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Cinnamaldehyde-loaded chitosan nanoparticles: characterization and antimicrobial activity

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ABSTRACT

Due to its wide spectrum activity and low toxicity, chitosan has shown to be a promising molecule to be applied in food science and technology. Meanwhile, cinnamaldehyde (the main component in cinnamon flavor) has shown potential uses as a strong antimicrobial compound. To improve the antimicrobial activity of chitosan, we have prepared N-acylated chitosan nanoparticles, with cinnamaldehyde as acylating reagent. The properties of the modified material were compared against the ones of the unmodified chitosan nanoparticles. Modification of the material was characterized by means of FT-IR spectroscopy with the identification of the imine group formed due to the addition of cinnamaldehyde (soft band appearance in the range of 1630-1660 cm^{-1}). Meanwhile, ^{1}H NMR analysis was used to quantify the modification. Nanoparticles of both, the modified and the unmodified chitosan, were characterized by TEM and DLS analysis showing a higher diameter size and a reduced zeta potential for the cinnamaldehyde loaded material than the chitosan nanoparticles. Finally, their activity was tested against *E. coli* and *L. monocytogenes* by measuring the minimal inhibitory concentration, showing a greater activity when the nanoparticles were functionalized (with an observed increase of the activity with the higher loading of cinnamaldehyde). This works evidence that the N-acylated chitosan nanoparticles have promising and potential use in food preservation.

Keywords: *nanoparticles, chitosan, cinnamaldehyde, antimicrobial activity.*

1. INTRODUCTION

Applied nanotechnology in the food industry has attracted much attention during the last decade since it can provide food products with better-quality characteristics and may improve their stability during storage and preparation, or even to work as sensors for food control [1] applications in the food sector may range from bioactive packaging to the design of new functional products [2–4]. Among such variety of applications, nanoparticles (NPs) offer a number of benefits, including retention of volatile compounds and their controlled release [5]. Several techniques to load bioactive compounds into nanoparticles, which include emulsification, spontaneous emulsification, complexation, coacervation, spray drying, ionotropic gelation, emulsion-ionic gelation, ionic crosslinking, high-pressure homogenization, among others, have been reported [6–9]. Most authors also pointed out that the selection of the proper method greatly depends on the required properties of the product as well as the nature of the core and wall materials. Nowadays, the use of chitosan nanoparticles has gained significant interest for diverse procedures including tissue engineering, drug delivery, and several food applications due to their suitable biocompatibility, biodegradability, nontoxicity, low cost, and abundance [10,11].

Particularly, nanoencapsulation of essential oils (EOs) has been considered in order to face the challenges for their application as natural antimicrobial and antioxidant compounds. Ghaderi-Ghahfarokhi et al. [12] studied the effect of chitosan nanoparticles loaded with thyme essential oil concerning to lipid oxidation, microbial spoilage, and sensory changes of beef burgers during chilled storage, observing important improvements in product quality and shelf-life. The potential application of nanoencapsulated essential oils as coating materials has also been explored; Mohammadi et al. [13] evaluated the antimicrobial activity of *Cinnamomum zeylanicum* essential oil (CEO) when encapsulated by chitosan nanoparticles (ChNPs), using those particles as protective films for cucumbers, assessing their effectiveness against *Phytophthora drechsleri.* Their results showed that coating with CEO-ChNPs improved microbiological and physicochemical quality of cucumbers extending for more than a week their shelf life; furthermore, coated cucumbers were firmer, maintained their color and water content while displaying lower microbial counts ($p<0.05$) throughout storage when compared to cucumbers not coated. Hu et al. [14] investigated the effectiveness of chitosan nanoparticles (loaded with cinnamon essential oil when applied in the package to preserve chilled pork. Low-density polyethylene (LDPE) film was the base material, and then active LDPE films coated with cinnamon essential oil -NPs of different sizes (112, 215 or 527 nm) were evaluated. According to obtained results, the size of such nanoparticles had a direct effect on their potential as natural preservatives to maintain the quality of meat and meat products. Thus, enhanced performance of essential oils or their components when encapsulated by chitosan nanoparticles can be achieved, depending on their final application; however, the size of nanoparticles needs to be also considered.

