International Journal of Biological Macromolecules 233 (2023) 123489

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Impact of tannic acid on nisin encapsulation in chitosan particles

Mihaela D. Leonida [a,*], Alice Benzecry [a], Bisera Lozanovska [a], Zainab Mahmoud [a], Ashley Reid [a], Sabrina Belbekhouche [b,*]

[a] Department of Chemistry and Biochemistry, Fairleigh Dickinson University, Teaneck, NJ 07666, USA
[b] Université Paris Est Créteil, CNRS, Institut Chimie et Matériaux Paris Est, UMR 7182, 2 Rue Henri Dunant, 94320 Thiais, France

ARTICLE INFO

Keywords:
Nisin
Tannic acid
Loaded chitosan particle

ABSTRACT

This study investigates the effect of addition of tannic acid on nisin encapsulated in chitosan matrices. Composite materials were prepared using a mild, environmentally friendly procedure, ionotropic gelation of chitosan by sodium tripolyphosphate in the presence of nisin (N) at different concentrations. In two parallel sets of preparations, tannic acid (TA) was added at 10:1 and 5:1 N:TA, respectively. The obtained particles were characterized by FTIR, SEM, size, zeta potential, encapsulation efficiency, loading capacity, and ratio of residual free amino groups. The kinetics of nisin release from the particles was studied to assess the role of TA as a potential modulator thereof. Its addition resulted in enhanced release, higher at lower N:TA ratio. An additional benefit was that TA, a strong antioxidant, imparted antioxidant activity to the composites. Antimicrobial turbidimetric tests were performed against one gram-positive bacterium (*Staphylococcus aureus*) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), all relevant for the food, pharmaceutical, and cosmetic industries. All the composites showed synergistic effects against all the bacteria tested. The positive coaction was stronger against the gram-negative species. This is remarkable since nisin by itself has not known activity against them.

1. Introduction

Nisin (Fig. 1A) is a bacteriocin, class of antimicrobial peptides, secreted by some gram-positive bacteria (*Lactococcus* and *Streptococcus* species – in the case of nisin) to protect themselves against similar bacterial [1]. Nisin (N) is obtained by fermentation of dairy or dextrose nutrients in the presence of *Lactococcus lactis* cultures. It contains 34 amino acids, has a molecular weight of 3.5 kDa, is synthesized in the ribosomes and modified posttranslationally to contain the less common amino acids mesolanthionine, 3-methyllanthionine, dehydroalanine, and dehydrobutyrine. Their presence in a peptide assigns it to the class of lantibiotics. Nisin is considered to be a Class A(I) lantibiotic [2].

Nisin is the only bacteriocin considered as a Generally Recognized As Safe (GRAS) ingredient and has been used as a natural preservative (not man-made) in the food industry in United States and other countries. It is active against a large spectrum of gram-positive bacteria, against spores, and it does not give cross-resistance in bacteria [3]. As a food preservative, nisin is able to control spoilage due to lactic acid bacteria in meats, fish, cheese, salad dressings, and fermented beverages. The main drawbacks of this lantibiotic are its lack of inhibitory action against gram-negative bacteria, yeasts, and molds, and the decrease of antibacterial activity in complex food matrices. This last feature is mainly due to low resistance to proteolysis, poor stability at pH values close to neutral, and unfavorable interactions with lipids leading to its fast release in lipophilic environments [4]. There are in the literature reports of different strategies targeting to overcome these challenges and to obtain synergistic effects [5,6]. Among these strategies, nisin combination with antimicrobial agents of plant origin has been reported with mixed outcomes [7–9]. Another shortcoming of nisin, when it is used to treat different medical conditions, is inactivation by digestive enzymes [10].

A solution to some of nisin's challenges has been encapsulation in different nanosized biopolymeric matrices which benefit from the advantages afforded by interacting at the nanolevel [11]. Encapsulation of nisin in chitosan has been reported in several studies [4,12,13]. The properties recommending chitosan (C, copolymeric *β–(1,4)-2-amino-2-deoxy-D-glucose and N-acetyl-D-glucosamine,* Fig. 1B) for applications in the food and pharmaceutical industries are: biodegradability, being approved for human consumption, mucoadhesivity, and low production cost from the abundant chitin [14]. The fact that it has numerous alcohol

* Corresponding authors.
E-mail addresses: mleonida@fdu.edu (M.D. Leonida), sabrina.belbekhouche@cnrs.fr (S. Belbekhouche).

https://doi.org/10.1016/j.ijbiomac.2023.123489
Received 5 September 2022; Received in revised form 25 January 2023; Accepted 27 January 2023
Available online 2 February 2023

groups [15] and that it is a cationic biopolymer allows its easy processing into several types of nanomatrices [15]. Additional benefits of chitosan which recommends it for use together with nisin are its antimicrobial activity against both gram-positive and gram- negative bacteria, some fungi, and its mild antioxidant activity [16].

When nisin is formulated into different composites, beside trying to overcome its shortcomings, synergistic effects are investigated as well. In this particular case, broadening its antimicrobial activity to gram-negative species and adding antioxidant effect may be reasonable targets.

Tannic acid (TA, Fig. 1C) is a polyphenol found in many vegetal sources. Its molecule contains a central glucose linked to ten galloyl moieties through degradable ester bonds. Due to the phenolic groups, it is water soluble and weakly acidic (pKa ~ 6). For a long time, it has been used in many fields such as making inks [17], leather tanning [18], and, more recently, in different polymeric composites [9,19,20] in wine and juice processing, and in membrane technologies [21]. Due to its structure tannic acid is a very versatile molecule and can become part of different composite materials. It has many phenolic groups which create a high potential for H-bonds [22]. In the presence of proteins this may lead to cross-linking and formation of soluble complexes [23]. Because it has hydrophobic parts in the molecule, it can establish hydrophobic interactions with nonpolar species [24]. Phenolic groups in tannic acid can complex metals [25] and, at pH values above its pKa, can deprotonate and establish electrostatic interactions with cationic species [26]. Available from many sources, tannic acid is inexpensive, nontoxic, and presents antibacterial [27] and strong antioxidant activity [28].

The present study targeted stabilizing nisin for use in complex matrices, broadening its antimicrobial spectrum, and modulating its release. To this end, it was encapsulated in chitosan based composites. Tannic acid was included in some of the particles targeting further enhancement in release kinetics and possible synergistic interactions (antimicrobial and/or antioxidant). It is, to our knowledge, the first report investigating the role of TA as modulator of nisin encapsulated in chitosan matrices.

2. Materials and Methods

2.1. Materials

Chitosan (C, DDA $\geq$ 75 %, molecular weight 190–235 kDa), obtained from chemically processed chitin from shrimp, and nisin from *Lactococcus lactis* (3354.07 g/mol, lyophilized powder, 900 IU/mg) were purchased from Millipore-Sigma. Tannic acid (TA, 1701.20 g/mol) sodium tripolyphosphate (TPP), poly(vinyl sulfate) potassium salt (PVSK), and all other chemicals were analytical grade, were purchased also from Millipore-Sigma and used without further purification. All solutions were made in deionized water (18.2 MΩ cm^{-1}).

2.2. Methods

2.2.1. Particles preparation

A method published in the literature [29], based on electrostatic interactions between chitosan and tripolyphosphate was adapted by us to prepare the composites particles. Solutions of 0.5 % chitosan in 1 % acetic acid were ultrasonicated for 30 min and left overnight under magnetic stirring. Next day, after adjusting the pH to 5.5, value at which chitosan is still positively charged (pKa 6.5) [30], nisin and both nisin and tannic acid were added, respectively, and the mixtures were ultrasonicated for 45 min. After adding 0.25 % solutions of TPP (2:1 *v*/v C:TPP or 1:1C:TPP) to the mixtures, they were left under magnetic stirring for 1 h. The preparations were centrifuged at 9500 rpm, for 1 h, at 25 °C. The supernatant was collected and saved for the determination of nisin/tannic acid present. The pellets were air-dried and ground using a mortar and pestle. Particles containing only chitosan and TPP (NC) were also prepared. The compositions of the synthetic mixtures are given in Table 1.

