Fucoidan as a carrier of antimicrobial peptide: Preparation and characterization of nisin-loaded particles

Rūta Gruškienė [a], Aistė Galinskaitė [a], Tatjana Kavleiskaja [b], Ramunė Stanevičienė [c], Elena Servienė [a,c], Jolanta Sereikaitė [a,*]

[a] Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
[b] Institute of Chemistry, Vilnius University, Vilnius, Lithuania
[c] Laboratory of Genetics, Nature Research Centre, Vilnius, Lithuania

ARTICLE INFO

ABSTRACT

Fucoidan is an anionic sulphated polysaccharide found in brown seaweed. Due to its various biological activities, biodegradability, and biocompatibility, the application of fucoidan is increasing in various fields. The research was aimed to use fucoidan for nisin encapsulation. Nisin-loaded fucoidan particles were prepared by the complexation method in the pH range of 4.0–7.0. The interaction of nisin and fucoidan was confirmed by FT-IR spectroscopy, dynamic light scattering method, thermogravimetric and differential scanning calorimetry analysis. The stability of the particles was analysed under storage at 4 °C. After one month of storage, the hydrodynamic diameter and zeta potential of particles changed insignificantly. Encapsulated nisin preserved its biological activity. The antimicrobial activity was tested against Gram-positive bacteria *Bacillus subtilis* and *Listeria innocua*, and Gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium*. Nisin-loaded-fucoidan particles exhibited antibacterial activity against Gram-positive bacteria *B. subtilis* and *L. innocua* comparable to free nisin. The application of fucoidan for the encapsulation of nisin is promising. A new nisin formulation could be used in the food industry for biopreservation.

1. Introduction

Fucoidan is a sulphated polysaccharide found in brown algae. Its chemical structure is heterogeneous and species-related and varies in monomer composition, sulphation pattern, and glycosidic linkage. Moreover, its heterogeneity might also depend on the fucoidan extraction method, the maturation of algae, the harvesting time, and geographical factors. Fucoidan is mainly composed of an L-fucopyranoside backbone linked by repeating α-(1 → 3) with α-(1 → 4) bonds. The fucose backbone can also be linked by α-(1 → 3) or α-(1 → 4) bonds only. Other sugar monomers, such as mannose, galactose, glucose, xylose, and glucuronic acid, are found in the composition of fucoidan. In addition, L-fucose molecule can be mono-or disulphated at C-2 and/or C-4 position and depending on the type of glycosidic linkage at C-3 position (Zayed, El-Aasr, Ibrahim, & Ulber, 2020; Zayed & Ulber, 2019). *Fucus vesiculosus* is often used as a source of fucoidan. Florez-Fernandez et al. (2023) found that it consists of 35 g/100g of fucose and approximately 8 g/100g of a mixture of xylose, galactose, and mannose. The

sulphate content is 27 g/100g. A similar content of total sugar (42 g/100g) and sulphates (22 g/100g) was also detected in another research (Rioux, Turgeon, & Beaulieu, 2007).

The marine algae-derived polysaccharides attract attention due to their potential for various applications. They are inexpensive, biodegradable, and biocompatible. First, fucoidan has biological activity, and different factors such as sugar composition, degree of sulphation, molecular weight, glycosidic linkage, branching sites, and purity affect its biological behaviour. However, the fucoidan structure-activity relationship is not fully understood (Zayed et al., 2020; Zayed & Ulber, 2019). Fucoidan exhibits anticoagulant, antiviral, antitumor, and anti-inflammatory activities. The anticoagulant properties of fucoidan are the most investigated (Barbosa, Coutinho, Costa Lima, & Reis, 2019; Luthuli et al., 2019). Furthermore, fucoidan has antioxidant activity and shows ferric reducing power and DPPH radical scavenging activity (Du et al., 2022; Li, Lu, Wei, & Zhao, 2008).

Fucoidan is used in the food industry as a dietary supplement. The US Food and Drug Administration approved fucoidan extracts of *Undaria*

---

pinnatifida and *Fucus vesiculosus* as generally recognized as safe (GRAS). In Europe, those extracts are recognized as a novel food. Oral consumption of fucoidan up to 250 mg/day is considered safe (Jayawardena et al., 2022; Lahteenmaki-Uutela et al., 2021).

Together with other polyelectrolytes, fucoidan-based nanoparticles have been developed. They find applications for drug delivery systems, tissue engineering, and magnetic resonance imaging systems (Dubashynskaya, Gasilova, & Skorik, 2023; Lu et al., 2017; Venkatesan, Murugan, & Seong, 2022).Furtthermore, fucoidan is an antimicrobial agent (Ayrapetyan et al., 2021; Mensah, Kanwugu, Panda, & Adadi, 2023) and its antimicrobial activity can be coupled with the effect of silver or gold nanoparticles by coating them with fucoidan. Fucoidan is also formulated with chitosan for antimicrobial applications (Rajasekaran & Viswanathan, 2023).

Nisin is a well-known peptide and is composed of 34 amino acid residues. Nisin is approved by the US Food and Drug Administration for its applications in food products. Additionally, it is synthesised by the food-grade lactic acid bacterium *Lactococcus lactis* subsp. *lactis*. The number E234 is assigned to nisin as a food additive in the EU. Nisin is active against Gram-positive bacteria, including *Clostridium botulinum* and *Listeria monocytogenes* (Gharsallaoui, Oulahal, Joly, & Degraeve, 2016). To improve the proteolytic stability of nisin, protect it from interaction with food components, and ensure homogeneous distribution in food products, various encapsulation technologies are used. Moreover, the constant consumer demand for safe and healthy food stimulates the development of biopreservation techniques (Eghbal, Viton, & Gharsallaoui, 2022; O'Connor et al., 2020).

Fucoidan could be explored as a carrier for antimicrobial peptides used in the food industry. Herein, fucoidan was used for the first time for the encapsulation of nisin. The preparation of nisin-loaded fucoidan particles by low-cost and simple complexation method was presented. The physicochemical and antimicrobial properties of the obtained particles were also described.

2. Materials and methods

2.1. Materials

Nisin (NisinZ™ P) was obtained from Handary S.A (Belgium). Fucoidan from *Fucus vesiculosus* (≥98 g/100 g) was purchased from Sigma-Aldrich. Its molecular weight was 95 kDa and the content of sulfur was 8.89 g/100 g (Hahn et al., 2016). Hydrochloric acid (32 g/100 g, p.a.), sodium hydroxide (≥98 g/100g, p.a.), and ortho-phosphoric acid (85 g/100 g, p.a.) were purchased from Carl Roth. Copper sulphate pentahydrate was obtained from Merck. Bicinchoninic acid solution was purchased from Sigma-Aldrich. All reagents were used without additional purification.

2.2. Preparation of nisin-loaded fucoidan particles

The solutions of nisin and fucoidan were prepared in deionized water. The concentration of both solutions was 2 mg/mL. The pH was then adjusted to the desired value (4, 5, 6, or 7) using 0.1 mol/L NaOH or 0.1 mol/L HCl. Subsequently, the solutions were filtered through 0.2 μm pore size cellulose acetate filters. For particle formation, the fucoidan solution was being stirred and a desired amount of peptide solution was added dropwise at 20 °C. Subsequently, the pH value of the mixture was also adjusted to 4, 5, 6, or 7. Before the addition of the peptide, if necessary, an appropriate amount of water was added to obtain the final fucoidan concentration of 0.4 mg/mL and the nisin concentration of 0–1.0 mg/mL. Subsequently, the samples were gently shaken at 150 rpm for 24 h at 20 °C. The particles obtained in the form of soluble complexes or deposits were stored at 4 °C for further analysis (Pachaleva, Gruskiene, Bockuviene, & Sereikaite, 2023).

2.3. Analysis of the efficiency of peptide encapsulation

The solution of 0.5 mL was centrifuged at $10,000 \times g$ and 4 °C for 15 min using ultrafiltration tubes (Amicon Ultra-0.5 centrifugal filter unit, 10 kDa MWCO, Merck Millipore Ltd). Then, the concentration of free peptide was measured in the solution of the outer tube by the bicinchoninic acid method (Noble & Bailey, 2009). The following equation was used for the calculation of the encapsulation efficiency (EE) of nisin: EE (%) = ((Total nisin – free nisin)/Total nisin) × 100.