Chitosan displays advantages when compared with other kinds of polymers for stabilizing food nanoparticles [6], due to its structure, charge, antimicrobial activity cost and high availability [15]. Chitosan antimicrobial activity has been studied against a wide variety of microorganisms, including fungi, algae, and

bacteria [16–18]. Further, essential oils and some of their components have displayed several advantages for the food industry, especially for microbial control, due to their important antimicrobial activities, a wide spectrum of activity, and low toxicities, which have been reported by several researchers [19–21]. On the other hand, essential oils of cinnamon, clove, and thyme, which contain a high concentration of phenolic compounds, exhibit effective antimicrobial activities, which have been attributed mainly to cinnamaldehyde, eugenol, and thymol, respectively [22–24]. However, the application of EOs as antimicrobial agents continues to face important challenges due to their interactions with the food matrix, which can induce important changes in the sensory characteristics of food products, as well as for their volatile characteristics [12].

In this work, we investigated the effect of chitosan nanoparticles loaded with cinnamaldehyde (CCLNPs) with different ratios (1:0.00, 1:0.50, 1:0.75, 1:1.00, or 1:1.50 w/w), obtained by ionotropic gelation and ultrasound as dispersion method, on their antimicrobial activity against *Escherichia coli* or *Listeria monocytogenes*. The minimal inhibitory concentration (MIC) against the two studied microbial strains was evaluated using different concentrations of chitosan nanoparticles loaded with cinnamaldehyde. Furthermore, the characterization of prepared and tested nanoparticles is also reported in order to provide useful information for future applications as antimicrobial agents.

2. MATERIALS AND METHODS

2.1. Materials.

Chitosan (deacetylation degree of 0.95 and molecular weight of 760 kDa), polyoxyethylene (20) sorbitan monooleate (Tween 80), sodium tripolyphosphate (TPP), potassium bromide (KBr), acetic acid, and cinnamaldehyde (>99%) were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Two bacterial strains were used for antimicrobial activity tests, *Escherichia coli* (ATCC 35218) and *Listeria monocytogenes* (Scott A), obtained from the Food Microbiology Laboratory of Universidad de las Américas Puebla. Trypticasein soy broth (TSB) and Trypticasein soy agar (TSA) were acquired from Difco (Difco, Detroit, MI, USA).

2.2. Methods.

2.2.1. Synthesis of chitosan nanoparticles loaded with cinnamaldehyde. The chitosan nanoparticles loaded with cinnamaldehyde (CCLNPs) were prepared according to the self-assembley method [25,26]. Briefly, 40 mL of a chitosan solution (0.3 % w/v) was prepared by dissolving chitosan in an aqueous solution of acetic acid (1% v/v) under agitation overnight at room temperature. Tween 80 (0.5 mL) was added and stirred at 60 ºC for 2 h to obtain a homogeneous mixture. Cinnamaldehyde was added into the stirring mixture, and agitation was carried out for 40 min at 25 °C to obtain a uniform mix. Different chitosan-cinnamaldehyde proportions (1:0.00, 1:0.50, 1:0.75, 1:1.00, or 1:1.50 w/w) were prepared. After the addition of cinnamaldehyde to the chitosan solution; 15 mL of sodium tripolyphosphate (TPP) solution (1% w/v) were added and continuously mixed during 40 min to induce ionotropic gelation between chitosan and TPP. Then, sonication at 20 kHz and 98 µm (Vibra Cell 250; Sonics and Materials, Danbury, CT, USA) was applied for 20 s using a 1.2 cm probe. Once sonication treatment was concluded, the solution was centrifuged (Marathon 21K/R, Fisher Scientific, Pittsburgh, PA, USA) for 10 min at 12,600 x g, and then the precipitate was washed twice with Tween 80 (1%) solution and centrifuged again. Finally, the precipitate was retrieved for freeze-drying (6L Freeze Dryer, Labconco Co., USA).