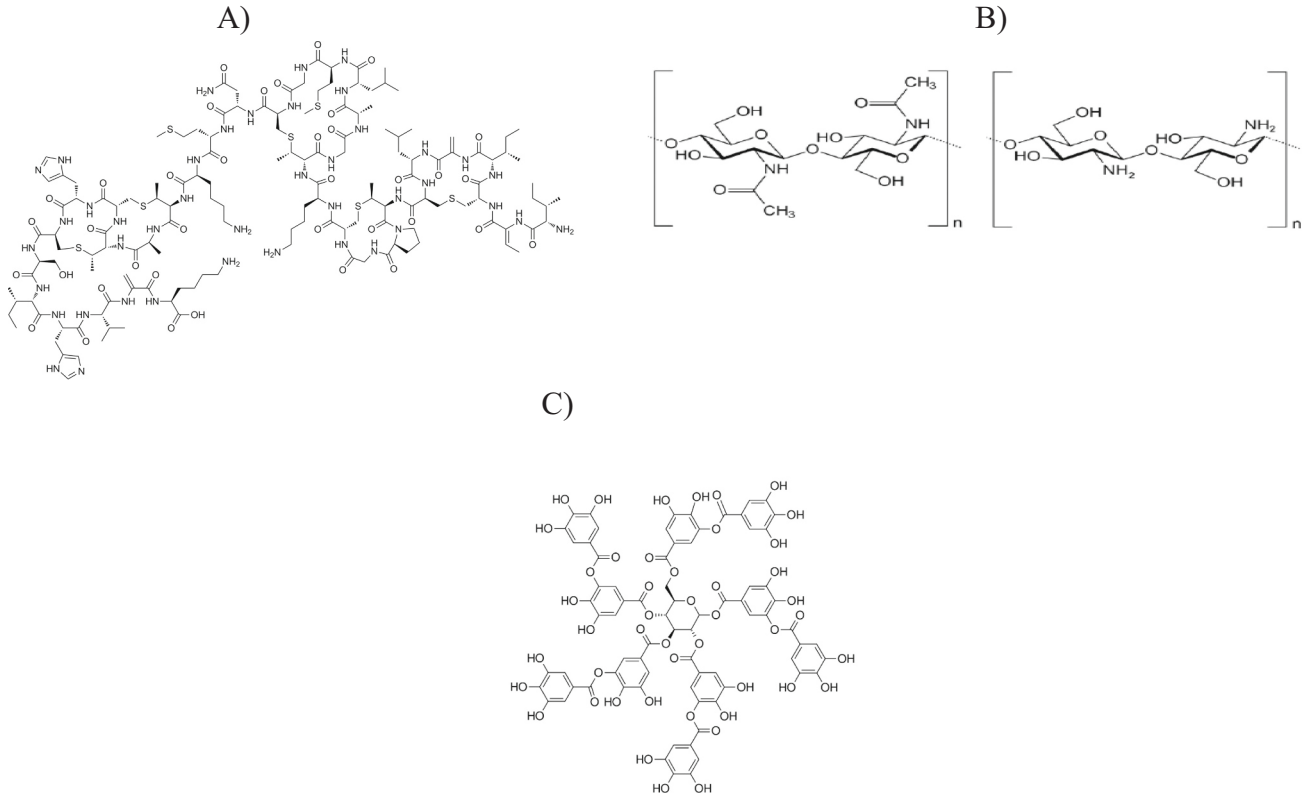

Fig. 1. Structure of A) nisin, B) chitosan and C) tannic acid.

Table 1
Composition of the synthetic mixtures.

Sample		Nisin concentration (mg/mL)	Chitosan:TPP (v/v)	Concentration of tannic acid (mg/mL)
Chitosan	NC		2:1	
Nisin Loaded Chitosan Particles	NCN 0.1	0.1	2:1	
	NCN 0.2	0.2	2:1	
	NCN 0.5	0.5	2:1	
	NCN 1	1	2:1	
Nisin Loaded Chitosan Particles with Tannic Acid	NCNTA 0.1 (10:1)	0.1	2:1	0.01
	NCNTA 0.2 (10:1)	0.2	2:1	0.02
	NCNTA 0.5 (10:1)	0.5	2:1	0.05
	NCNTA 1 (10:1)	1	2:1	0.1
	NCNTA 0.1 (5:1)	0.1	2:1	0.02
	NCNTA 0.31 (5:1)	0.31	2:1	0.06
	NCNTA 0.15 (5:1)	0.15	1:1	0.03
	NCNTA 0.5 (5:1)	0.5	2:1	0.1
	NCNTA 1 (5:1)	1	2:1	0.2

2.2.2. Particle characterization

2.2.2.1. The ratio of free amino groups (RRAG). To evaluate the extent of the cross-linking in the particles, colloidal titration with poly(vinyl sulfate) potassium salt (PVSK) using toluidine blue as indicator was used as in reference [31]. All titrations were done in triplicate and the RRAG was calculating using Eq. (1).

$$\%\text{RRAG} = \left[\frac{\text{amino groups/g NP (after cross}-\text{linking))}}{\text{(total amino groups/g C (before cross}-\text{linking)}}\right] \times 100 \quad (1)$$

The number of amino groups per gram of particles (NP)/per gram of chitosan was calculated according to Eq. (2):

$$\text{Amino groups/g of NP/C} = \Delta V \times M \times \frac{N_a}{SM} \quad (2)$$

where: ΔV is the net volume of PVSK (L) consumed, M is molar concentration of PVSK (mol/L), N_a is Avogadro's number (6.023×10^{23} molecules/mol), and SM is the mass of NP/ in the sample (g).

2.2.2.2. Nisin and tannic acid content in the composites. The nisin and tannic acid contents in the composites were determined indirectly as in [32]. The nisin/tannic acid content in the supernatants from centrifugation (Section 3.1) was measured at 260 nm [33,34] (Fig. SI.1) / 278 nm [35], respectively, in triplicate, using a DU 640 UV spectrometer. The content of nisin/tannic acid in the particles was determined by difference, from the amount used in the preparation (Table 1). The encapsulation efficiency (EE) and loading capacity (LC) were calculated according to Eqs. (3) and (4).

$$\text{EE}\ (\%) = \frac{\text{Amount of N/TA entrapped}}{\text{Total amount of N/TA}} \times 100 \quad (3)$$

$$\text{LC}\ (\%) = \frac{\text{Amount of N/TA entrapped}}{\text{Total mass of particles}} \times 100 \quad (4)$$

2.2.2.3. Fourier Transform Infrared (FTIR) spectra. Fourier Transform Infrared (FTIR) spectra were recorded on a Bruker Tensor 27 instrument (Bruker Optik GmbH, Germany). Ground samples were placed on the crystal of the ATR accessory and 32 co-added scans at 4 cm^{-1} resolution were taken on each sample.

2.2.2.4. Scanning Electron Microscopy (SEM). Scanning Electron Microscopy (SEM) was performed on a LEO1530 microscope equipped with InLens and Secondary Electron detectors using low accelerating voltage (3 kV). It was used to observe the shape of the obtained materials (in Leo Elektronenmikroskopie GmbH, Oberkochen, Germany). The non-conducting surface of the hybrid materials was covered with palladium, by vapor deposition, before analysis.

2.2.2.5. Size and zeta potentials measurement. Size and zeta potential measurements were performed on a Zetasizer Nano-ZS (Zetasizer 4700 Malvern Instruments, Brookhaven Instruments Corporation, USA) with the particles suspended in acetate buffer 0.5 g/L, pH 4.The intensity–intensity time correction function was analyzed by the cumulative method. Each measurement was repeated eight times. The mean value of the hydrodynamic diameter was calculated from the diffusion measurement using the Stokes–Einstein equation. In all cases, standard deviation was always lower than 5 % (experiment data were interpreted with statistical software (GraphPad®).

2.2.3. Kinetics of the nisin release from the particles

0.1 g samples of finely ground particles were dissolved in 30 ml phosphate buffer pH 5.0 and left under magnetic stirring at room temperature. At different times 3 ml volumes were withdrawn (and replaced by 3 ml of fresh buffer), filtered, and analyzed for protein content at 280 nm, using phosphate buffer pH 5.0 as blank. The % mass of nisin released at time t was calculated as in Eq. (5):

$$\%\text{mass of nisin released} = \left(\frac{\text{total mass released}}{\text{total mass of nisin in the nanoparticle sample}}\right) \times 100\% \quad (5)$$

The total mass released at a time t was calculated as in Eq. (6):

$$\text{Total Mass released} = (CtV + \Sigma Ct - 1Vs) \times MW \quad (6)$$

where: C_t - concentration of nisin released in the solution at time t; V - total volume of the solution (30 mL); V_s - volume of the sample (3 mL); MW – molar mass of nisin (3354.07 g/mol)

2.2.4. Antioxidant assays

The antioxidant activities of the composites were evaluated in duplicate using the Cayman Antioxidant Kit [36]. A BioTek® PowerWave HT Microplate Spectrophotometer was used to take readings for the chromophore at 750 nm.

2.2.5. Antimicrobial assays

The antimicrobial activities of chitosan, nisin and tannic acid were tested individually, in combination with each other, and as particulate composites containing different amounts of both nisin and tannic acid (Table 1). The minimal inhibitory concentrations (MIC), as defined by the National Committee for Clinical Laboratory Standards (1991), were determined against the gram-positive *Staphylococcus aureus* (ATCC 25923), the gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

Combination solutions of individual components were prepared as follows:

- (C + N) − 1 mg/mL C + 1 mg/ml N
- (C + N + TA) − 1 mg/mL C + 1 mg/ml N + 1 mg/ml TA
- (N + TA, 10:1) − 1 mg/mL N + 0.1 mg/ml TA
- (N + TA, 5:1) − 1 mg/mL N + 0.2 mg/ml TA
- (NC + N) − 1 mg/mL NC + 1 mg/ml N
- (NC + N + TA) − 1 mg/mL `NC + 1 mg/ml N + 1 mg/ml TA

Test and control samples were first dissolved in 1 % acetic acid to obtain a 1 mg/mL stock solution. Solution pH was adjusted to 5.5. Subsequently, dilution series were made (500 μg/mL, 250 μg/mL, 125 μg/mL, 62 μg/mL, and 31 μg/mL) using 1 % acetic acid and cationic adjusted Miller-Hilton broth. Test media containing controls and composites, respectively, were autoclaved at 121 °C / 15 psi for 15 min.

Cultures were grown and prepared following the Clinical and Laboratory Standards Institute (CLSI) protocols for turbidimetric assays [37]. Bacterial cultures used for test inoculations were first grown on agar surface for 24 h, then suspended into 0.9 % sterile saline solution to obtain McFarland standard of 0.5 (approximately 1.5×10^8 CFU/ml). Each test tube containing 10 ml medium was inoculated with 0.01 mL of bacteria solution and incubated at 37 °C for 24 to determine MIC. Assays were done in triplicate. To confirm the turbidimetric results, microdilution 96 well-plates were prepared, in triplicate, incorporating resazurin as an indicator of cell metabolic activity, as in [38].