2.4. Characterization of nisin-loaded fucoidan particles

The hydrodynamic diameter of the particles was determined using the Zetasizer Nano ZS instrument (Malvern Instruments). It was equipped with a 4 mV HeNe laser at a wavelength of 633 nm. At an angle of 173°, the intensity of the scattered light was registered. All measurements were performed at 20 °C (Krivorotova et al., 2016). Malvern Zetasizer software 7.03 was used for data analysis. The zeta potential of particles was determined using the same instrument.

Fourier-transform infrared (FT-IR) spectra were recorded with a PerkinElmer Frontier 65 spectrometer using Universal ATR Sampling Accessory. All spectra were an average of 100 scans from 4000 to 700 cm^{-1} at a resolution of 2 cm^{-1}. The solutions of nisin, fucoidan, and nisin-loaded fucoidan complex were freeze-dried and subsequently dissolved in D$_2$O and freeze-dried. The procedure was repeated once again to ensure a complete H/D exchange.

Thermogravimetric and differential scanning calorimetry (TG-DSC) analysis were performed using an STA 6000 Simultaneous Thermal Analyzer (PerkinElmer, Waltham, MA, USA). An amount of 5 mg of the freeze-dried sample was heated from 25 °C to 300 °C at a rate of 10 °C/min. A nitrogen atmosphere was used at a flow rate of 40 mL/min (You, Liu, & Zhao, 2018).

Scanning electron microscopy (SEM) was performed using a Hitachi SU-70 (FE-SEM, Hitachi, Tokyo, Japan) scanning electron microscope. SEM images were examined at an acceleration voltage of 1.0 kV. The nisin-loaded fucoidan complex was prepared at pH 5. The final concentration of fucoidan and nisin was 0.4 mg/mL. The solution was then diluted 5 times with deionized water, dropped onto a glass slide sputter-coated with 10-nm silver layer, and gently dried at room temperature.

2.5. In vitro release of nisin from nisin-loaded fucoidan particles

A volume of 0.5 mL of particles prepared at the nisin and fucoidan concentrations of 0.4 mg/mL was transferred to the dialysis bag (cut-off 6–8 kDa) and dialyzed against 4 mL of 25 mmol/L phosphate buffer at pH 2, 6 and 8 containing 0, 0.1, 0.2 and 0.5 mol/L NaCl. Dialysis was performed at 20 °C and 37 °C with shaking at 150 rpm. At the time points of 1, 24, 72, and 96 h, 50 μL of the sample was withdrawn and the amount of released nisin was determined by the bicinchoninic acid method (Noble & Bailey, 2009). The volume taken was replaced by a 25 mmol/L phosphate solution of the appropriate pH value and ionic strength. The relative amount of released nisin (%) was calculated according to the following equation (Brum, dos Santos, Santos, & Brandelli, 2022):

$$CR\ (\%) = (C_n/C_t) \times 100$$

where CR (%) is the percentage of nisin released, C_n, and C_t are the concentrations of nisin of the nth sample and the total concentration of nisin, respectively.

2.6. Analysis of sensitivity of bacteria to nisin-loaded fucoidan particles by agar-diffusion method

Gram-positive bacteria *Bacillus subtilis* ATCC 6633 (obtained from Vilnius University) and *Listeria innocua* CECT 910T (kindly provided by

Maria Joao Fraqueza from the University of Lisbon) as well as Gram-negative bacteria *Salmonella typhimurium* LT2 (*pyrF146*) (kindly provided by Jaunius Urbonavičius, Vilnius Gediminas Technical University, Lithuania) and *Escherichia coli* BL21 [F-dcm ompT hsdS(rB-mB-) gall (DE3)] (obtained from ThermoFisher Scientific) were used. For the propagation of bacteria, Luria-Bertani (LB) medium composed of 2 g/dL tryptone, 2 g/dL yeast extract and 1 g/dL NaCl was used. Bacteria were cultivated under shaking at 37 °C for 16–18 h.

For the agar-diffusion assay, 20 μL of nisin-fucoidan nanoparticles along with free nisin samples were spotted on the LB-agar plates seeded with bacteria grown overnight either *B. subtilis*, *L. innocua*, *E. coli* or *S. typhimurium* (2x10^7 cells/plate). Gram-negative bacteria *E. coli* and *S. typhimurium* were additionally treated with the permeabilization solution containing 0.8 mol/L of sorbitol, 4 mmol/L of DTT, 0.5 mol/L of EDTA, and 10 mmol/L of Tris-HCl for 30 min at pH 7.5 and 37 °C. The plates were incubated at 37 °C for 24 h and growth inhibition zones were measured (Gruskiene et al., 2021).

2.7. Statistical analysis

Three independent experiments were performed unless otherwise specified and data are presented as the mean ± standard deviation. To define statistically significant data, a one–way analysis of variance (ANOVA, $P < 0.05$) was used.

3. Results and discussion

3.1. Formation of nisin-loaded fucoidan particles

Nisin-loaded fucoidan particles were prepared using the complexation strategy. The approach based on the self-assembly method is simple and cost-efficient. The particles were fabricated at pH values from 4.0 to 7.0 having in mind their potential application in various food matrices of different acidity. Fucoidan is a strong polyelectrolyte with a pK$_a$ value of 1.0–2.5 (Barbosa et al., 2019). Nisin is a cationic peptide that has an isoelectric point above 8.5 (Tai, Joshi, & McGuire, & Neff, 2008). Therefore, complex formation was expected at neutral and slightly acid pH values (Amara, Kim, Oulahal, Degraeve, & Gharsallaoui, 2017) (Fig. 1).

To study the nisin-fucoidan interaction and the formation of the complex, FT-IR measurements were performed. To better separate the absorption bands of nisin and highlight structural changes during complex formation, the exchange of hydrogen atoms by deuterium was used. The extent of deuterium exchange allows assessing the accessibility of the OH and NH (NH$_2$) groups and conformational changes of peptides (Barth, 2000; de Meutter & Goormaghtigh, 2021; Militello et al., 2004). In the nisin spectrum (Fig. 2A), the band at 3260 cm^{-1}
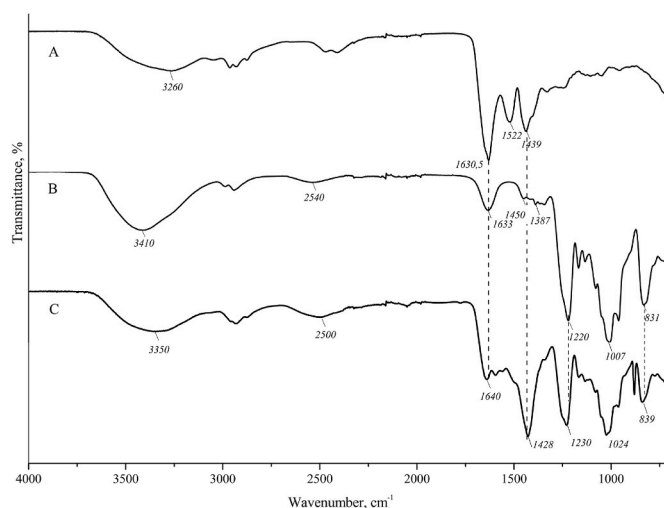

Fig. 2. FT-IR spectra of nisin (A), fucoidan (B) and nisin-fucoidan complex (C).

