LWT - Food Science and Technology 72 (2016) 518–524

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Preparation and characterization of nisin-loaded pectin-inulin particles as antimicrobials

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ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form
29 April 2016
Accepted 7 May 2016
Available online 9 May 2016

Keywords:
Pectin
Degree of esterification
Inulin
Nisin
Particles
Antimicrobial activity

ABSTRACT

Nisin is a known bacteriocin approved as a food additive for food preservation. To protect the bacteriocin from its interaction with food components, nisin-loaded pectin-inulin particles were prepared. For particles preparation, pectins with different degree of esterification were used. The combination of inulin with pectin increased the efficiency of nisin loading as compared with nisin-pectin particles. For all tested pectins in the pH range of 4.0–7.0 and in the range of nisin concentration of 0.1–1.0 mg/mL the loading efficiency was equal to 100%. The combination of inulin and pectin for particles preparation slightly but not significantly increased the antimicrobial activity of nisin comparing to nisin-pectin particles. The antimicrobial activity of nisin-loaded pectin-inulin particles was dependent on the degree of pectin esterification. The particles with low degree of pectin esterification or with not esterified pectic acid demonstrated the higher activity as compared with the particles of high degree of pectin esterification. Due to the high efficiency of nisin-loading and similar antimicrobial activity comparing to nisin-pectin particles, the combination of pectin and inulin has the perspectives for the antimicrobial particles preparation and their application in food industry.

1. Introduction

Food safety is an important problem not only for developing countries, but also for economically strongest countries in the world. Especially, it is actual for the growing consumer demands for foods that are ready to eat. Therefore, the importance of food preservation and storage has increased over the years. In recent years, there has been an increasing interest for higher quality and natural foods without chemical preservatives. The application of natural antimicrobials for the production of safe and healthy food is a promising perspective (Gyawali & Ibrahim, 2014). Bacteriocins that are produced by different groups of bacteria are being examined as biopreservatives (Mills, Stanton, Hill, & Ross, 2011; Prudencio, dos Santos, & Vanetti, 2015; Yang, Lin, Sung, & Fang, 2014). Lactic acid bacteria that are regarded as safe have a widespread ability to produce bacteriocins (Cintas, Casaus, Herranz, Nes, & Hernandez, 2001; Pisano et al., 2015).

Nisin is produced by *Lactococcus lactis* subsp. *lactis* and is the first bacteriocin granted GRAS status in 1988 (Arthur, Casera, & Chikindas, 2014). Moreover, nisin is also recognized as a food additive in EU. It is a small cationic peptide of 34 amino acid residues. Nisin belongs to lantibiotics and contains unusual amino acid residues dehydrobutyrine, dehydroalanine, lanthionine and β-methyl lanthionine (Cheigh & Pyun, 2005). The bacteriocin is widely used in food industry (Balciunas et al., 2013). Therefore, to protect the bacteriocin from its interaction with food components, there is a great demand for low cost and effective delivery systems. The incorporation of bacteriocins into delivery systems is a rather new and rapidly expanding field (Cavera, Arthur, Kashtanov, & Chikindas, 2015). For nisin encapsulation, various natural polymers as alginate/cellulose, alginate/pectin, alginate/guar gum, alginate/starch are used (Hosseini et al., 2014; Huq, Riedl, Bouchard, Salmieri, & Lacroix, 2014; Khaksar et al., 2014; Narsaiah, Jha, Wilson, Mandge, & Manikantan, 2014). For nisin, liposomal delivery systems (Imran et al., 2015; Silva Malheiros, Serafini

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http://dx.doi.org/10.1016/j.lwt.2016.05.022
0023-6438/

Micheletto, Pesce da Silveira, & Brandelli, 2010) as well as bioactive caseinate (Calderon-Aquirre et al., 2015) and pullulan films (Pattanayaiying, Kittikun, & Cutter, 2015), and nisin-nanoclay system (Meira, Jardim, & Brandelli, 2015) were also developed.

The encapsulation of nisin overcomes the limitations related to its interaction with food components, the reduction of antimicrobial activity due to enzymatic degradation of peptide or due to electrostatic repulsion induced by divalent cations associated with bacterial cell wall surfaces (Khan & Oh, 2016). As an example, nisin encapsulated in alginate-chitosan-pluronic composite nanoparticles exhibited bacteriostatic effect on tomato juice for 6 months compared to 5 months for free nisin (Bernela, Kaur, Chopra, & Thakur, 2014). Nisin-loaded chitosan/alginate nanoparticles were able to decrease the population of *S. aureus* and *Listeria monocytogenes* up to five- and sevenfold on a logarithmic scale in comparison with free nisin in ultra filtered Feta cheese (Zohri et al., 2013).

Previously, we prepared nisin-loaded pectin particles and showed that they exhibit antimicrobial activity and have a potential to be applied in food industry for food preservation (Krivorotova et al., 2016). Moreover, we found that the antimicrobial activity of particles is dependent on the degree of pectin esterification. Pectins with free carboxyl groups or of low degree of esterification are the most suitable for particles preparation (Krivorotova, Staneviciene, Luksa, Serviene, & Sereikaite, 2016). Here, we present the preparation of nisin-loaded pectin-inulin particles and their biological characteristics. Inulin consists of linear β (2 → 1) linked fructofuranosyl units terminated by a glucose residue through a sucrose type linkage (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015a) Inulin was approved of the Generally Recognized As Safe (GRAS) status by the United States Food and Drug Administration in 2002 (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015b). Inulin is widely used in food industry as a soluble dietary fiber and fat or sugar replacement (Meyer, Bayarri, Tarrega, & Costell, 2011). Moreover, inulin finds new applications in pharmaceutical industry in the areas of protein stabilization and the modification of drug delivery systems (Mensink et al., 2015b).