2.2.6. Statistical analysis

The statistical analyses were done with a GraphPad Prism software, version 5.01 (USA) statistical package. Data were analyzed using the Kruskal-Wallis test.

3. Results and discussion

Chitosan being approved for human consumption, being biodegradable, and antibacterial is a good candidate to encapsulate proteins using green processes which allow easy self-assembly in mild condition of temperature and pH [39,40]. Based on our previous experience preparing chitosan particles encapsulating bioactive agents [15,16,32], we encapsulated nisin in chitosan matrices together with tannic acid. The intended role of the TA was to improve the interactions of nisin within the structures, to possibly modulate its release from particles, and to have a positive coaction with the antimicrobial and antioxidant activities of nisin and chitosan. The preparations used ionic gelation due to electrostatic interactions between the positively charged amine groups of chitosan and negatively charged TPP. In such a procedure it is difficult to control the crosslinking which results in products with variable compositions.

Nisin is an amphiphilic peptide, containing both hydrophilic and hydrophobic regions and with an isoelectric point of 8.8 [41]. At 5.5 pH, the positively charged nisin competes with chitosan for the negative charges of TPP. In the composites TA is present as well, through its numerous phenolic groups, it can establish H-bonds with both chitosan and nisin. Additionally, due to the presence of nonpolar regions on TA, hydrophobic interactions may occur with nonpolar area on nisin [42].

3.1. Characterization of the chitosan based composite particles

Interactions between components, as discussed above, are present in the FTIR spectra (Fig. 2) which are proof of chitosan particulate structures formation and incorporation of cargoes inside chitosan matrix. Starting chitosan (Figure 2Aa) spectrum shows two vibrations at 1658 and 1562 cm^{-1}, assigned, respectively, to amide I and amide II vibrations [43,44]. Amine deformation vibrations are usually correlated to bands which are strong in the 1638–1575 cm^{-1} region. Based on Lawrie et al. work [44], the band at 1562 cm^{-1} has been assigned to the N—H bending vibration overlapping the amide II vibration and the band at 1658 cm^{-1} has been attributed to the amide I vibration. Vibrations of C—N stretching are seen in the 1000 cm^{-1} region and overlapped the vibrations from the carbohydrate ring. N—H stretching due to amine group is observed in the 3300 cm^{-1} region overlapping the O—H stretching from the carbohydrate ring. In the spectrum of chitosan particles (NC, Fig. 2), two new peaks are observed, one at 1637 cm^{-1} and another one at 1543 cm^{-1} due to interactions with TPP. The broader band starting in the 3300 cm^{-1} region in NP, compared to starting chitosan, may correlated to an enhancement in hydrogen bonding [29]

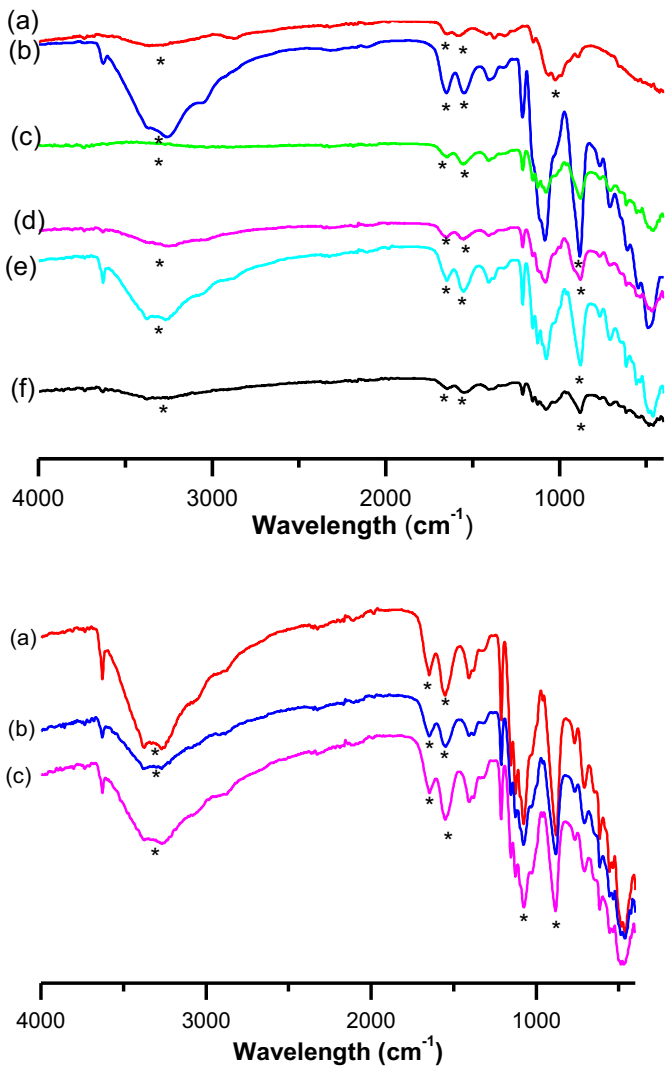

Fig. 2. FTIR spectra of: (A) (a) C, (b) NC, (c) NCN 0.1, (d) NCN 0.2, (e) NCN 0.5, (f) NCN 1.0 and B) (a) NCN 0.5, (b) NCNTA 0.5 (10:1) and (c) NCNTA 0.5 (5:1). Curves are offset for clarity.

and also to the hydroxyl groups due to TPP. Moreover after treatment with the TPP, the presence of the peaks at above1200 cm^{-1} (P═O stretching) and 887 cm^{-1} - 891 cm^{-1} for P—O bending, clearly evidence crosslinking with chitosan.

When nisin was added during the process of fabrication of the chitosan particles, the amounts of chitosan and TPP were maintained constant (Section 3.1 and Table 1). The spectra in Figures 2Ac/d/e/f show all peaks of the unloaded chitosan particles (NC) being present in all chitosan particles encapsulating nisin (NCN). Expected peaks due to incorporation of nisin overlap with chitosan peaks [45]. For example the peak at 1515 cm^{-1} attributed to NH_3^+ of nisin overlapped with N—H bending vibration overlapping the amide II vibration of chitosan, or the expected peak of amide band of nisin at 1650 cm^{-1} [46,47] overlapped with peak due to amine deformation in chitosan, i.e. the band at 1658 cm^{-1} attributed to the amide I vibration. Successful incorporation of nisin was evidenced by the presence of sulfur detected by energy dispersive X-ray (EDX) analysis during the SEM analysis and by estimation of the nisin loading content inside each hybrid chitosan particles (Table 2, discussed further).

Same procedure was used when composites containing chitosan, nisin and tannic acid were prepared. From spectra shown in Fig. 2B, it is observed that all peaks of the chitosan particles (NC) are seen in all resulting particles (NCNTA). Expected peaks due to incorporation of

Table 2
Characterization of the particles (EE, LC, RRAG).

Samples	Encapsulation efficiency for nisin (%)	Loading capacity for nisin (%)	Encapsulation Efficiency for tannic acid (%)	Loading capacity for tannic acid (%)	RRAG (%)
NC	N/A	N/A	N/A	N/A	53.85
NCN 0.1	55.56	1.31	N/A	N/A	53.85
NCN 0.2	88.31	5.54	N/A	N/A	44.23
NCN 0. 5	92.01	11.41	N/A	N/A	48.08
NCN 1	96.36	28.07	N/A	N/A	46.15
NCNTA 0.1 (10:1)	81.86	2.43	72.25	0.21	44.23
NCNTA 0.2 (10:1)	83.48	5.38	71.4	0.46	40.38
NCNTA 0.5 (10:1)	93.95	12.70	88.5	1.2	36.54
NCNTA 1 (10:1)	94.67	26.30	90.66	2.52	38.46
NCNTA 0.1 (5:1)	58.49	1.49	63.22	0.32	44.20
NCNTA 0.31 (5:1)	83.35	4.57	73.91	0.81	42.31
NCNTA 0.15 (5:1)[a]	64.52	6.69	59.01	1.22	30.77
NCNTA 0.5 (5:1)	90.96	12.52	91.35	2.51	36.44
NCNTA 1 (5:1)	94.79	25.62	96.65	5.22	35.08

(% values for RRAG, EE and LC: standard deviation was always lower than 5 %, data interpreted using statistical software (GraphPad®Prism, $p < 0.01$ Kruskal-Wallis test)

[a] Different C:TPP ratio.

tannic acid overlap with the already present peaks of chitosan (e.g. aromatic C═C bending of tannic acid is expected to be seen at above 1500 cm^{-1}, place where peaks of the chitosan are already observed. Successful incorporation of tannic was also demonstrated by estimation of its content inside each hybrid chitosan particles (Table 2).

In the FTIR spectra, all samples show an important feature between 3500 and 3000 cm^{-1}. This band is attributed to the OH and NH stretching modes [48]. This band extends beyond 3000 cm^{-1}, obscuring the -CH_2 stretching band at ~2900 cm^{-1} and displaying a loss of small features. We attribute the broadening of this band in the composites to increase in bonding between NH and OH groups (numerous in tannic acid). Two maxima at approximately 3250 cm^{-1} and 3370 cm^{-1} are exhibited by all the NCNTA particles. This band broadening is similar to that reported in the literature at 3180 cm-1 for chitosan-TTP particles and for chitosan succinlyated particles [49] and is attributed to the formation of extensive hydrogen bonded networks. The intensity of this band decreases upon TA addition (TA:N 1:10), at the same nisin content, but the two peaks shift slightly to 3245 cm^{-1} and 3374 cm^{-1} Higher TA content (TA:N 1:5) affords an increase in the same band while preserving the 2 small features. Due to the comparatively low TA content its presence cannot be more clearly documented from IR spectra.