corresponds to O–H and N–H stretching vibrations. The bands at 1630.5 cm^{-1} and 1522 cm^{-1} are attributed to Amide I and Amide II, respectively. The Amide I band corresponds to carbonyl stretching vibrations, and the Amide II band is assigned to C–N stretching and in-plane N–H bending vibrations. The band at 1439 cm^{-1} is attributed to a new Amide II' band that appeared due to H/D exchange and the shift of vibrations to lower wavenumbers (Gruskiene et al., 2021; Sosa Morales, Juarez, Montich, & Alvarez, 2019). In the fucoidan spectrum, the broad band at 3410 cm^{-1} and the band at 2540 cm^{-1} correspond to the stretching of the O–H and O-D groups after H/D exchange, respectively (Fig. 2B). The band at 1633 cm^{-1} is assigned to the structural water deformation band. The molecules of water are in well-defined positions of the polysaccharide interlayer (Dong et al., 2019; Synytsya et al., 2010). The absorption bands at 1387 cm^{-1} and 1450 cm^{-1} are associated with symmetric CH$_3$ deformations from the fucose 6C methyl group. The intense band at 1220 cm^{-1} with a shoulder at 1254 cm^{-1} suggests the presence of the S═O stretching of sulphate groups. The intense band at 1007 cm^{-1} is a typical band of polysaccharides observed due to the coupling of the C–O or C–C stretching vibrations with the C–O–H bending vibrations. The band at 831 cm^{-1} corresponds to the C–O–S of the sulphate group and the glycosidic bonds in α-conformation between fucopyranose units of fucoidan (Dorschmann, Kopplin, & Roider, 2023; Synytsya et al., 2010). After the complexation, the bands at 3410 cm^{-1} and 2540 cm^{-1} shift to a lower wavenumber at 3350 cm^{-1} and 2500 cm^{-1}, respectively, and indicate the formation of hydrogen bonds in the complex (Fig. 2C). Both bands of Amide I and Amide II decrease in the
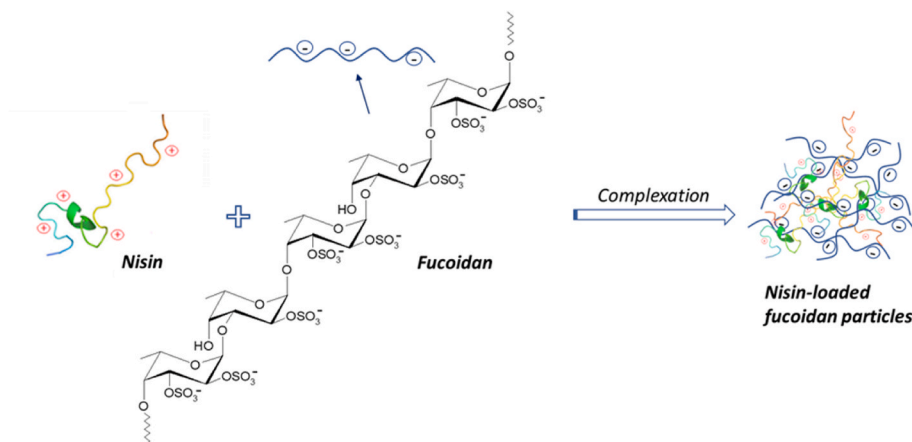

Fig. 1. Schematic presentation of nisin-loaded fucoidan particle formation.

intensity compared to the pure nisin spectrum. Furthermore, the band of Amide I shifts to a higher frequency from 1630.5 cm^{-1} to 1640 cm^{-1} and shows a weakening of intramolecular hydrogen bonds in nisin complexed with fucoidan (Sosa Morales et al., 2019). The intensity of the amide II band increases, suggesting the higher accessibility of protons to H/D exchange and nisin conformational changes during complex formation (Militello et al., 2004). The spectral shift of the Amide II' stretching deformation from 1438 cm^{-1} to 1428 cm^{-1} also confirms the interaction between the peptide and the polysaccharide (Fael & Demirel, 2020). After complexation, the bands at 1220 cm^{-1} and 831 cm^{-1} belonging to sulphate group in the fucoidan spectrum shift to 1230 cm^{-1} and 839 cm^{-1}, respectively, and indicate the electrostatic interaction between $-NH_3^+$ groups of nisin and sulphate groups of fucoidan. The changes are in line with previously published data. The blue shift of both S=O and C–O–S stretching due to electrostatic interactions was reported for the complexation of heparin with chitosan or *N, N, N*-trimethyl chitosan (Martins, Piai, Schuquel, Rubira, & Muniz, 2011). Moreover, the broad band at 1007 cm^{-1} shifts to 1024 cm^{-1} and indicates steric arrangement changes of complexed fucoidan (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000).

As seen from Table 1, the EE of nisin was practically equal to 100% in the nisin concentration range of 0–0.6 mg/mL and was independent of pH value. Only at the highest nisin concentration of 1 mg/mL the EE was lower by a few percent. However, at the neutral pH value of 7.0, the EE was the same as at the lower nisin concentration. Previously, it was demonstrated that not only electrostatic forces and hydrogen bonding were involved in the interaction of nisin with polyanionic polysaccharides. For complex formation, hydrophobic forces are also significant and are especially evident at neutral pH values. Despite the reduction in the positive charge of nisin by histidine deprotonation, at high concentrations of nisin, the EE is higher at neutral pH values than at lower pH values (Gruskiene et al., 2021; Krivorotova et al., 2016). Furthermore, the nisin-loaded fucoidan particles were stable under storage at 4 °C. Overall, the release of nisin was not observed and the EE did not change.

Previously, fucoidan was combined with zein to develop composite nanoparticles. The interaction of components was based mainly on electrostatic attraction and hydrogen bonding. Composite nanoparticles were used for curcumin encapsulation with a high encapsulation efficiency of 81.07% (Zhang et al., 2021). Pinheiro et al. (2015) showed that multilayer nanocapsules can be prepared through layer-by -layer assembly using fucoidan and chitosan. Two polysaccharides formed layers because of the electrostatic interaction between the opposite charges of the components. The nanocapsules served as a nanocarrier system for poly-L-lysine, a cationic peptide with antimicrobial activity.

3.2. Physicochemical characterization of particles

The zeta potential of the particles is shown in Fig. 3. As seen, the zeta potential of fucoidan is negative and varies from −25.6 at pH 4.0 to −57.0 at pH 7.0. The addition of positively charged nisin should reduce the negative surface charge of fucoidan. Indeed, at the high nisin concentration of 1 mg/mL, the zeta potential of the particles becomes even positive (Fig. 3A, B, C). However, at low concentrations of nisin and all pH values, the interaction of nisin with fucoidan results in a more negative surface charge of the complex compared to fucoidan. The same trend was observed in the case of nisin complexation with ulvan (Gruskiene et al., 2021) and could be related to fucoidan conformational changes. For associative interactions and complex formation, not only electrostatic attraction but also the flexibility of macromolecules is important (Reynolds & Perez, 2011; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). Afterward, the negative surface charge of the complex continuously decreased with increasing nisin concentration. As seen in Fig. 3, the particles were stable under storage at 4 °C for 4 weeks. In most cases, the zeta potential did not change significantly. At all pH values and a nisin concentration of 0.6 mg/mL or lower, the absolute value of the zeta potential of the particles was greater than 30 mV. Usually, a surface charge of ±30 mV prevents the particles from aggregation (Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013).

As seen in Fig. 4, at all pH values, the hydrodynamic diameter of the particles was in the range of 200–600 nm when the concentration of encapsulated nisin varied from 0 to 0.6 mg/mL. Statistical analysis showed that at higher pH values of 6.0 and 7.0 and low nisin concentrations (0–0.3 mg/mL at pH 6.0 and 0–0.4 mg/mL at pH 7.0), the difference in size was insignificant compared to pure fucoidan. The same phenomenon was observed in the complexation of nisin with ulvan (Gruskiene et al., 2021) and could be related to conformation changes of polysaccharide molecules through peptide binding. At the higher nisin concentration of 1 mg/mL, the particles were large and the hydrodynamic diameter varied from 2600 to 5500 nm. As seen, in most cases the changes in particle size were insignificant in storage for 4 weeks at 4 °C.

The SEM image shows that nisin-loaded fucoidan particles are not

Table 1

The dependence of nisin encapsulation efficiency on the pH value of solution[a, b, c d].