2.2.2. Characterization of chitosan nanoparticles loaded with cinnamaldehyde. Fourier-transform infrared (FT-IR) spectra were obtained with a Varian 800 Scimitar series with Fourier transform (Varian Inc. Chicago, IL, USA), forming a KBr pellet. Each KBr disk was scanned over a wave number region of 400 - 4000 cm^{-1} at 20°C using 64 explorations at a resolution of 4 cm^{-1} at 20°C. The bonds' analysis was carried out with a Varian proton nuclear magnetic resonance (^{1}H NMR) spectrophotometer (Varian Inc. Chicago, IL, USA) at 200 MHz; samples (10 mg) were placed in Eppendorf tubes (1 mL capacity) and dissolved in 500 µL of deuterated water, keeping in an ultrasonic bath for 5 minutes. Then, an aliquot of the solution was taken and added to a quartz tube of 5 mm diameter. Analysis with the transmission electron microscopy (TEM) was carried out in a JEOL H-7650, Hitachi High-Technologies Corp. (Philips, NJ. USA) at an acceleration voltage of 100 kV. The nanoparticle solutions were sonicated for 1 min to produce better particle dispersion and to prevent nanoparticle agglomeration on the copper grid. After this, a drop of the nanoparticles solution was spread onto a copper grid, which was then dried at room temperature. The zeta potential and particle diameter were measured by dynamic light scattering (DLS) at 20°C, using a Nanotrac Wave (Nikkiso Co. Ltd. Largo, FL, USA) equipped with a diode laser at 3.0 mV and 780 nm, preparing samples by taking 1 mg of the lyophilized nanoparticles powder into 10 mL of distilled water. Each determination was performed by triplicate except for zeta potential and particle diameter that was replicated ten times.

2.2.3. Antimicrobial activity of chitosan nanoparticles loaded with cinnamaldehyde. Experiments were conducted with two different microbial strains, *Escherichia coli* (ATCC 35218) and *Listeria monocytogenes* (Scott A). Both bacteria were cultivated in TSB at 30ºC during 24 h. The minimal inhibitory concentration (MIC) for the two microbial strains was assessed using different concentrations of both: CCLNPs and non-loaded chitosan nanoparticles (ChNPs). For each case, the nanoparticles were dissolved in 1% (v/v) acetic acid and added to TSB to obtain a final concentration of 3.0, 3.1, 3.2, 3.3, up to 7.0 µg/mL. Subsequently, 100 µL of *E. coli* or *L. monocytogenes* with a concentration of 2.0 x 10^6 CFU/mL were inoculated; the inoculated tubes were incubated at 37ºC during 24 h. The MIC was defined as the concentration where the tubes presented no bacterial growth (not turbid, optical density change less than 0.05). For those concentrations were no-growth was observed, 100 µL from each tube was taken and surface platted on TSA, and after incubation (35°C for 24 h) plates were observed and counted.

Those concentrations without bacterial growth and containing the lowest tested concentration were defined as the minimal bactericidal concentration (MBC). Each determination was performed by triplicate.

2.2.4. Statistical analysis. Minitab 17 (Minitab Inc., State College, PA, USA) software was used to perform analysis of variance (ANOVA) and the statistical significance of differences between means was evaluated by Tukey's tests ($p<0.05$).

3. RESULTS

3.1. Particle size and distribution.

Morphology and size distribution of the ChNPs and CCLNPs was determined with TEM and DLS studies. In figure 1, TEM images showed spherical nanoparticles with an average size of 5 to 20 nm. In the same figure, it can also be observed that some of the particles were already aggregated. DLS studies gave information about the size distribution of the nanoparticles. ChNPs showed an average diameter of 6.5 nm while the CCLNPs nanoparticles showed a larger value of the average diameter, with 65.1 nm. In addition, it can be observed that their diameter is increased proportionally to their cinnamaldehyde content (Table 1). The variation between the observed results in TEM and the DLS is also reported by Keawchaoon and Yoksan [25] where they loaded chitosan NPs with carvacrol. They related this variation to the fact that in DLS it is measured the hydrodynamic diameter and the aggregation of the NPs in aqueous solution.

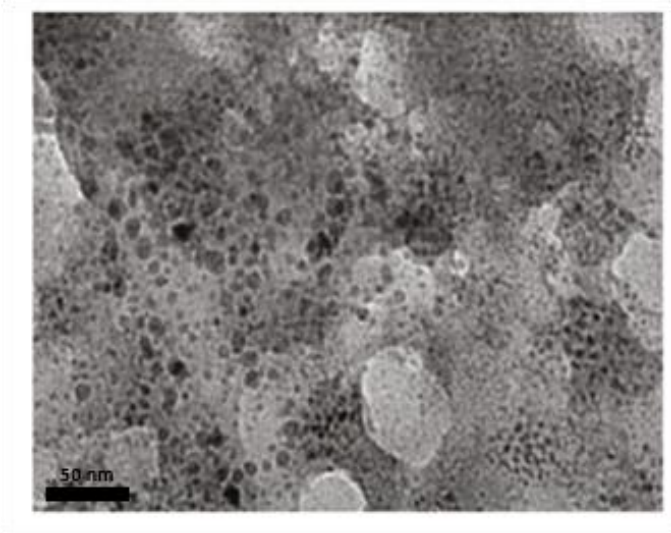

Figure 1. Transmission electron microscopy of chitosan loaded cinnamaldehyde (1:0.50) nanoparticles for particle size determination.