The main objectives of the present study were to prepare nisin-loaded pectin-inulin particles and to compare their antimicrobial activity with nisin-loaded pectin particles.

2. Materials and methods

2.1. Materials

Nisin Z (NisinZ™ P) was purchased from Handary S.A. (Brussels, Belgium). Both high methoxyl pectin (HMP, M_w 30000-1000000, degree of esterification 60%) and low methoxyl pectin (LMP, M_w not determined, degree of esterification ≤ 26%) from citrus peel were purchased from Fluka and Sigma, respectively. All materials were used without additional purification. Pectic acid (PecA, M_w 30,000) was purchased from Serva. Prior to use, PecA was dissolved in distilled water by adjusting the pH of solution to the value of 8.0 with 0.1 M NaOH. The solution was dialyzed against distilled water containing 0.03% of sodium azide with a 3000 molecular weight cut off dialysis membrane to remove Na ions. Dodecyl pectic acid (DoPecA) with the degree of dodecyl substitution of 25% was synthesized as previously described (Krivorotova et al., 2016). Inulin from dahlia tubers (M_w ~ 5000) was purchased from Sigma.

2.2. Preparation and physicochemical characteristics of nisin-loaded pectin-inulin particles

The stock solutions of pectin, inulin and nisin were prepared by dissolving 250, 500 or 1000 mg of each compound in 250 mL of deionized water. Prior to use, the solutions were filtered through 0.2 μm pore size filters. For the formation of particles, a volume of nisin solution in the range of 5–30 mL at the concentration of 1 mg/mL (or 20 mL and 25 mL at the concentration of 2 mg/mL) was added dropwise to the pectin-inulin solution under constant stirring at room temperature. Prior to the addition of nisin, the solutions of pectin and inulin were mixed and diluted with water to obtain a final mixture at the pectin and inulin concentration of 0.4 mg/mL, and the nisin concentration in the range of 0.1–1 mg/mL. Finally, the desired pH value in the range of 4.0–7.0 was adjusted with 0.01 or 0.1 M NaOH solution.

The hydrodynamic radius of nisin-loaded particles, their zeta-potential and nisin-loading efficiency were determined, and the scanning electron microscopy of particles was performed as previously described (Krivorotova et al., 2016). Fourier transform-infrared (FT-IR) analyses was performed using a FT-IR spectrophotometer (Perkin Elmer Frontier FT-IR). FT-IR spectra of dried samples were recorded from wave number 500–4000 cm^{-1}.

2.3. Bacterial strains and culture conditions

Arthrobacter sp. and *Bacillus subtilis* bacteria were used as Gram-positive indicator strains, and *Escherichia coli* and *Klebsiella* sp. bacteria were used as Gram-negative indicator strains. *Bacillus subtilis, Escherichia coli* and *Klebsiella* sp. were propagated in Luria-Bertani (LB) medium (2% tryptone, 2% yeast extract, 1% NaCl) at 37 °C and *Arthrobacter* sp. at 30 °C, respectively, for 24 h.

2.4. Analysis of viability of bacteria in contact with nisin-loaded pectin-inulin particles

For the estimation of bacteria sensitivity to nisin-inulin-pectin particles, the bacteria *B. subtilis*, *E. coli*, *Klebsiella* sp. and *Arthrobacter* sp. were grown overnight at 37 °C and 30 °C, respectively. 8×10^8 cells/sample of Gram-positive bacteria *B. subtilis* and 4×10^9 cells/sample of *Arthrobacter* sp. were collected by centrifugation at 3000x*g* for 5 min. In the case of Gram-negative bacteria *E. coli* and *Klebsiella* sp. 2×10^7 cells/sample were collected and additionally treated with the permeabilization solution containing 0.8 M sorbitol, 4 mM DTT, 0.5 M EDTA and 10 mM Tris-HCl, pH 7.5 for 30 min at 37 °C. Then, bacterial cells were mixed with 1 mL of nisin-inulin-pectin particle solution or appropriate pectin or pectin-inulin only as control (concentrations of nisin, pectin and inulin were equal to 0.4 mg/mL). Samples were incubated at room temperature (20 °C) for 24 h with gentle agitation. Serial dilutions were performed in 0.9% NaCl and 50 μL of each solution was spread onto LB-agar plates with following incubation overnight at 37 °C for *B. subtilis*, *E. coli*, *Klebsiella* sp. and 30 °C for *Arthrobacter* sp. bacteria. After incubation, colonies were counted as CFU (colony forming units), and then the mean value of CFU/mL was calculated.

2.5. Statistical analysis

For the analysis of antimicrobial activity, three independent experiments with three replicates in parallel were conducted, and the mean values ± standard deviations were defined. All data were analyzed using Statistica v.9 (Tulsa, USA), and significant differences in survived cells ($P < 0.05$) was determined using paired-*t*-test (two-tailed with equal variance).

3. Results and discussion

3.1. Preparation and physicochemical characteristics of nisin-loaded pectin-inulin particles

Nisin-loaded pectin-inulin particles were prepared at different