The concentration of nisin, mg/mL	Encapsulation efficiency, %							
	pH 4		pH 5		pH 6		pH 7	
	t_0	t_1	t_0	t_1	t_0	t_1	t_0	t_1
0.1	96.7 ± 3.6[Aa1]	98.8 ± 1.5[Aa1]	98.1 ± 1.8[Aa1]	99.8 ± 0.4[Ab1]	97.3 ± 3.9[ABa1]	98.7 ± 1.5[Aa1]	97.3 ± 2.1[Aa1]	99.0 ± 1.1 [Aa1]
0.2	97.6 ± 2.9[Aa1]	99.1 ± 1.1[Aa1]	98.1 ± 2.6[ABa1]	99.1 ± 3.5[ABCa1]	97.8 ± 2.2[Aa1]	98.5 ± 1.2[Aa1]	98.3 ± 1.0 [Aa1]	97.8 ± 2.1 [ABa1]
0.3	97.3 ± 1.9[Aa12]	99.4 ± 0.7[Ab1]	99.0 ± 1.1[ABa1]	99.0 ± 0.9[ABa1]	97.4 ± 1.3[Aa2]	96.1 ± 1.5[Ba2]	98.3 ± 1.0 [Aa12]	98.9 ± 0.7 [Aa1]
0.4	98.2 ± 1.4[Aa1]	99.1 ± 0.7[Aa1]	99.1 ± 1.6[ABa1]	98.7 ± 0.5[Ba1]	98.6 ± 0.9[Aa1]	99.0 ± 0.6[Aa1]	98.2 ± 1.2 [Aa1]	97.8 ± 1.6 [ABa1]
0.5	98.8 ± 1.1[Aa12]	98.5 ± 1.2[Aa12]	99.6 ± 0.4[Ba1]	98.9 ± 0.6[Bb1]	98.9 ± 0.6[Aa2]	98.4 ± 1.5[Aa12]	98.6 ± 0.7 [Aa2]	97.9 ± 0.7 [Bb2]
0.6	98.8 ± 1.1[Aa1]	99.3 ± 0.6[Aa1]	99.2 ± 0.8[ABa1]	98.7 ± 0.5[Ba1]	98.8 ± 1.0[Aa1]	97.5 ± 0.9[Ab2]	98.6 ± 0.3 [Aa1]	97.0 ± 1.6 [Bb2]
1.0	92.8 ± 0.9[Ba1]	95.7 ± 1.8[Bb1]	92.2 ± 8.5[Aa12]	90.3 ± 8.1[Ca1]	93.5 ± 2.3[Ba1]	93.9 ± 1.4[Ca1]	98.2 ± 0.5 [Aa2]	97.5 ± 0.4 [Bb2]

[a] Data are presented as mean values ± standard deviations of 5–8 independent experiments.
[b] t_0 and t_1 are the initial time of particle preparation and the time after 4 weeks of storage at 4 °C, respectively.
[c] Different superscript capital letters represent significant differences in the mean within each column; different superscript lowercase letters represent significant differences in the mean of each pair t_0 and t_1; different numbers represent significant differences in the mean of the parameter at different pH values, but at the same time and the same concentration of nisin (P < 0.05).
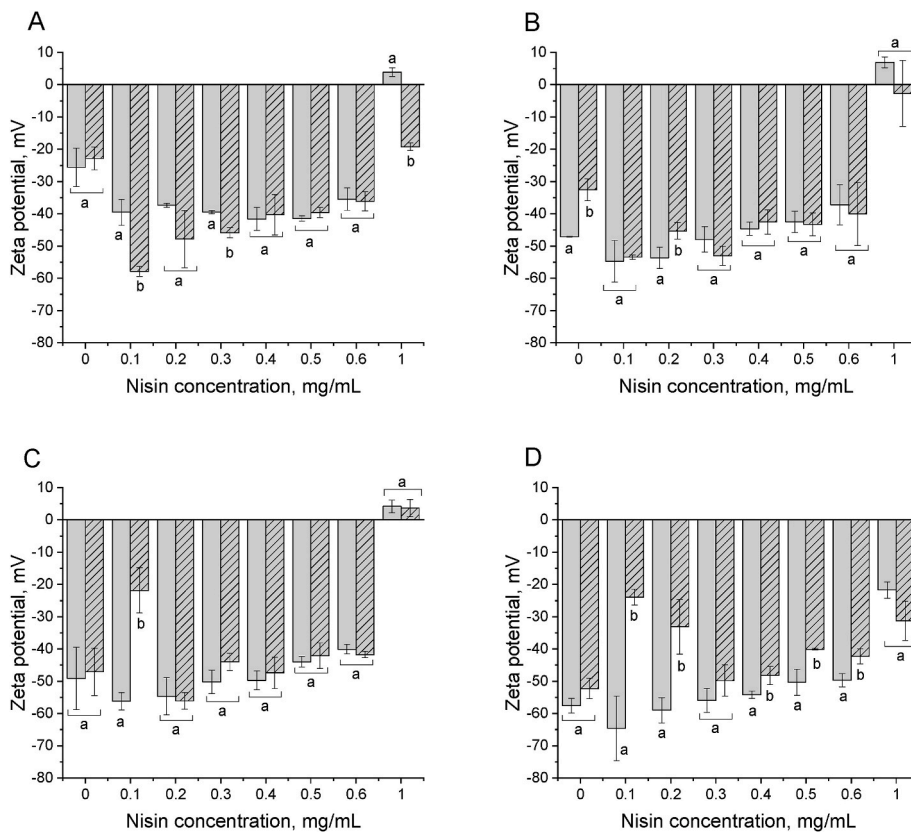[d] The concentration of fucoidan was equal to 0.4 mg/mL.

Fig. 3. Zeta potential of nisin-loaded fucoidan particles at different pH values (A, pH 4; B, pH 5; C, pH 6; D, pH 7) depending on nisin concentration. Dashed bars indicate the zeta potential of particles after 4 weeks of storage at 4 °C.
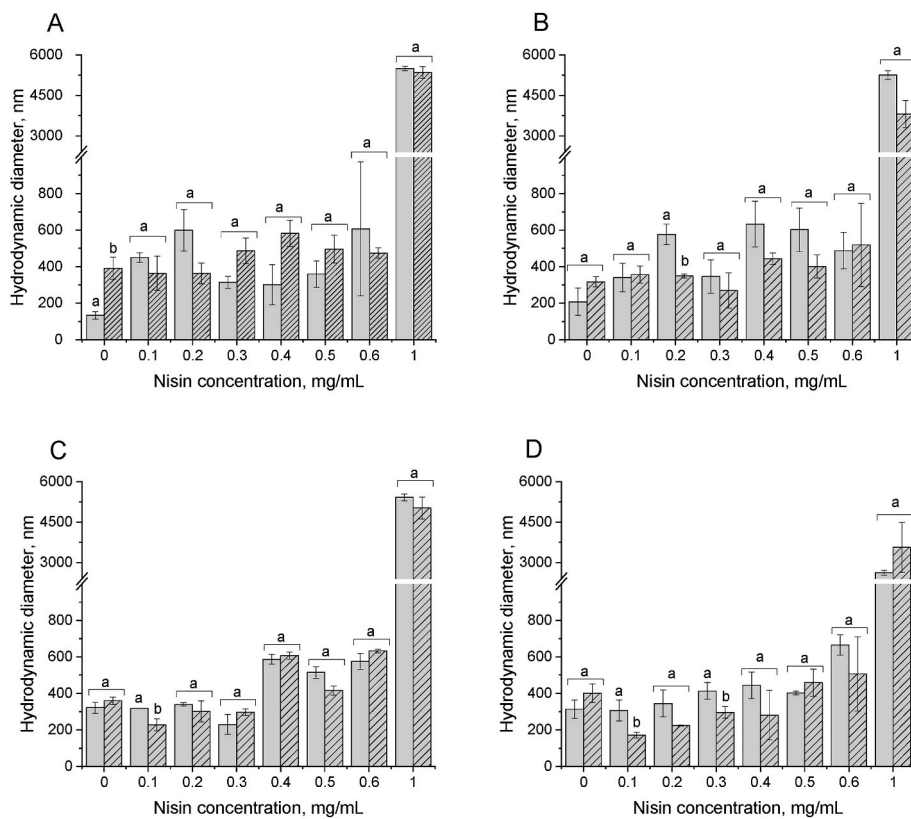

Fig. 4. Size of nisin-loaded fucoidan particles at different pH values (A, pH 4; B, pH 5; C, pH 6; D, pH 7) depending on nisin concentration. Dashed bars indicate the size of particles after 4 weeks of storage at 4 °C.

uniform. Most of them have irregular and angular shape. However, some of them exhibit a sphere-like shape (Fig. 5). It can be observed that the particle size is smaller than that measured by the Zetasizer Nano. The same phenomenon was observed for chitosan/γ-PGA encapsulated nisin nanoparticles. The Zetasizer Nano instrument measures the hydrodynamic diameter of particles swelled in aqueous solution while SEM gives the size of particle in the dry state. It could be the reason of the observed discrepancy (Wu et al., 2016).