Chitosan nanoparticles depicted a Zeta potential value of 43.21 mV, which implies a particle surface positively charged (Table 1). The Zeta potential was reduced to values ranging from 24.5 to 29.9 mV for chitosan-cinnamaldehyde nanoparticles. This reduction of the potential is also in agreement with the TEM and DLS results.

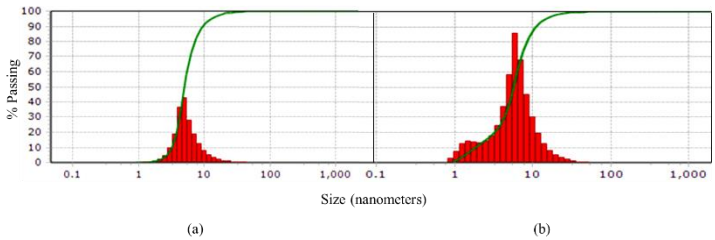

Figure 2. Dynamic light scattering particle size and distribution determination for (a) chitosan nanoparticles or (b) chitosan loaded cinnamaldehyde (1:0.50) nanoparticles.

The observed decrease reflects that the cinnamaldehyde charge reduces the positive charge on the surface of the nanoparticle and therefore reduces the dispersion stability of particles in water; therefore, nanoparticles will agglomerate as shown by the increased diameter observed by TEM and DLS.

This reduction in the value of Zeta potential can be the result of the coating of cinnamaldehyde on the surface of the particles. Keawchaoon and Yoksan [25] reported that chitosan-tripolyphosphate particles were expanded and the surface positively charge was reduced when the content of carvacrol was increased; these authors prepared and tested chitosan nanoparticles loaded with carvacrol with diverse chitosan to carvacrol ratios including 1:00, 1:0.25, 1:0.50, 1:0.75, 1:1.00, or 1:1.25.

Table 1. Diameter and Zeta potential of studied chitosan nanoparticles loaded or not with cinnamaldehyde

Chitosan: cinnamaldehyde (w:w)	Diameter* (nm)	Zeta Potential* (mV)
01:0.0	6.5 ± 0.3^{a}	43.2 ± 0.4^{a}
01:0.5	8.5 ± 0.2^{b}	29.4 ± 0.8^{b}
01:0.7	23.7 ± 0.5^{c}	27.5 ± 0.9^{c}
01:1.0	45.4 ± 0.2^{d}	25.3 ± 0.4^{d}
01:1.5	65.1 ± 0.6^{e}	24.5 ± 0.6^{d}

* Results reported as average of measurements ± standard deviation, n=10. Different lowercase letters in the same column indicate significant difference ($p<0.05$).

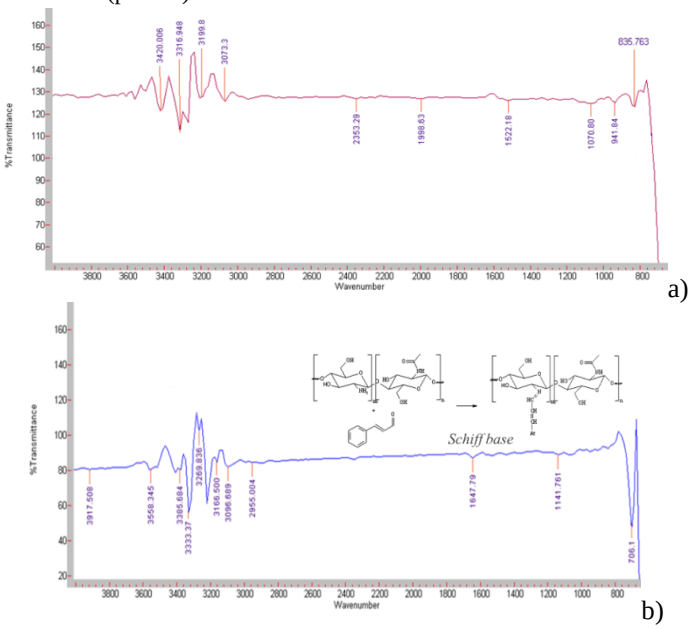

Figure 3. Fourier-transform infrared (FT-IR) spectra for a) chitosan nanoparticles, or b) chitosan loaded cinnamaldehyde (1:0.50) nanoparticles.