The same pattern of interactions is evidenced by the data in Table 2. When an anionic crosslinking agent is added to chitosan (positively charged at 5.5 pH), electrostatic interactions form and the number of free amino groups decreases. Table 2 shows the number of residual amino groups in samples determined by colloidal titration with PVSK and calculated as in Eq. (1) (Section 2.2.2.1). When nisin is present in the composites, a decrease is expected and shown in the table (comparison with unloaded chitosan) although the variation with increase in protein content is less clear cut. The presence of TA, expected to increase crosslinking, is well illustrated by our samples which show lower RRAG values compared to those with nisin only, at the same concentration (NCN 1–46.15 %, NCNTA 1 (10:1) – 38.46 %, NCNTA 1 (5:1) – 35.08 %). The increase in nisin content affords a decrease in RRAG, at each N:TA ratio, due to interactions N:TA and increase in crosslinking, as discussed above. For the NCNTA 0.15 (5:1) in which C:TPP ratio is lower (1:1 compared to 2:1 in all other samples), RRAG is the lowest (30.77 %) due to enhanced crosslinking, as expected.

The encapsulation of an active agent in a polymeric matrix has been typically discussed in the literature in terms of encapsulation efficiency (EC) and loading capacity (LC) [50,51] and calculated as in Eqs. (3) and (4) (Section 2.2.2.2). Our results show a clear and direct dependance between nisin content and EE for all sets of particles. When only nisin is present in chitosan, EE increases from 55.56 % for NCN 0.1 to 96.36 % for NCN 1.0. These values are close to those reported by us for two catalytic proteins (histaminase and catalase which are both bigger macromolecules) and show the same dependance on protein concentration [32]. Nisin, a cationic protein (isoelectric point >8.5 [1]) highly charged at pH 5.0–5.5, at the same ratio chitosan:TPP in all the preparations, competes with chitosan for the same number of negatively charged TPP groups. Higher nisin concentrations make nisin a stronger competitor and afford higher %EE values. The same effect was mentioned by Sahu and Prusty [52]. When TA is present in the particles, EE values are slightly lower, more so at higher TA:N ratio, but they show the same trend as the NCN particles, increase when N concentration increases. EE values for TA show increase when N concentration increases and are similar for the two N:TA ratios. The loading capacity for nisin in the composite shows increase in value when nisin content increases for NCN and for NCNTA. A higher content of TA in the particles results in a slightly lower value of LC for nisin, at the same N concentration (LC for NCN 1–28.07 %, for NCNTA 1 (10:1) -26.30 %, and for NCNTA 1 (5:1) – 25.62 %). The same dependence of LC on encapsulated protein concentration is reported by some authors [52,53], while others report an opposite trend [32] or an indetermined one [54]. At the same nisin concentration, LC for TA shows the same trend, increase upon increase in nisin content.

The morphological characteristics of the chitosan-based particles were investigated via SEM technique. Fig. 3 shows that, following crosslinking, centrifugation, drying, and grinding chitosan particles were obtained as agglomerates in all cases as reported in [32]. The SEM pictures do not present significant morphological difference between the unloaded chitosan particles (Fig. 3, NC) and chitosan particles loaded with nisin (Fig. 3, NCN0.5). Agglomerates of larger sizes were obtained for the NCNTA 0.5 (10:1) and NCNTA 0.5 (5:1) composites containing both nisin and tannic acid compared to those encapsulating nisin alone. From these SEM pictures, it seems that the interactions between formed chitosan particles and the cargoes promote aggregation following encapsulation. Moreover, smoother surface structure could be seen in composite particles compared to unloaded chitosan particle (NC) (these smoother surface structures are more pronounced in the presence of both nisin and tannic acid). This is in accordance with the successful loading of cumin into chitosan particles reported by Amiri et al. [55].

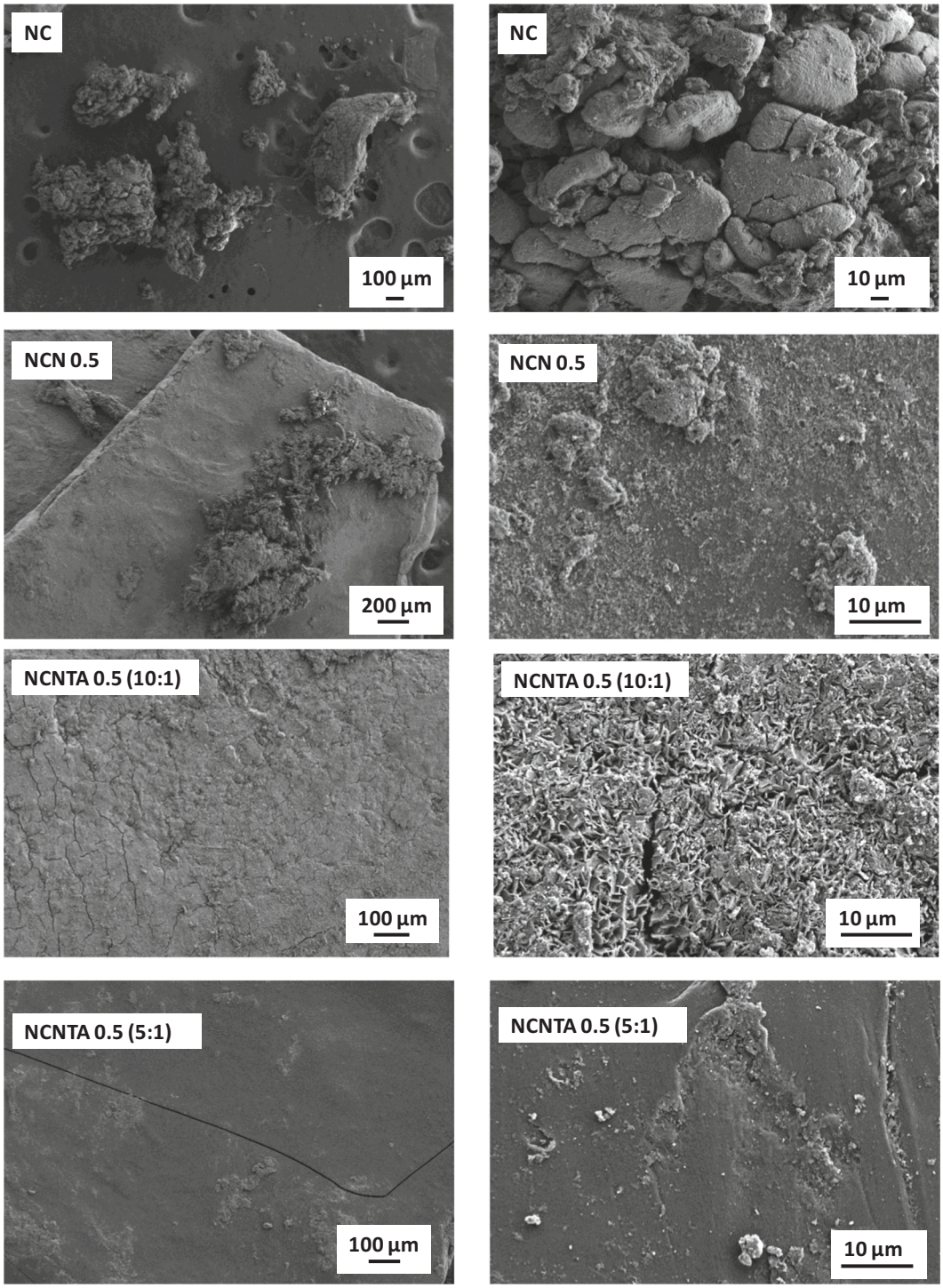

Fig. 3. SEM pictures of the hybrid chitosan particles without prior suspension.

Table 3 contains all the measured sizes (*Z*-average) and average zeta potential values for all the particles loaded with either nisin or nisin and tannic acid. No significant variation of the average zeta potential values is observed with or without cargoes, i.e. values remain around +20 mV. These results indicate that free amino groups (noncrosslinked, positively charged) remained on the particles surface for NC (amino group from chitosan) [19] and for NCN and NCNTA (amino groups from chitosan and/or nisin). No impact due to incorporation of tannic acid on the average zeta potential values is seen. This may be due to the neutral character of tannic acid at pH 4.0. Because antibacterial property is targeted herein, keeping positively charged character is then crucial since this might contribute to the antibacterial effect, i.e. the positively charged particle can interact with the bacterial cell surface by binding to its surface (negatively charged), which will lead to a disruption of the normal functions of the bacterial membrane [53,56,57]. This possible consequence will be investigated further, when the results of the antimicrobial assays will be discussed.

Compared to pure chitosan particles (NC), a decrease in particles size can be noticed in Table 3 due to nisin incorporation (NCN 0.1 to NCN 1). Such decrease may be correlated to an increase in crosslinking density (chitosan-NH_2/TPP and nisin-NH_2/TPP) leading to a reduction of particle diameters. However, no clear correlation between the increase in

Table 3
Size and average zeta potential values for particles suspended at 0.5 g/L in acetate buffer pH 4.