In general, the formation of polyion complexes is becoming one of the popular techniques for nisin encapsulation. Previously, polyanionic polysaccharides such as pectin (Krivorotova et al., 2016), negatively charged natural polysaccharide gum Arabic (Gong et al., 2018), or soluble soybean polysaccharide (Luo et al., 2019) were used for that purpose. Recently, nisin– carboxymethylcellulose polyion complex nanoparticles have been developed (Celen, Anumudu, Miri, Onyeaka, & Fernandez-Trillo, 2023). The complexation is a simple method and results in the nisin-loaded particles with negative surface charge. Their hydrodynamic diameter varies depending on the mass ratio of nisin and polyanionic polysaccharides, and the pH values.

The TG and DSC thermograms of nisin, fucoidan, and its complex are presented in Fig. 6. All samples exhibited two-stage weight loss. In the DSC thermograms, broad endothermic transitions were recorded at 79, 72, and 58 °C for nisin, fucoidan, and nisin-loaded particles, respectively. The peaks were also seen on the TG thermograms and were related to the slight loss of dehydration mass. The second nisin degradation step started at 260 °C and was seen in both TG and DSC thermograms (Fig. 6A) (Niaz et al., 2018). For fucoidan, an intensive sharp exothermic transition at 247 °C and extensive weight loss were observed in DSC and TG thermograms, respectively (Fig. 6B) (Bonaccorso et al., 2021; Cheow, Kiew, & Hadinoto, 2015; Mou et al., 2020). However, for nisin-loaded particles, a broad exothermic transition at 256 °C associated with the decomposition of the complex was observed (Fig. 6C). The TGA - DSC analysis indicated that the temperature of thermal decomposition of the complex changed, and was related to intermolecular interactions between nisin and fucoidan (Bonaccorso et al., 2021).

3.3. In vitro release of nisin from nisin-loaded fucoidan particles

The release of nisin from the particles was analysed at different pH values of 2.0, 6.0, and 8.0 and at two different temperatures of 20 °C and 37 °C. Furthermore, the influence of the ionic strength of the solution on the release of nisin was examined. As seen in Fig. 7, at both temperatures, the greatest influence of ionic strength on nisin release was observed at pH 2.0. The percentage of released nisin increased with
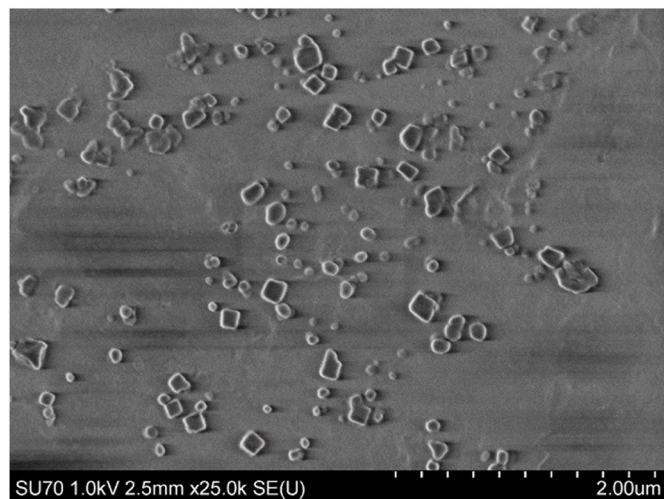

Fig. 5. SEM image of nisin-loaded fucoidan particles prepared at pH 5. The final concentration of fucoidan and nisin was 0.4 mg/mL.

increasing concentration of salt. At pH 6.0, on the contrary, higher concentrations of NaCl (0.5 mol/L and 2 mol/L) decreased the amount of nisin. The same tendency was also observed at pH 8.0. At pH 2.0, it seems that the electrostatic forces of interaction between nisin and fucoidan are dominant. At neutral or slightly alkaline pH value, the hydrophobic forces are important for nisin - fucoidan complexation. At high ionic strength, hydrophobic interactions increase (Graziano, 2009) and consequently, the release of nisin from the complex decreases. As shown above, the effect of salt at those pH values is consistent with the encapsulation efficiency data at a high concentration of nisin. Wu et al. (2016) previously described a pH-dependent pattern of nisin release from chitosan/poly-γ-glutamic acid. The pH value of the environment also affected the release of nisin from pectin-chitosan nanoparticles (Wang, Yang, & Sun, 2017). Moreover, the impact of hydrophobic interactions between nisin and polysaccharide on the release control at higher pH values was also observed (Wu et al., 2016). After 100 h the amount of released peptide is lower at the higher temperature of 37 °C compared to 20 °C. It could also be related to the effect of temperature on hydrophobic interactions. With increasing temperature, hydrophobic forces become stronger (Sun, 2022). In general, a slower sustained release of the peptide was observed from nisin-fucoidan complexes compared to alginate-chitosan-pluronic composite nanoparticles or pectin-chitosan complexes (Bernela, Kaur, Chopra, & Thakur, 2014; Wang et al., 2017).

3.4. Antibacterial activity of particles

To analyse the antibacterial activity of the nisin-loaded fucoidan particles, Gram-positive bacteria *B. subtilis* and *L. innocua* were used, as well as Gram-negative bacteria *E. coli* and *S. typhimurium* (Fig. 8). The most effective inhibiting activity of encapsulated nisin was detected against *B. subtilis*, where the largest lysis zones (11–13 mm) were observed (Fig. 8). The antibacterial action against *L. innocua* was weaker in comparison to *B. subtilis*. As seen from Fig. 8, the size of the lysis zones was similar but the transparency was reduced. The analysis of antimicrobial activity revealed the important characteristic of particles, i.e., nisin complexed with fucoidan preserves its biological activity against Gram-positive bacteria. Moreover, the antibacterial activity of encapsulated nisin does not decrease significantly as compared to the same concentration of free nisin. As previously found, nisin-loaded ulvan particles also exhibited antibacterial activity against *B. subtilis* and *L. innocua* comparable to free nisin (Gruskiene et al., 2021). In general, nisin is more active against *B. subtilis* than *L. innocua* (Olasupo, Fitzgerald, Narbad, & Gasson, 2004). The same tendency was preserved for encapsulated nisin. Nevertheless, the active nisin-loaded fucoidan particles against such foodborne pathogenic bacteria as *Listeria* are promising to be used as biopreservatives. The efficiency of nisin-loaded fucoidan particles against additionally permeabilized Gram-negative bacteria *E. coli* and *S. typhimurium* was negligible. Semi-transparent lysis zones of 7–8 mm in diameter were detected only at a concentration of 0.4 mg/mL of encapsulated or free nisin. This peptide is generally not active against *E. coli* and *S. typhimurium,* as previously demonstrated (Khan, Vu, Riedl, & Lacroix, 2015). The outer membrane of these bacteria is a barrier for the penetration of nisin and reaching the cytoplasmic membrane (Khan et al., 2022).

The antimicrobial activity of some nisin-loaded polyion complexes were evaluated in real food systems. Soybean-polysaccharide-based nanoparticles were used for tomato juice biopreservation. The slow and sustained release of nisin resulted in a high antimicrobial capacity of the nanoparticles for 60 days (Luo et al., 2019). The antimicrobial activity of nisin – carboxymethylcellulose nanoparticles against *Staphylococcus aureus* was demonstrated in skimmed milk (Celen et al., 2023).

