sciences*, **92**, 900–910.

Goh, C.H., Heng, P.W.S. & Chan, L.W. (2012). Alginates as a useful natural polymer for microencapsulation and therapeutic applications. *Carbohydrate Polymers*, **88**, 1–12.

Gombotz, W.R. & Wee, S. (1998). Protein release from alginate matrices. *Advanced Drug Delivery Reviews*, **31**, 267–285.

Hosseini, S.M., Hosseini, H., Mohammadifar, M.A. *et al.* (2013). Incorporation of essential oil in alginate microparticles by multiple emulsion/ionic gelation process. *International Journal of Biological Macromolecules*, **62**, 582–588.

Hosseini, S.M., Hosseini, H., Mohammadifar, M.A. *et al.* (2014). Preparation and characterization of Alginate and alginate-resistant starch microparticles containing nisin. *Carbohydrate Polymers*, **103**, 573–580.

Islan, G.A., De Verti, I.P., Marchetti, S.G. & Castro, G.R. (2012). Studies of ciprofloxacin encapsulation on alginate/pectin matrixes and its relationship with biodisponibility. *Applied Biochemistry and Biotechnology*, **167**, 1408–1420.

Jin, T., Liu, L., Zhang, H. & Hicks, K. (2009). Antimicrobial activity of nisin incorporated in pectin and polylactic acid composite

films against *Listeria monocytogenes*. *International Journal of Food Science and Technology*, **44**, 322–329.

Jozala, A.F., Silva, D.P., Vicente, A.A., Teixeira, J.A., Pessoa Junior, A. & Penna, T.C. (2011). Processing of byproducts to improve nisin production by *Lactococcus lactis*. *African Journal of Biotechnology*, **10**, 14920–14925.

Laridi, R., Kheadr, E.E., Benech, R.O., Vuillemard, J.C., Lacroix, C. & Fliss, I. (2003). Liposome encapsulated nisin Z: optimization, stability and release during milk fermentation. *International Dairy Journal*, **13**, 325–336.

Li, Z., Percival, S.S., Bonard, S. & Gu, L. (2011). Fabrication of nanoparticles using partially purified pomegranate ellagitannins and gelatin and their apoptotic effects. *Molecular Nutrition and Food Research*, **55**, 1096–1103.

Moebus, K., Siepmann, J. & Bodmeier, R. (2012). Novel preparation techniques for alginate-poloxamer microparticles controlling protein release on mucosal surfaces. *European Journal of Pharmaceutical Sciences*, **45**, 358–366.

Paula, H.C.B., Sombra, F.M., Cavalcante, R.D.F., Abreu, F.O.M.S. & De Paula, R.C.M. (2011). Preparation and characterization of chitosan/cashew gum beads loaded with Lippia sidoides essential oil. *Materials Science and Engineering C-Materials for Biological Applications*, **31**, 173–178.

Prombutara, P., Kulwatthanasal, Y., Supaka, N., Sramala, I. & Chareonpornwattana, S. (2012). Production of nisin-loaded solid lipid nanoparticles for sustained antimicrobial activity. *Food Control*, **24**, 184–190.

Register, F. (1988). Nisin preparation: affirmation of GRAS status as a direct human food ingredient. *Federal Register*, **53**, 11247–11251.

Ritger, P.L. & Peppas, N.A. (1987). A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *Journal of Controlled Release*, **5**, 37–42.

Rodriguez, J. (1996). Review: antimicrobial spectrum, structure, properties and mode of action of nisin, a bacteriocin produced by *Lactococcus lactis*. *Food Science and Technology International*, **2**, 61–68.

Salmaso, S., Elvassore, N., Bertucco, A., Lante, A. & Caliceti, P. (2004). Nisin-loaded poly-L-lactide nano-particles produced by CO2 anti-solvent precipitation for sustained antimicrobial activity. *International Journal of Pharmaceutics*, **287**, 163–173.

Sartori, C. (1997). The characterisation of alginate systems for biomedical applications. Brunel University School of Engineering and Design PhD Theses. http://bura.brunel.ac.uk/handle/2438/7399.

Silva Malheiros, P.D., Serafini Micheletto, Y.M., Pesce Da Silveira, N. & Brandelli, A. (2010). Development and characterization of phosphatidylcholine nanovesicles containing the antimicrobial peptide nisin. *Food Research International*, **43**, 1198–1203.

Singthong, J., Ningsanond, S., Cui, S.W. & Douglas Goff, H. (2005). Extraction and physicochemical characterization of Krueo Ma Noy pectin. *Food Hydrocolloids*, **19**, 793–801.

Su, R., Zhu, X.-L., Fan, D.-D., Mi, Y., Yang, C.-Y. & Jia, X. (2011). Encapsulation of probiotic *Bifidobacterium longum* BIOMA 5920 with alginate–human-like collagen and evaluation of survival in simulated gastrointestinal conditions. *International Journal of Biological Macromolecules*, **49**, 979–984.

Xiao, D. & Zhong, Q. (2011). *In vitro* release kinetics of nisin as affected by Tween 20 and glycerol co-encapsulated in spray-dried zein capsules. *Journal of Food Engineering*, **106**, 65–73.

Zamboni, W.C. (2005). Liposomal, nanoparticle, and conjugated formulations of anticancer agents. *Clinical Cancer Research*, **11**, 8230–8234.

Zohri, M., Alavidjeh, M.S., Haririan, I. *et al.* (2010). A comparative study between the antibacterial effect of nisin and nisin-loaded chitosan/alginate nanoparticles on the growth of *Staphylococcus aureus* in raw and pasteurized milk samples. *Probiotics and Antimicrobial Proteins*, **2**, 258–266.

Zou, Y., Jung, L.-S., Lee, S.H., Kim, S., Cho, Y. & Ahn, J. (2013). Enhanced antimicrobial activity of nisin in combination with allyl isothiocyanate against *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella Typhimurium* and *Shigella boydii*. *International Journal of Food Science and Technology*, **48**, 324–333.