Sample	Average zeta potential values[a]	*Z*-Average[a]
NC	22 mV	10,880 nm PDI = 1.0
NCN 0.1	21.6 mV	3978 nm PDI = 0.3
NCN 0.2	22.1 mV	9397 nm PDI = 1.0
NCN 0. 5	19.7 mV	6060 nm PDI = 0.5
NCN 1	18 mV	5289 nm PDI = 0.6
NCNTA 0.1 (10:1)	23.4 mV	1950 nm PDI = 0.5
NCNTA 0.2 (10:1)	21.6 mV	2454 nm PDI = 0.3
NCNTA 0.5 (10:1)	20 mV	1140 nm PDI = 1.0
NCNTA 1 (10:1)	18.1 mV	3191 nm PDI = 0.3
NCNTA 0.1 (5:1)	22.5 mV	739 nm PDI = 0.7
NCNTA 0.31 (2.5:1)	22 mV	987 nm PDI = 1.0
NCNTA 0.15 (5:1)	22.1 mV	670 nm PDI = 0.8
NCNTA 0.5 (5:1)	20.5 mV	579 nm PDI = 0.5
NCNTA 1 (5:1)	19 mV	5524 nm PDI = 1.0

[a] Standard deviation was always lower than 5 %, experimental data interpreted with statistical software (GraphPad® Prism, $p < 0.01$ Kruskal-Wallis test).

nisin concentration and the size variation could be made within the same series (NCN 0.1 to NCN 1).

Addition of tannic acid leads to a higher size decrease compared to that afforded by addition of nisin alone, from 10,880 nm (NC) to 2 μm after small addition of tannic acid (NCNTA 0.1 (10:1)). The higher the tannic acid content, the lower the size is for NCNTA 0.1 (10:1) to NCNTA 0.5 (5:1) (size variation from ~2 μm to ~600 nm). This may be correlated to matrix interactions between the chitosan matrix and tannic acid which increase the crosslinking density leading to a reduction of particle diameters. These may also be due to hydrophobic interactions and some covalent linkages which may also occur as suggested by Zhang et al. who observed a size decrease due to incorporation of catechin in hybrid chitosan particle by using the sodium tripolyphosphate ionic crosslinking technique [19]. For NCNTA 1 (5:1), the comparatively high quantity of nisin may mask the effect linked to tannic acid leading to particle aggregation. Note that the PDI is another factor that represents the dispersion homogeneity. We found polydispersity index (PDI) ranging from 0.3 to 1. PDI values lower than 0.5 show homogeneous particle solution while PDI values above 0.5 indicate a heterogeneous system. High PDI values have been reported for other chitosan-based particles [58].

3.2. Kinetics of the nisin release from the particles

The kinetics of protein release was investigated by monitoring the concentration of nisin released in phosphate buffer pH 5.0 over several days (Fig. 4A). The value of the pH was chosen to be close to values of interest for the intended applications (processed meats and skin care/treatment formulations) [59,60]. In the case of the NCN composites, the release is slower (Graphs available in Supplementary information, Figure SI2) than in other reported cases of nisin loaded into chitosan but follows the same direct dependence on the concentration of the protein, as expected in a Fickian diffusion case [61]. However, nisin alone encapsulated in chitosan, while addressing the problem of too fast release in lipophilic environments, displays too slow release (~0.3 % over a week, for particles with relatively high nisin content, Fig. 4B) which is not effective for its intended use as a preservative. The sharp increase appearing after a week may be correlated to matrix degradation.

TA is known and used as a complexing agent for proteins. [59] Their interaction is a result of H-bonding and hydrophobic interactions [60]. The formation of tannin–protein complexes depends on numerous factors among which: presence of amino acids with hydrophobic side chains in the protein, N to TA ratio, protein isoelectric point (8.5 for N), pH, and presence of other compounds in the solution [59]. The presence of TA in the composites resulted in an increase in nisin release into the buffer. The fact that all NCNTA samples in Fig. 4 display an initial burst in release over the first 2 h is, probably, due to the release of some free nisin adsorbed on the surface of particles. In all cases, a sustained release followed the burst. Fig. 4B displays sustained release from NCNTA reaching effective levels (up to 5.5 % over more than a week) without signs of noticeable matrix degradation. As in the release from NCN particles, an increase in N content in the NCNTA composites resulted in increased release of N from the particles at a high N:TA ratio (10:1). At a higher TA content in the composites (N:TA 5:1), the amount of nisin released increased with increased N concentration in the composites reaching a maximum and then showing a decline at higher N content. This situation has been reported in [62] and is ascribed to the increase in polyphenol binding sites which result in bigger complexes/aggregates with slower diffusion, hence release, out of the matrix. For both N:TA ratios tested, TA modulated favorably the sustained release of nisin from particles.

3.3. Biological activity of the composite particles

3.3.1. Antioxidant activity

The composite particles were assayed for antioxidant activity and the results were compared to the starting components. The results are presented in Table 4. While chitosan has a mild antioxidant activity [63], the particulate chitosan shows an important enhancement thereof. Nisin, like chitosan, has a modest antioxidant effect itself [64]. In combination with natural antioxidants (avocado seeds and avocado peel) nisin showed positive coaction (antimicrobial and antioxidant) [65]. The presence of nisin in the composites does not enhance this effect although increase in nisin concentration is beneficial. What was surprising was the fact that tannic acid, a potent antioxidant itself [28] did not show positive coaction with chitosan. However, it is risky to make a definite statement since the amount of free tannic acid in each assay is sensibly higher than the total TA amount used in the preparation of each type of particles [36]. The results in Table 4 clearly show (with one exception) that a higher TA:N ratio in the composites affords an enhancement in the antioxidant effect. Increased crosslinking, due to a higher TPP:C ratio in the preparation (sample NCNTA 0.15 (5:1)), resulted in a sizable increase in antioxidant activity. The lack of clear correlation between the variation in TA concentration and the antioxidant activities of the composites may be due to the different time scales of the diffusion of the active components out of the particles and that of the antioxidant assay. This factor which impacts the actual concentration of tannic acid in the assay mixture makes our results lower than those presented in [66] where the polymeric particles had been degraded by being kept for 15 days in buffer, under shaking, prior to the assays. At pH 5.5, as our assays maintained, there is very little degradation of the polymeric samples.

3.3.2. Antibacterial activity of the particles

The particulate materials prepared were tested for antibacterial activity and possible synergistic effects against both gram-positive and gram-negative species, following the method described in Section 2.2.5. Results of a subset of the three species tested (a) *Staphylococcus aureus* (ATCC 25923), (b) *Escherichia coli* (ATCC 25922) and (c) *Pseudomonas*

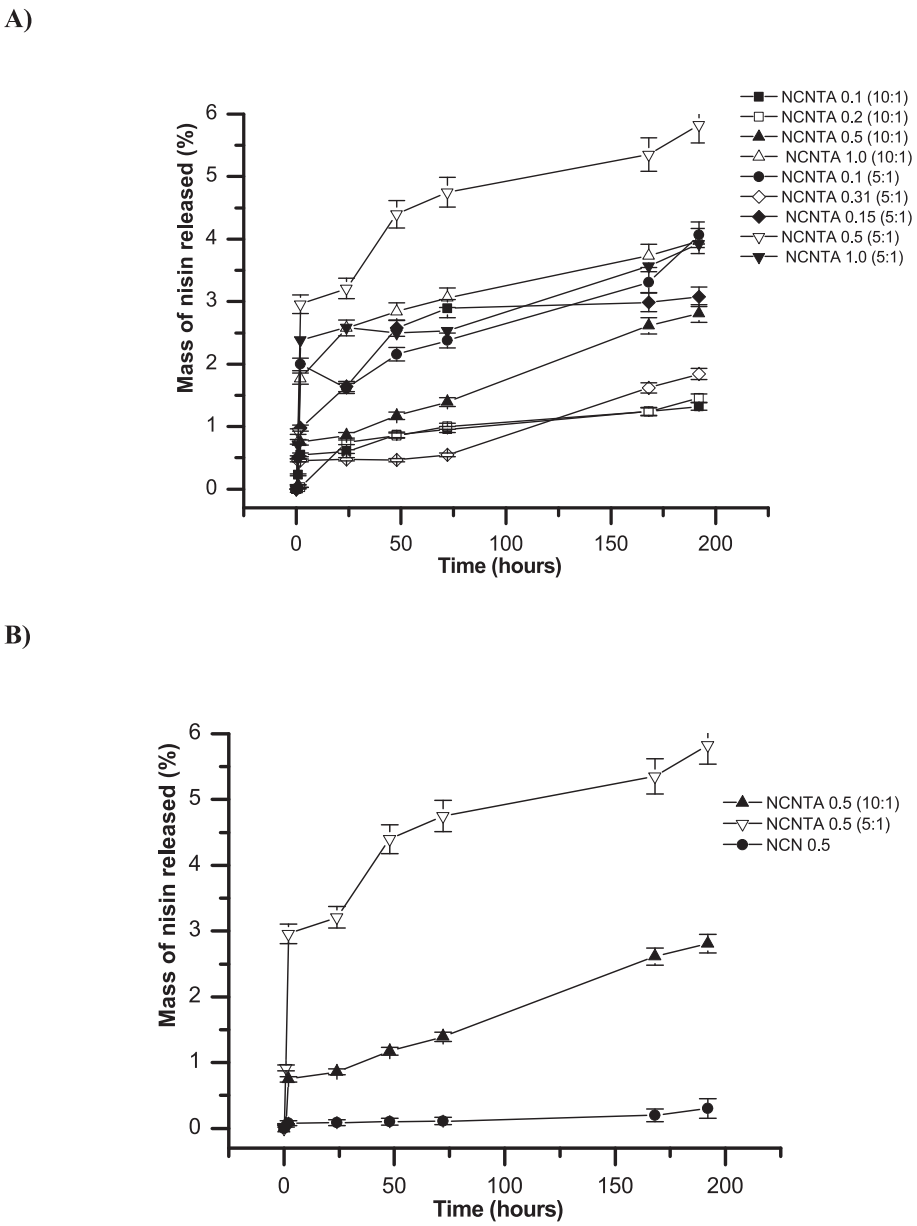

Fig. 4. A) Kinetics of the nisin release from the composite particles; B) Nisin release from composites with same amount of nisin in preparation without TA or with different N:TA ratios ("% mass of nisin released: standard deviation was always lower than 5 %, data interpreted using the statistical software (GraphPad® Prism, $p <$ 0.01 Kruskal-Wallis test).