4. Conclusions

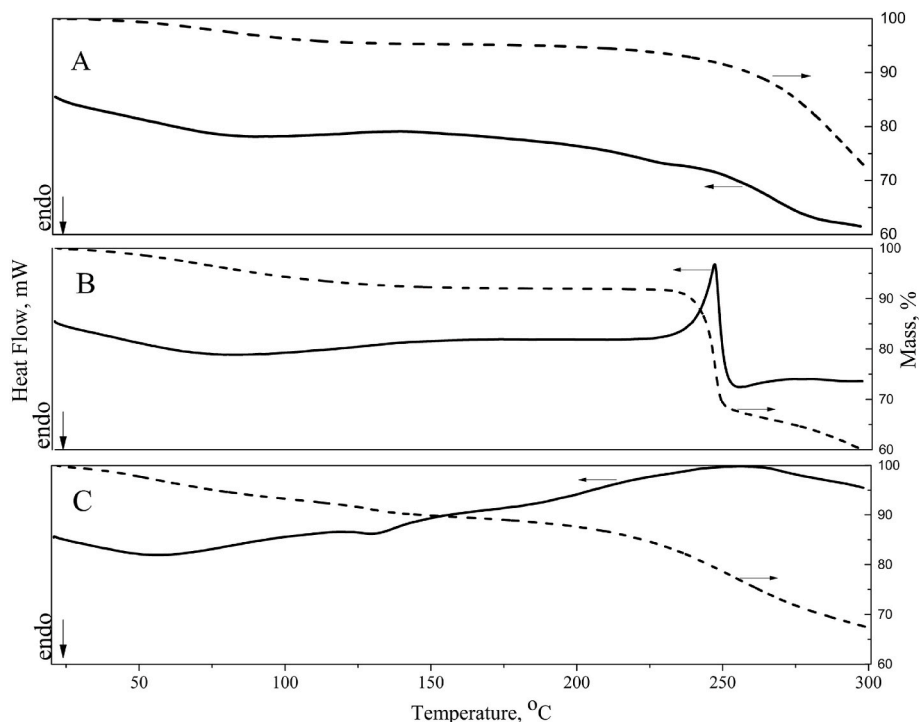Nisin-loaded fucoidan particles were prepared by the complexation

Fig. 6. TG-DSC thermograms of nisin (A), fucoidan (B) and nisin-fucoidan complex (C); DSC (—), TG (——).
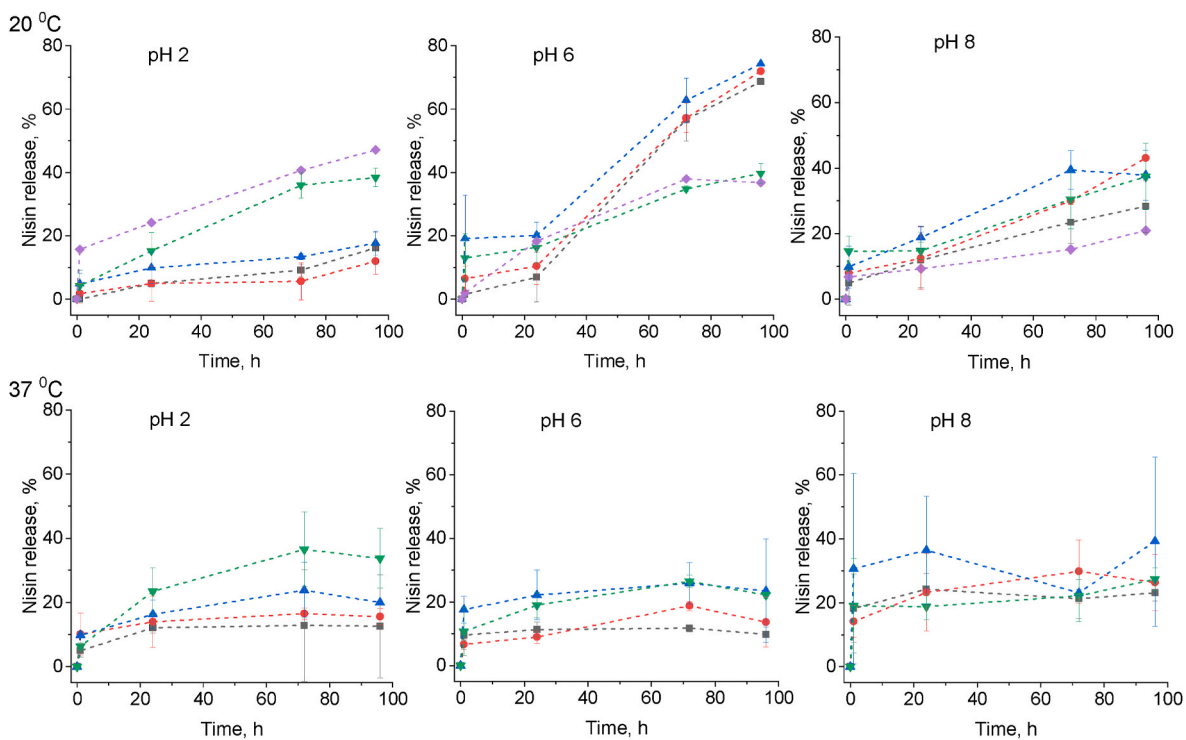

Fig. 7. Release of nisin from nisin-loaded fucoidan particles at the pH values of 2, 6 and 8 and at the temperature of 20 °C and 37 °C in the absence (– ■ –) and in the presence of 0.1 M (– ● –), 0.2 M (– ▲ –), 0.5 M (— ▼ —) and 2 M NaCl (— ◆ —).

method. Anionic fucoidan and cationic nisin self-assembled by electro-static interactions. For the complex formation, the importance of hydrogen bonding and hydrophobic forces was also revealed. The formation of complexes was shown by FT-IR spectroscopy. The analysis of thermal properties by thermogravimetric and differential scanning calorimetry also demonstrated the interaction between fucoidan and
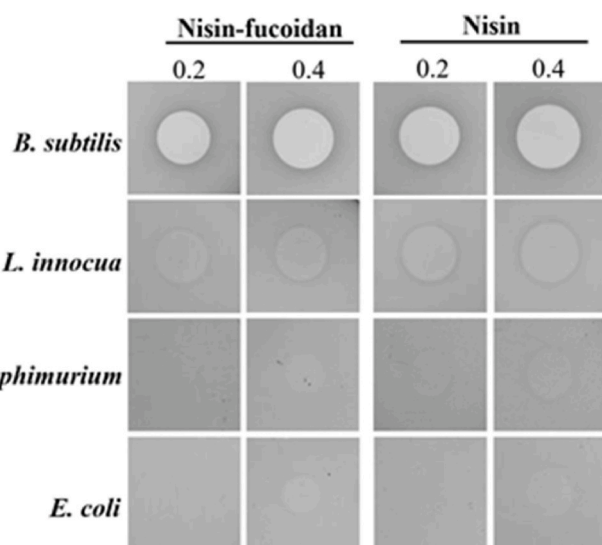
Fig. 8. Analysis of bacteria sensitivity to nisin-loaded fucoidan particles. The inhibitory activity of encapsulated nisin was tested in an agar-diffusion assay by measuring growth inhibition zones. Free nisin concentration was 0.2 and 0.4 mg/mL. Nisin-loaded fucoidan particles were prepared using the same concentration of nisin.

nisin. The particles were stable for four weeks in storage at 4 °C. The size and zeta potential of the particles did not change significantly. Nisin complexed with fucoidan preserves its antimicrobial activity. Therefore, the application of fucoidan for the encapsulation of nisin is promising. The formulation of nisin using fucoidan could be used in the food industry for biopreservation.

CRediT authorship contribution statement

Rūta Gruškienė: Writing – original draft, Investigation, Conceptualization, Validation. **Aistė Galinskaitė:** Investigation. **Tatjana Kavleiskaja:** Investigation, Visualization. **Ramunė Stanevičienė:** Investigation, Visualization. **Elena Servienė:** Writing – original draft, Conceptualization, Validation. **Jolanta Sereikaitė:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

References

Amara, C. B., Kim, L., Oulahal, N., Degraeve, P., & Gharsallaoui, A. (2017). Using complexation for the microencapsulation of nisin in biopolymer matrices by spray-drying. *Food Chemistry, 236*, 32–40. https://doi.org/10.1016/j.foodchem.2017.04.168

Ayrapetyan, O. N., Obluchinskaya, E. D., Zhurishkina, E. V., Skorik, Y. A., Lebedev, D. V., Kulminskaya, A. A., et al. (2021). Antibacterial properties of fucoidans from the brown algae *Fucus vesiculosus* L. of the Barents sea. *Biology, 10*, Article 67. https://doi.org/10.3390/biology10010067

Barbosa, A. I., Coutinho, A. J., Costa Lima, S. A., & Reis, S. (2019). Marine polysaccharides in pharmaceutical applications: Fucoidan and chitosan as key players in the drug delivery match field. *Marine Drugs, 17*, Article 654. https://doi:10.3390/md17120654.