3.2. Fourier-Transform Infrared Spectroscopy (FT-IR).

FT-IR spectra for CLNPs presented differences in relation to the spectrum of chitosan alone, analyzed under the same conditions (Figure 3). Most important changes in these spectra are observed in the bands appearing around 3100-3500 cm^{-1} that correspond to the stretching movement of groups O-H and N-H.

These bands tend to show a sharper figure in the spectrum as a result of the chitosan-cinnamaldehyde reaction; indicating the strong interaction with amino groups present in chitosan, which have suffered an important decrease or modification. Furthermore, changes can also be observed in the region close to 1550-1600 cm^{-1} corresponding to the band generated by the presence of amides (C = O), it is evident an intensity decrease in the spectra of Figure 3 (b). Another band that reflects a significant modification is the one placed at 3269 cm^{-1} that corresponds with angular deformation of N-H group that form the amines present in the compound. Besides, in the spectra of Figure 3 (b) it is observed the formation of a new low-intensity band in the region of 1630-1660 cm^{-1} which is typical of the bond C=N that is present in Schiff's bases, which is generally difficult to identify due to the reduced intensity or overlapping with other bands of bigger size [27].

3.3. Spectroscopy Analysis through ^{1}H NMR.

Spectra obtained after the analysis of a chitosan sample and its N-acylated derivative loaded with cinnamaldehyde are shown in Figure 4. Signals that are typical of this kind of polymer can be clearly identified; such as the observed in the region of 2 ppm, attributed to the proton of the methyl that conforms the acetamide group; the signal located at 3.1 ppm, generated by the proton found in position 2 of the glucosamine ring. The signals in the region of 3.7 ppm, corresponding to hydrogen nucleus located in positions 5 and 6; while the signal located at 3.9 ppm is generated by the hydrogen of the carbon atoms situated in positions 3, 4, and 6 [28].

3.4. Determination of the substitution degree through ^{1}H NMR technique.

The spectrum (Figure 4b) obtained for the loaded sample allowed determining the substitution degree for the analyzed polymeric chain. This was possible by establishing a relation between the areas of the signal located at 7.4 ppm in reference to the aromatic hydrogen nucleus (A_{H-7}) and the area of the signal located in 2 ppm, corresponding to the hydrogen nucleus of the deacetylated units (A_{H-2}); this relation is summarized in the proposed equation by Keawchaoon and Yoksan [25]:

$$SD = \left(\frac{A_{H-7}}{3A_{H-2}}\right) \times 100$$

The obtained substitution degree (SD) after using the equation was of 58%. The stability and physical-chemical characteristics of the chitosan-cinnamaldehyde particles can be affected as a result of the ionic interactions [29,30] and the biological behavior of the molecules that could adhere as molecules with antimicrobial activity.

3.5. Antimicrobial activity.

The minimum inhibitory concentrations (Table 2) of chitosan (ChNPs) and chitosan-cinnamaldehyde nanoparticles (CCLNPs) against two tested bacterial strains *(E. coli* and *L. monocytogenes*) were determined. Chitosan nanoparticles and chitosan-cinnamaldehyde nanoparticles with concentrations below 5.3 µg/mL did not inhibit the growth of *E. coli* while with concentrations below of 4.3 µg/mL did not inhibit *L. monocytogenes*. On the other hand, for CCLNPs, the strains did not grow when concentration was 4.6 µg/mL for *E. coli* or 3.9 µg/mL for *L. monocytogenes* (Table 2) for those nanoparticles prepared with 1:0.50 chitosan:cinamaldehyde proportion. The concentration needed to inhibit bacterial growth decrease as the proportion of cinnamaldehyde in the nanoparticle increase (Table 2).