aeruginosa (ATCC 27853), assayed on microdilution 96 well-plates incorporating resazurin as an indicator of cell metabolic activity can be observed in Fig. 5. Therein, red color indicates the presence of metabolic activity, while the dark blue/black color indicates microbial death. Minimum inhibitory concentration (MIC) was determined as the first concentration displaying dark blue/black color.

The average of the triplicate turbidimetric and microdilution 96 well-plates MIC values are shown in Table 5.

The starting nisin and chitosan show each a MIC value of 500 μg/ml for the gram-positive bacterium and of 1000 μg/mL for the gram-negative ones. Tannic acid showed a MIC of 1000 μg/mL for both gram-positive and gram-negative bacteria. Combining nisin with chitosan did not result in an increase in inhibitory action against the gram-positive *Staphylococcus aureus.* Antimicrobial activities against the gram-negative *E. coli* and *P. aeruginosa* increased by 25 %, respectively, reducing their MIC values from 1000 μg/ml to 750 μg/ml. The particulate chitosan, benefitting from the advantages of the size, decreased the MIC 4 times for *S. aureus* and even more for the gram-negative species (8 times for *E. coli* and 5.6 times for *P. aeruginosa*). Encapsulating nisin into chitosan afforded as expected, based on previous results [67], higher antimicrobial activities against both gram-positive and gram-negative bacteria despite the much lower amounts used compared to the simple combination of the two elements. NCN 0.1 and NCN 0.2 showed MIC values of 62 μg/ml for *Staphylococcus aureus* (~ 88 % increase) and *E. coli* (~92 % increase), respectively, and 125 μg/ml, vs. *P. aeruginosa* which represents an increase in activity of roughly 84 %. Nisin release test showed insignificant amount protein released during the first 48 h. This is a proof of effective encapsulation but also of strong synergistic action between chitosan and the nisin entrapped in the matrix. As the nisin content in the composites and the extent of the crosslinking increased, the release was higher during the same interval (~0.1 %) and the synergy shown is lower, reducing the antimicrobial activities of NCN 0.5 and of NCN 1 to values higher only by 75 % / 83 % (against gram-positive and gram-negatives species, respectively) compared to

Table 4
Antioxidant activities expressed as Equivalent Trolox Concentration.

Sample	Equivalent Trolox Concentration (mM)
NC	0.781
NCN 0.1	0.054
NCN 0.2	0.064
NCN 0.5	0.227
NCNTA 0.1 (10:1)	0.112
NCNTA 0.2 (10:1)	0.027
NCNTA 0.5 (10:1)	0.044
NCNTA 1.0 (10:1)	0.103
NCNTA 0.1 (5:1)	0.032
NCNTA 0.31 (5:1)	0.139
NCNTA) 0.15 (5:1)[a]	0.257
NCNTA 0.5 (5:1)	0.240
NCNTA 1.0 (5:1)	0.362
Acetic acid	0
Nisin	0.164
Tannic acid	0.680
Chitosan	0.134

(% values for Equivalent Trolox Concentration: standard deviation was always lower than 5 %, data interpreted with the statistical software (GraphPad® Prism, $p < 0.01$ Kruskal-Wallis test)
[a] Chitosan:TPP 1:1 (v/v).

starting components.

When testing the combination of the individual components nisin and tannic acid, we observed some synergistic effects between those two substances as well. Two different ratios of N:TA were used in these assays: 10:1 and 5:1, respectively. For 10:1 we observed an increase of 63 % to 75 % in the antibacterial activity against both gram-negative and gram-positive bacteria, when compared to nisin alone. The increase in TA content (5:1 N:TA) afforded an increase in activity against gram-negative microbes of 75 % for *P. aeruginosa* and 82 % for *E. coli,* when compared to nisin alone. Curiously, the antimicrobial activity against the gram-positive *Staphylococcus aureus* was reduced to 50 % compared with that of 10:1 N:TA.

In an intent to modulate nisin release from particles and increase the effectiveness of the nisin-chitosan particles over a longer period of time, tannic acid was added to chitosan and nisin during preparation (resulting in NCNTA composites). Considering the % mass of nisin released in the buffer (Fig. 4), the presence of tannic acid increases the release of nisin over time. NCNTA 0.1 (10:1) showed a release of nisin mass between 2 % and 2.15 % between 2 h and 48 h and MIC values of 178 μg/mL for *Staphylococcus aureus,* and 250 μg/mL *for E. coli* and *Pseudomonas aeruginosa*. These values represent decreases in inhibitory effectiveness of 50 to 65 % compared to NCN particles with the same nisin content. Similar results were observed for the other particulate composites irrespective of the concentration of nisin in the synthetic mixtures.

The NCNTA (5:1) displayed higher release over time. Having more TA and more extensive crosslinking than the NCNTA (10:1), at equal concentrations of nisin, more peptide interacts with TA, making it less available to interact with the gram-positive bacterial cell wall. Results show that, regardless of the amount of nisin present in the particulate complex, the antimicrobial effect against gram-positive *Staphylococcus aureus* is the same (MIC 500 μg/mL) as that of starting chitosan, or those of C + N, and of chitosan + tannic acid added separately in solution. The MIC values of NCNTA (5:1) against gram-negative *E. coli* and *P. aeruginosa,* between 250 μg/ml and 500 μg/ml, showed the same effectiveness as the combination of individual 1 mg/mL NC + 1 mg/mL N + 1 mg/mL TA. Taking into account that the amounts of each component in the composite samples is several times lower than those in the solution of individual components, the NCNTA (5:1) composites still show enhancement but a more modest effect than NCNTA (10:1) and a much lower one than the NCN samples. The difference in effectiveness of the two sets of NCNTA against the gram-positive and the gram-negative species may be correlated to the different extent of complexation of nisin by the tannic acid within the matrices. This seems to result in stronger penetration of the gram-negative outer membrane, making the composites effective against those microbes and confirming the findings of Boziaris and Adams [68], Adams [69], and Alakomi et al. [70]

From the data in Table 5, it appears that bonding of small amounts of nisin only (as in NCN 0.1 and NCN 0.2) to chitosan within a composite structure results in higher synergistic effects as demonstrated by the lowest MIC values against both gram-positive and gram-negative bacteria. In this case, the antimicrobial action seems to be achieved by the complex itself, and not by its components individually. The nisin release test showed that nisin was not released from the complex within the first

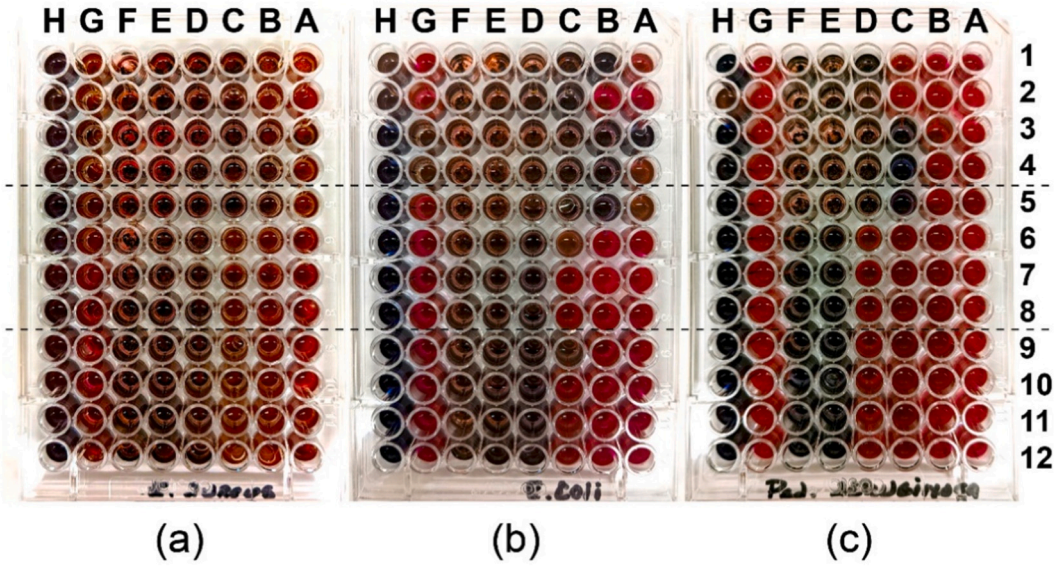

Fig. 5. Results for (a) *Staphylococcus aureus* (ATCC 25923), (b) *Escherichia coli* (ATCC 25922) and (c) *Pseudomonas aeruginosa* (ATCC 27853). Microdilution 96 well-plates incorporating resazurin as an indicator of cell metabolic activity (red color). Column H negative control, Column G positive control, Columns F-A contain particles in serial dilution starting at 1000 μg/mL. Rows contain different particles as follows: (1) NCN 0.1; (2) NCN 0.2; (3) NCN 0. 5; (4) NCN 1; (5) NCNTA 0.1 (10:1); (6) NCNTA 0.2 (10:1); (7) NCNTA 0.5 (10:1); (8) NCNTA 1 (10:1); (9) NCNTA 0.1 (5:1); (10) NCNTA 0.31 (5:1); (11) NCNTA 0.15 (5:1); (12) NCNTA 0.5 (5:1).