Barth, A. (2000). The infrared absorption of amino acid side chains. *Progress in Biophysics and Molecular Biology, 74*, 141–173. https://doi.org/10.1016/S0079-6107(00)00021-3

Bernela, M., Kaur, P., Chopra, M., & Thakur, R. (2014). Synthesis, characterization of nisin loaded alginate-chitosan-pluronic composite nanoparticles and evaluation against microbes. *LWT - Food Science and Technology, 59*, 1093–1099. https://doi.org/10.1016/j.lwt.2014.05.061

Bonaccorso, A., Carbone, C., Tomasello, B., Italiani, P., Musumeci, T., Puglisi, G., et al. (2021). Optimization of dextran sulfate/poly-L-lysine based nanogels polyelectrolyte complex for intranasal ovalbumin delivery. *Journal of Drug Delivery Science and Technology, 65*, Article 102678. https://doi.org/10.1016/j.jddst.2021.102678

Brum, L. F. W., dos Santos, C., Santos, J. H. Z., & Brandelli, A. (2022). Structured silica materials as innovative delivery systems for the bacteriocin nisin. *Food Chemistry, 366*, Article 130599. https://doi.org/10.1016/j.foodchem.2021.130599

Celen, T., Anumudu, C., Miri, T., Onyeaka, H., & Fernandez-Trillo, P. (2023). Nisin: Carboxymethylcellulose polyion complex (PIC) nanoparticles. Preparation and antimicrobial activity. *Carbohydrate Polymers, 317*, Article 121032. https://doi.org/10.1016/j.carbpol.2023.121032

Cheow, W. S., Kiew, T. Y., & Hadinoto, K. (2015). Amorphous nanodrugs prepared by complexation with polysaccharides: Carrageenan versus dextran sulfate. *Carbohydrate Polymers, 117*, 549–558. https://doi.org/10.1016/j.carbpol.2014.10.015

Dong, Y., Wen, C., Li, T., Wu, C., Qi, H., Liu, M., et al. (2019). The effects of amino acids on the gel properties of potassium iota carrageenan. *Food Hydrocolloids, 95*, 378–384. https://doi.org/10.1016/j.foodhyd.2019.04.023

Dorschmann, P., Kopplin, G., Roider, J., & Klettner, A. (2023). Interaction of high-molecular weight fucoidan from *Laminaria hyperborea* with natural functions of the retinal pigment epithelium. *International Journal of Molecular Sciences, 24*, Article 2232. https://doi.org/10.3390/ijms24032232

Dubashynskaya, N. D., Gasilova, E. R., & Skorik, Y. A. (2023). Nano-sized fucoidan interpolyelectrolyte complexes: Recent advances in design and prospects for biomedical applications. *International Journal of Molecular Sciences, 24*, Article 2615. https://doi.org/10.3390/ijms24032615

Du, B., Zhao, Q., Cheng, C., Wang, H., Liu, Y., Zhu, F., et al. (2022). A critical review on extraction, characteristics, physicochemical activities, potential health benefits, and industrial applications of fucoidan. *eFood, 3*, Article e19. https://doi.org/10.1002/efd2.19

Eghbal, N., Viton, C., & Gharsallaoui, A. (2022). Nano and microencapsulation of bacteriocins for food applications: A review. *Food Bioscience, 50*, Article 102173. https://doi.org/10.1016/j.fbio.2022.102173. Part B.

Fael, H., & Demirel, A. L. (2020). Nisin/polyanion layer-by-layer films exhibiting different mechanisms in antimicrobial efficacy. *RSC Advances, 10*, 10329–10337. https://doi:10.1039/C9RA10135G.

Florez-Fernandez, N., Pontes, J. E., Guerreiro, F., Afonso, I. T., Lollo, G., Torres, M. D., et al. (2023). Fucoidan from *Fucus vesiculosus*: Evaluation of the impact of the sulphate content on nanoparticle production and cell toxicity. *Marine Drugs, 21*, Article 21. https://doi.org/10.3390/md21020115

Gharsallaoui, A., Oulahal, N., Joly, C., & Degraeve, P. (2016). Nisin as a food preservative: Part 1: Physicochemical properties, antimicrobial activity, and main uses. *Critical Reviews in Food Science and Nutrition, 56*, 1262–1274. https://doi.org/10.1080/10408398.2013.763765

Gong, F., Qian, J., Chen, Y., Yao, S., Tong, J., & Guo, H. (2018). Preparation and properties of gum Arabic cross-link binding nisin microparticles. *Carbohydrate Polymers, 197*, 608–613. https://doi.org/10.1016/j.carbpol.2018.05.080, 2018.

Graziano, G. (2009). Role of salts on the strength of pairwise hydrophobic interaction. *Chemical Physics Letters, 483*, 67–71. https://doi.org/10.1016/j.cplett.2009.10.040

Gruskiene, R., Kavleiskaja, T., Staneviciene, R., Kikionis, S., Ioannou, E., Serviene, E., et al. (2021). Nisin-loaded ulvan particles: Preparation and characterization. *Foods, 10*, Article 1007. https://doi.org/10.3390/foods10051007

Hahn, T., Zayed, A., Kovacheva, M., Stadtmuller, R., Lang, S., Muffler, K., et al. (2016). Dye affinity chromatography for fast and simple purification of fucoidan from marine brown algae. *Engineering in Life Sciences, 16*, 78–87. https://doi.org/10.1002/elsc.201500044

Jayawardena, T. U., Nagahawatta, D. P., Fernando, I. P. S., Kim, Y.-T., Kim, J.-S., Kim, W.-S., et al. (2022). A review on fucoidan structure, extraction techniques, and its role as an immunomodulatory agent. *Marine Drugs, 20*, Article 755. https://doi.org/10.3390/md20120755

Kacurakova, M., Capek, P., Sasinkova, V., Wellner, N., & Ebringerova, A. (2000). FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers, 43*, 195–203. https://doi.org/10.1016/S0144-8617(00)00151-X

Khan, F., Singh, P., Joshi, A. S., Tabassum, N., Jeong, G.-J., Bamunuarachchi, N. I., et al. (2022). Multiple potential strategies for the application of nisin and derivatives. *Critical Reviews in Microbiology*. https://doi.org/10.1080/1040841X.2022.2112650

Khan, A., Vu, K. D., Riedl, B., & Lacroix, M. (2015). Optimization of the antimicrobial activity of nisin, Na-EDTA and pH against gram-negative and gram-positive bacteria. *LWT - Food Science and Technology, 61*, 124–129. https://doi.org/10.1016/j.lwt.2014.11.035

Krivorotova, T., Cirkovas, A., Maciulyte, S., Staneviciene, R., Budriene, S., Serviene, E., et al. (2016). Nisin-loaded pectin nanoparticles for food preservation. *Food Hydrocolloids, 54*, 49–56. https://doi.org/10.1016/j.foodhyd.2015.09.015

Lahteenmaki-Uutela, A., Rahikainen, M., Camarena-Gomez, M. T., Piiparinen, J., Spilling, K., & Yang, B. (2021). European Union legislation on macroalgae products. *Aquaculture International, 29*, 487–509. https://doi.org/10.1007/s10499-020-00633-x

Li, B., Lu, F., Wei, X., & Zhao, R. (2008). Fucoidan: Structure and Bioactivity. *Molecules, 13*, 1671–1695. https://doi:10.3390/molecules13081671.

Lu, K.-Y., Li, R., Hsu, C.-H., Lin, C.-W., Chou, S.-C., Tsai, M.-L., et al. (2017). Development of a new type of multifunctional fucoidan-based nanoparticles for anticancer drug delivery. *Carbohydrate Polymers, 165*, 410–420. https://doi.org/10.1016/j.carbpol.2017.02.065

Luo, L., Wu, Y., Liu, C., Huang, L., Zou, Y., Shen, Y., et al. (2019). Designing soluble soybean polysaccharides-based nanoparticles to improve sustained antimicrobial activity of nisin. *Carbohydrate Polymers, 225*, Article 115251. https://doi.org/10.1016/j.carbpol.2019.115251

Luthuli, S., Wu, S., Cheng, Y., Zheng, X., Wu, M., & Tong, H. (2019). Therapeutic effects of fucoidan: A review on recent studies. *Marine Drugs, 17*, Article 487. https://doi:10.3390/md17090487.