Table 5
Average results for MIC (μg/mL) using Miller Hilton broth at pH 5.5.

Sample	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Chitosan	500	1000	1000
Nisin	500	1000	1000
Tannic acid	1000	1000	1000
C + N	500	750	750
C + N + TA	750	500	500
Nisin + TA 10:1	125	375	250
Nisin + TA 5:1	250	178	250
NC + N	125	250	500
NC + TA	500	500	500
NC + N + TA	178	250	500
NC	125	125	178
NCN 0.1	62	62	125
NCN 0.2	62	62	125
NCN 0. 5	125	125	125
NCN 1	178	178	250
NCNTA 0.1 (10:1)	178	250	250
NCNTA 0.2 (10:1)	250	125	250
NCNTA 0.5 (10:1)	178	125	250
NCNTA 1 (10:1)	178	125	250
NCNTA 0.1 (5:1)	500	250	380
NCNTA 0.5 (5:1)	500	250	380
NCNTA 1 (5:1)	500	500	500

(% values for average results for MIC: standard deviation was always lower than 5 %, data interpreted using statistical software (GraphPad® Prism, $p < 0.01$ Kruskal-Wallis test)

24 h, making the nisin-chitosan complex very effective. Once we increased the amount of nisin to 0.5 mg/mL and 1 mg/mL, respectively, during the preparative procedure, some of the nisin (0.09 % and 0.12 % respectively) was released from the complex making it less effective and increasing the MIC values for both gram-positive and gram-negative bacteria. The addition of tannic acid as a modulator of nisin release from the matrices seems to steadily increase release over time. The more nisin is released from the complex, the smaller the synergistic action of the composite, as indicated by the antimicrobial actions of the particles containing tannic acid (NCNTA), is. The nisin being released from the complex appears to have lost some of its antimicrobial activity. This may be due to multiple causes. The most obvious is the much lower concentrations of nisin released in the kinetics test (within the first 48 h) compared to those used in the antimicrobial assays of the mixtures of separate components. Another cause may be, based on the findings of Chan et al. [71], nisin fragmentation processes which could lead to a loss of potence between 10 and 100 fold. Karam et al. [41] also discuss the possibility of nisin fragmentation and the creation of either a hydrophilic or hydrophobic subunit which prevents its bonding to either the peptidoglycan or the outer membrane of the bacterial cell wall. Yet another cause may be the fact that nisin can act as a specific antagonist of the growth-inhibitory effects of nisin. Despite these facts, the particles still maintain certain stability and antimicrobial activity similar or better than their individual components. The fact that this is stronger against the gram-negative microbes is remarkable since nisin itself is not active against them.

4. Conclusions

We report here the preparation of composite particles containing nisin encapsulated in chitosan, with and without tannic acid present, by using an inexpensive, green method, ionotropic gelation. Such particles may be used in the food, pharmaceutic, and cosmetic industries where the antimicrobial and antioxidant activities of the components are of interest. Also, nisin being from a natural source, is preferred by some consumers to chemically obtained preservatives. Encapsulation in the hydrophilic structure of chitosan protects nisin again proteolysis and addresses the early leakage problem found when it is used as preservative in matrices with high fat content. The presence of tannic acid in the particles added another protective layer to nisin through complex interactions. Acting as a modulator of nisin release, tannic acid brought nisin in the medium at effective levels over an extended time period. An unforeseen consequence was decrease in nisin antibacterial action, possibly due to extensive complexation.

Our study sought to broaden the characteristics of particles containing nisin encapsulated in chitosan while investigating possible synergistic effects in the composites since all components were bringing desirable features: chitosan – biodegradable/active against gram-positive, gram-negative species/mild antioxidant; nisin – bacteriocin with strong activity against gram-positive species; tannic acid – strong antioxidant with documented antibacterial activity. The materials prepared by us showed positive antimicrobial coaction enhancing the effectiveness of nisin against *S. aureus* and extending the activity to gram-negative species (*E. coli and P. aeruginosa*) against which nisin does not have documented activity. More investigations are needed to find an optimal concentration of nisin in the preparative procedure which would lead to optimal crosslinking in a particle effective against both gram-positive and gram-negative bacteria.

Addition of very low amounts of tannic acid to the preparations imparted antioxidant activity to the composites. This effect was stronger at higher TA:N ratio. The combination of a natural preservative (nisin) with a strong antioxidant (tannic acid) co-entrapped in a biopolymeric, inexpensive matrix led to synergistic antimicrobial activity which may lead to decrease in amounts of nisin used in industrial applications hence cost reductions. Future investigations should study the release of nisin from composites containing tannic acid as nisin enhancer in high fat content media, similar to those used in foods, ointments and skin care products and extend the activity assays to molds and fungi. Also, in order to bring the materials reported herein closer to the intended applications, future research should assay their antimicrobial and antioxidant activities in vivo as well.

Credit authorship contribution statement

Mihaela D. Leonida prepared the particles, performed measurements and calculations for encapsulation, performed the antioxidant assays and wrote ~78 % of the manuscript.

Alice Benzecry performed the antibacterial assays and wrote the discussion of the results.

Bisera Lozanovska performed the kinetic measurements and calculations for nisin release.

Zainab Mahmoud and Ashley Reid performed the colloidal titrations and calculations of RRAG.

Sabrina Belbekhouche performed particles characterization (FTIR, DLS, zeta potential measurements and SEM) and wrote one part of the manuscript.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2023.123489.

References

[1] A. Gharsallaoui, et al., Nisin as a food preservative: part 1: physicochemical properties, antimicrobial activity, and Main uses, Crit. Rev. Food Sci. Nutr. 56 (8) (2016) 1262–1274.
[2] K.D. Entian, C. Klein, Lantibiotics, a class of ribosomally synthesized peptide antibiotics, Naturwissenschaften 80 (10) (1993) 454–460.
[3] E. Cunha, et al., in: Nisin Influence on the Antimicrobial Resistance Ability of Canine Oral Enterococci 9, 2020, p. 12.
[4] C. Wu, et al., Formation, characterization and release kinetics of chitosan/γ-PGA encapsulated nisin nanoparticles, RSC Adv. 6 (52) (2016) 46686–46695.
[5] L.A. Ibarra-Sánchez, et al., Invited review: advances in nisin use for preservation of dairy products, J. Dairy Sci. 103 (3) (2020) 2041–2052.
[6] J. David, Expectations and applications of natural antimicrobials to foods: a guidance document for the supplier, user, Research and Development, and regulatory agencies, Food Prot. Trends 33 (4) (2013) 241–250.
[7] X. Zhao, et al., Effect of nisin and perilla oil combination against listeria monocytogenes and Staphylococcus aureus in milk, J. Food Sci. Technol. 53 (6) (2016) 2644–2653.
[8] H. Chen, Q. Zhong, Lactobionic acid enhances the synergistic effect of nisin and thymol against listeria monocytogenes Scott a in tryptic soy broth and milk, Int. J. Food Microbiol. 260 (2017) 36–41.
[9] J.I. Yoon, V.K. Bajpai, S.C. Kang, Synergistic effect of nisin and cone essential oil of Metasequoia glyptostroboides Miki ex hu against listeria monocytogenes in milk samples, Food Chem. Toxicol. 49 (1) (2011) 109–114.
[10] J.M. Shin, et al., Biomedical applications of nisin, J. Appl. Microbiol. 120 (6) (2016) 1449–1465.
[11] H.A. Fahim, A.S. Khairalla, A.O. El-Gendy, Nanotechnology: a valuable strategy to improve bacteriocin formulations, Front. Microbiol. 7 (2016) 1385.
[12] P.K. Sidhu, K. Nehra, Bacteriocin-nanoconjugates as emerging compounds for enhancing antimicrobial activity of bacteriocins, J. King Saud Univ. Sci. 31 (4) (2019) 758–767.
[13] Y. Pranoto, S.K. Rakshit, V.M. Salokhe, Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin, LWT Food Sci. Technol. 38 (8) (2005) 859–865.
[14] M.D. Leonida, I. Kumar, Bionanomaterials for Skin Regeneration, Springer, 2016.
[15] M.D. Leonida, et al., Antibacterial hop extracts encapsulated in nanochitosan matrices, Int. J. Biol. Macromol. 120 (2018) 1335–1343.
[16] M.D. Leonida, et al., Nanocomposite materials with antimicrobial activity based on chitosan, Int. J. Nano Biomater. 3 (4) (2011) 316–334.
[17] M.D. Leonida, The Materials and Craft of Early Iconographers, Springer Science & Business Media, 2014.
[18] L. Falcão, M.E.M. Araújo, Tannins characterization in historic leathers by complementary analytical techniques ATR-FTIR, UV-vis and chemical tests, J. Cult. Herit. 14 (6) (2013) 499–508.
[19] L. Zhang, S.L. Kosaraju, Biopolymeric delivery system for controlled release of polyphenolic antioxidants, Eur. Polym. J. 43 (7) (2007) 2956–2966.
[20] B. Kaczmarek, Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials—A minireview, Materials 13 (14) (2020) 3224.
[21] W. Yan, et al., Applications of tannic acid in membrane technologies: a review, Adv. Colloid Interf. Sci. 284 (2020), 102267.
[22] M.C. Etter, Encoding and decoding hydrogen-bond patterns of organic compounds, Acc. Chem. Res. 23 (4) (1990) 120–126.
[23] K.J. Siebert, N.V. Troukhanova, P.Y. Lynn, Nature of polyphenol– protein interactions, J. Agric. Food Chem. 44 (1) (1996) 80–85.
[24] D. Chandler, Interfaces and the driving force of hydrophobic assembly, Nature 437 (7059) (2005) 640–647.
[25] G. Ambrosi, et al., Polynuclear metal complexes of ligands containing phenolic units, Coord. Chem. Rev. 252 (10–11) (2008) 1121–1152.
[26] J. Wang, et al., Tannic acid adsorption on amino-functionalized magnetic mesoporous silica, Chem. Eng. J. 165 (1) (2010) 10–16.
[27] G. Dong, et al., Antimicrobial and anti-biofilm activity of tannic acid against Staphylococcus aureus, Nat. Prod. Res. 32 (18) (2018) 2225–2228.
[28] R.G. Andrade Jr., et al., The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals, Arch. Biochem. Biophys. 437 (1) (2005) 1–9.
[29] L. Qi, et al., Preparation and antibacterial activity of chitosan nanoparticles, Carbohydr. Res. 339 (16) (2004) 2693–2700.
[30] A.K. Singla, M. Chawla, Chitosan: some pharmaceutical and biological aspects–an update, J. Pharm. Pharmacol. 53 (8) (2001) 1047–1067.
[31] S.W. Ali, S. Rajendran, M. Joshi, Synthesis and characterization of chitosan and silver loaded chitosan nanoparticles for bioactive polyester, Carbohydr. Polym. 83 (2) (2011) 438–446.
[32] M. Leonida, et al., Enzyme nanovehicles: histaminase and catalase delivered in nanoparticulate chitosan, Int. J. Pharm. 557 (2019) 145–153.
[33] W. Liu, J.N. Hansen, Enhancement of the chemical and antimicrobial properties of subtilin by site-directed mutagenesis, J. Biol. Chem. 267 (35) (1992) 25078–25085.
[34] L.W. Place, T. Helmonds, S.F. Filocamo, Development and Characterization of Methods to Encapsulate Nisin for Use as an Antimicrobial Agent, Army Natick Soldier Research Development and Engineering Center MA, 2018.
[35] L.C. Katwa, M. Ramakrishna, M.R.R. Rao, Spectrophotometric assay of immobilized tannase, J. Biosci. 3 (2) (1981) 135–142.
[36] https://www.caymanchem.com/pdfs/709001.pdf.
[37] F.R. Cockerill, et al., Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, in: Approved Standard 32, 2012.
[38] S.D. Sarker, L. Nahar, Y. Kumarasamy, Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals, Methods 42 (4) (2007) 321–324.
[39] N. Csaba, M. Köping-Höggård, M.J. Alonso, Ionically crosslinked chitosan/tripolyphosphate nanoparticles for oligonucleotide and plasmid DNA delivery, Int. J. Pharm. 382 (1–2) (2009) 205–214.
[40] H. Zhang, et al., Recent advancements in encapsulation of chitosan-based enzymes and their applications in food industry, Crit. Rev. Food Sci. Nutr. (2022) 1–19.
[41] L. Karam, et al., Nisin adsorption on hydrophilic and hydrophobic surfaces: evidence of its interactions and antibacterial activity, J. Pept. Sci. 19 (6) (2013) 377–385.
[42] E. Breukink, et al., Binding of nisin Z to bilayer vesicles as determined with isothermal titration calorimetry, Biochemistry 39 (33) (2000) 10247–10254.
[43] Z. Osman, A.K. Arof, FTIR studies of chitosan acetate based polymer electrolytes, Electrochim. Acta 48 (8) (2003) 993–999.
[44] G. Lawrie, et al., Interactions between alginate and chitosan biopolymers characterized using FTIR and XPS, Biomacromolecules 8 (8) (2007) 2533–2541.
[45] T. Krivorotova, et al., Nisin-loaded pectin nanoparticles for food preservation, Food Hydrocoll. 54 (2016) 49–56.
[46] S.M. Hosseini, et al., Preparation and characterization of alginate and alginate-resistant starch microparticles containing nisin, Carbohydr. Polym. 103 (2014) 573–580.
[47] S. Shang, L. Zhu, J. Fan, Intermolecular interactions between natural polysaccharides and silk fibroin protein, Carbohydr. Polym. 93 (2) (2013) 561–573.
[48] Y. Hu, et al., Self-aggregation and antibacterial activity of N-acylated chitosan, Polymer 48 (11) (2007) 3098–3106.
[49] M.R. Rekha, C.P. Sharma, pH sensitive succinyl chitosan microparticles: a preliminary investigation towards oral insulin delivery, Trends Biomater. Artif. Organs 21 (2008) 107.
[50] E. Curotto, F. Aros, Quantitative determination of chitosan and the percentage of free amino groups, Anal. Biochem. 211 (2) (1993) 240–241.
[51] W. Zhang, X. He, Encapsulation of Living Cells in Small (~100 μm) Alginate Microcapsules by Electrostatic Spraying: A Parametric Study, 2009.
[52] S.K. Sahu, A.K. Prusty, Design and evaluation of a nanoparticulate system prepared by biodegradable polymers for oral administration of protein drugs, Die Pharmazie 65 (11) (2010) 824–829.
[53] Y. Xu, Y. Du, Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles, Int. J. Pharm. 250 (1) (2003) 215–226.
[54] Q. Gan, T. Wang, Chitosan nanoparticle as protein delivery carrier—systematic examination of fabrication conditions for efficient loading and release, Colloids Surf. B: Biointerfaces 59 (1) (2007) 24–34.
[55] A. Amiri, et al., Fabrication of cumin loaded-chitosan particles: characterized by molecular, morphological, thermal, antioxidant and anticancer properties as well as its utilization in food system, Food Chem. 310 (2020), 125821.
[56] I. Helander, et al., Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria, Int. J. Food Microbiol. 71 (2–3) (2001) 235–244.
[57] N. Sudarshan, D. Hoover, D. Knorr, Antibacterial action of chitosan, Food Biotechnol. 6 (3) (1992) 257–272.
[58] Y. Hu, et al., Formation and optimization of chitosan-nisin microcapsules and its characterization for antibacterial activity, Food Control 72 (2017) 43–52.
[59] M. Haddad, et al., Molecular interactions of tannic acid with proteins associated with SARS-CoV-2 infectivity, Int. J. Mol. Sci. 23 (5) (2022) 2643.
[60] K. Asano, K. Shinagawa, N. Hashimoto, Characterization of haze-forming proteins of beer and their roles in chill haze formation, J. Am. Soc. Brew. Chem. 40 (4) (1982) 147–154.
[61] L.M. Marvdashti, M. Yavarmanesh, A. Koocheki, Controlled release of nisin from polyvinyl alcohol - alyssum homolocarpum seed gum composite films: nisin kinetics, Food Biosci. 28 (2019) 133–139.
[62] K.J. Siebert, Effects of protein–polyphenol interactions on beverage haze, stabilization, and analysis, J. Agric. Food Chem. 47 (2) (1999) 353–362.
[63] V. Jarmila, E. Vavříková, Chitosan derivatives with antimicrobial, antitumour and antioxidant activities–a review, Curr. Pharm. Des. 17 (32) (2011) 3596–3607.
[64] C.A. dos Santos, et al., Bacterial nanocellulose membranes combined with nisin: a strategy to prevent microbial growth, Cellulose 25 (11) (2018) 6681–6689.
[65] M. Calderón-Oliver, et al., Optimization of the antioxidant and antimicrobial response of the combined effect of nisin and avocado byproducts, LWT Food Sci. Technol. 65 (2016) 46–52.
[66] N. Sahiner, et al., Biocompatible and biodegradable poly(Tannic Acid) hydrogel with antimicrobial and antioxidant properties, Int. J. Biol. Macromol. 82 (2016) 150–159.
[67] M.D. Leonida, BA, Antimicrobials for Food Preservation Delivered in Nanosized Matrices. Proceedings of the 42nd ARA Conference, 108-112, Cluj-Napoca, Romania, 2018. May 23–26.
[68] I.S. Boziaris, M.R. Adams, Effect of chelators and nisin produced in situ on inhibition and inactivation of gram negatives, Int. J. Food Microbiol. 53 (2–3) (1999) 105–113.
[69] P.G. Adams, et al., Lipopolysaccharide-induced dynamic lipid membrane reorganization: tubules, perforations, and stacks, Biophys. J. 106 (11) (2014) 2395–2407.
[70] H.L. Alakomi, et al., Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane, Appl. Environ. Microbiol. 66 (5) (2000) 2001–2005.
[71] W.C. Chan, et al., Structure-activity relationships in the peptide antibiotic nisin: antibacterial activity of fragments of nisin, FEBS Lett. 390 (2) (1996) 129–132.