Martins, A. F., Piai, J. F., Schuquel, I. T. A., Rubira, A. F., & Muniz, E. C. (2011). Polyelectrolyte complexes of chitosan/heparin and *N,N,N*-trimethyl chitosan/heparin obtained at different pH: I. Preparation, characterization, and controlled release of heparin. *Colloid and Polymer Science, 289*, 1133–1144. https://doi.org/10.1007/s00396-011-2437-5

Mensah, E. O., Kanwugu, O. N., Panda, P. K., & Adadi, P. (2023). Marine fucoidans: Structural, extraction, biological activities and their applications in the food industry. *Food Hydrocolloids, 142*, Article 108784. https://doi.org/10.1016/j.foodhyd.2023.108784

de Meutter, J., & Goormaghtigh, E. (2021). Evaluation of protein secondary structure from FTIR spectra improved after partial deuteration. *European Biophysics Journal, 50*, 613–628. https://doi.org/10.1007/s00249-021-01502-y

Militello, V., Casarino, C., Emanuele, A., Giostra, A., Pullara, F., & Leone, M. (2004). Aggregation kinetics of bovine serum albumin studied by FTIR spectroscopy and light scattering. *Biophysical Chemistry, 107*, 175–187. https://doi.org/10.1016/j.bpc.2003.09.004

Mou, J., Li, Q., Shi, W., Qi, X., Song, W., & Yang, J. (2020). Chain conformation, physicochemical properties of fucosylated chondroitin sulfate from sea cucumber *Stichopus chloronotus* and its *in vitro* fermentation by human gut microbiota. *Carbohydrate Polymers, 228*, Article 115359. https://doi.org/10.1016/j.carbpol.2019.115359

Niaz, T., Shabbir, S., Noor, T., Rahman, A., Bokhari, H., & Imran, M. (2018). Potential of polymer stabilized nano-liposomes to enhance antimicrobial activity of nizin Z against foodborne pathogens. *LWT - Food Science and Technology, 96*, 98–110. https://doi.org/10.1016/j.lwt.2018.05.029

Noble, J. E., & Bailey, M. J. A. (2009). Quantitation of protein. *Methods in Enzymology, 463*, 73–95. https://doi.org/10.1016/S0076-6879(09)63008-1

O'Connor, P. M., Kuniyoshi, T. M., Oliveira, R. P. S., Hill, C., Ross, R. P., & Cotter, P. D. (2020). Antimicrobials for food and feed; a bacteriocin perspective. *Current Opinion in Biotechnology, 61*, 160–167. https://doi.org/10.1016/j.copbio.2019.12.023

Olasupo, N. A., Fitzgerald, D. J., Narbad, A., & Gasson, M. J. (2004). Inhibition of *Bacillus subtilis* and *Listeria innocua* by nisin in combination with some naturally occurring organic compounds. *Journal of Food Protection, 67*, 596–600. https://doi.org/10.4315/0362-028X-67.3.596

Pachaleva, J., Gruskiene, R., Bockuviene, A., & Sereikaite, J. (2023). The application of pectin and chitooligosaccharides for the preparation of nisin-loaded nanoparticles with different surface charges. *Cellulose, 30*, 8985–8996. https://doi.org/10.1007/s10570-023-05380-9

Pinheiro, A. C., Bourbon, A. I., Cerqueira, M. A., Maricato, E., Nunes, C., Coimbra, M. A., et al. (2015). Chitosan/fucoidan multilayer nanocapsules as a vehicle for controlled release of bioactive compounds. *Carbohydrate Polymers, 115*, 1–9. https://doi.org/10.1016/j.carbpol.2014.07.016

Rajasekaran, J., & Viswanathan, P. (2023). Anti-bacterial and antibiofilm properties of seaweed polysaccharide-based nanoparticles. *Aquaculture International.* https://doi.org/10.1007/s10499-023-01111-w

Reynolds, M., & Perez, S. (2011). Thermodynamics and chemical characterization of protein-carbohydrate interactions: The multivalency issue. *Comptes Rendus Chimie, 14*, 74–95. https://doi.org/10.1016/j.crci.2010.05.020

Rioux, L.-E., Turgeon, S. L., & Beaulieu, M. (2007). Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers, 69*, 530–537. https://doi.org/10.1016/j.carbpol.2007.01.009

Sosa Morales, M. C., Juarez, A. C., Montich, G. G., & Alvarez, R. M. S. (2019). Interaction of the antibiotic peptide nisin with anionic membranes in different phase-states: A vibrational study. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 389–397. https://doi.org/10.1016/j.saa.2019.03.009, 2015.

Sun, Q. (2022). The hydrophobic effects: Our current understanding. *Molecules, 27*, Article 7009. https://doi.org/10.3390/molecules27207009

Synytsya, A., Kim, W.-J., Kim, S.-M., Pohl, R., Synytsya, A., Kvasnicka, F., et al. (2010). Structure and antitumor activity of fucoidan isolated from sporophyll of Korean brown seaweed *Undaria pinnatifida*. *Carbohydrate Polymers, 81*, 41–48. https://doi.org/10.1016/j.carbpol.2010.01.052

Tai, Y. C., Joshi, P., McGuire, J., & Neff, J. A. (2008). Nisin adsorption to hydrophobic surfaces coated with the PEO-PPO-PEO triblock surfactant Pluronic® F108. *Journal of Colloid and Interface Science, 322*, 112–118. https://doi.org/10.1016/j.jcis.2008.02.053

Tamjidi, F., Shahedi, M., Varshosaz, J., & Nasirpour, A. (2013). Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. *Innovative Food Science and Emerging Technologies, 19*, 29–43. https://doi.org/10.1016/j.ifset.2013.03.002

Turgeon, S. L., Beaulieu, M., Schmitt, C., & Sanchez, C. (2003). Protein – polysaccharide interactions: Phase-ordering kinetics, thermodynamic and structural aspects. *Current Opinion in Colloid & Interface Science, 8*, 401–414. https://doi.org/10.1016/S1359-0294(03)00093-1

Venkatesan, J., Murugan, S. S., & Seong, G. H. (2022). Fucoidan-based nanoparticles: Preparation and applications. *International Journal of Biological Macromolecules, 217*, 652–667. https://doi.org/10.1016/j.ijbiomac.2022.07.068

Wang, H., Yang, B., & Sun, H. (2017). Pectin-chitosan polyelectrolyte complex nanoparticles for encapsulation and controlled release of nisin. *American Journal of Polymer Science and Technology, 3*, 82–88. https://doi:10.11648/j.ajpst.20170305.11.

Wu, C., Wu, T., Fang, Z., Zheng, J., Xu, S., Chen, S., et al. (2016). Formation, characterization and release kinetics of chitosan/γ-PGA encapsulated nisin nanoparticles. *RSC Advances, 6*, 46686–46695. https://doi.org/10.1039/C6RA06003J

You, G., Liu, X. L., & Zhao, M. M. (2018). Preparation and characterization of hsian-tsao gum and chitosan complex coacervates. *Food Hydrocolloids, 74*, 255–266. https://doi.org/10.1016/j.foodhyd.2017.08.004

Zayed, A., El-Aasr, M., Ibrahim, A.-R. S., & Ulber, R. (2020). Fucoidan characterization: Determination of purity and physicochemical and chemical properties. *Marine Drugs, 18*, Article 571. https://doi:10.3390/md18110571.

Zayed, A., & Ulber, R. (2019). Fucoidan production: Approval key challenges and opportunities. *Carbohydrate Polymers, 211*, 289–297. https://doi.org/10.1016/j.carbpol.2019.01.105

Zhang, H., Jiang, L., Tong, M., Lu, Y., Ouyang, X.-K., & Ling, J. (2021). Encapsulation of cucurmin using fucoidan stabilized nanoparticles: Preparation, characterization, and in vitro release performance. *Journal of Molecular Liquids, 329*, Article 115586. https://doi.org/10.1016/j.molliq.2021.115586