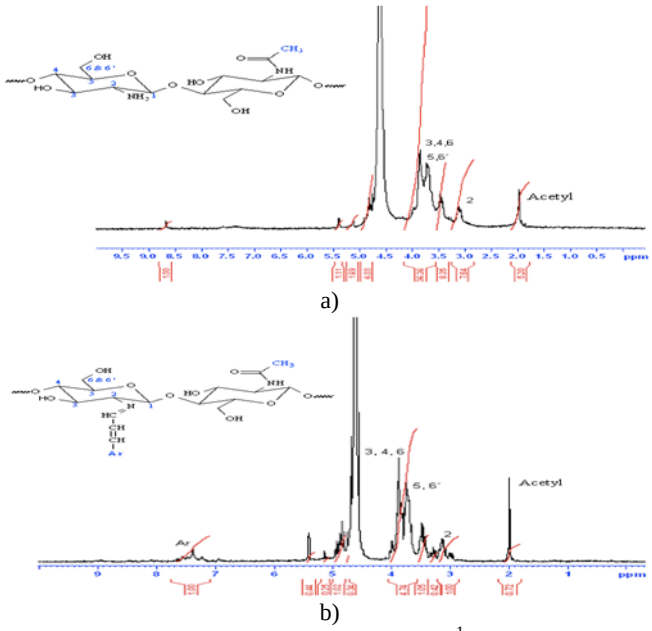

Figure 4. Proton nuclear magnetic resonance (^{1}H NMR) spectra for a) chitosan nanoparticles or b) chitosan loaded cinnamaldehyde (1:0.50) nanoparticles.

These results suggest that chitosan-cinnamaldehyde nanoparticles have an important antimicrobial activity. Furthermore, the minimal bactericidal concentrations of studied nanoparticles (prepared with 1:0.50 chitosan:cinnamaldehyde proportion) against *E. coli* and *L. monocytogenes* were 6.3 µg/mL and 5.3 µg/mL, respectively. Ghaderi-Ghahfarokhi et al. [12] reported successful encapsulation of thyme essential oil in chitosan nanoparticles, which maintained their antimicrobial activity when added to meat and improved shelf life of studied burgers.

Table 2. Minimal inhibitory concentration (MIC, µg/mL) and minimal bactericidal concentration (MBC, µg/mL) of tested compounds and studied nanoparticles against *Escherichia coli* and *Listeria monocytogenes*.

Sample	*E. coli*		*L. monocytogenes*	
	MIC	MBC	MIC	MBC
Chitosan (0.5%)	-*	-	-	-
Acetic acid (1%)	-	-	-	-
Chitosan/Cinnamaldehyde (w:w)				
01:0.0	5.3 ± 0.1 ^{a}	6.7 ± 0.1 ^{a}	4.3 ± 0.1 ^{a}	5.5 ± 0.1 ^{a}
01:0.5	4.6 ± 0.1 ^{b}	6.3 ± 0.1 ^{b}	3.9 ± 0.1 ^{b}	5.3 ± 0.1 ^{a,b}
01:0.7	4.3 ± 0.1 ^{c}	6.2 ± 0.1 ^{b}	3.5 ± 0.1 ^{c}	5.2 ± 0.1 ^{b,c}
01:1.0	4.0 ± 0.1 ^{d}	5.8 ± 0.1 ^{c}	3.4 ± 0.1 ^{c}	5.1 ± 0.1 ^{b,c}
01:1.5	3.8 ± 0.1 ^{d}	5.3 ± 0.1 ^{d}	3.0 ± 0.1 ^{d}	5.0 ± 0.1 ^{c}

* (-) No antimicrobial effect observed. Different lowercase letters in the same column indicates significant difference ($p<0.05$).

Our results proved that chitosan-cinnamaldehyde nanoparticles can be used as antimicrobial agents against *E. coli* and *L. monocytogenes*. Their antimicrobial activities could be explained

as result of the interaction of lipophilic cinnamaldehyde and the components of tested microorganisms' phospholipids layer, which can cause drastic changes in their membrane structure, affected by the destabilization and increase in the fluidity of the membrane, which increases passive permeability. MIC and MBC values for CCLNPs show that *L. monocytogenes* was inhibited to a higher degree than *E. coli*. In other words, the chitosan-cinnamaldehyde nanoparticles are more effective for Gram-positive than for Gram-negative bacteria.

This could be the result of a lower anti-bactericidal susceptibility of hydrophobic compounds against Gram-negative microorganisms, due to the restricted diffusion of these compounds through the lipopolysaccharide matrix that covers their outer membrane; this membrane does not exist in the outer layers of Gram-positive bacteria.

Furthermore, even though particle agglomeration was observed with a higher loading of cinnamaldehyde, it was noticed that the increase in the loading of the aldehyde was the main factor affecting the antimicrobial activity of the nanoparticles. It was noticed that the bactericidal activity was also proportional to the Zeta potential, decreasing this potential from values of 29.4 to 24.5 mV when cinnamaldehyde proportion was varied from 1:0.50 to 1:1.50, respectively; and with an average nanoparticle diameter between 8.5 and 65 nm.

4. CONCLUSIONS

Nanoparticles synthesis was accomplished by means of ionic gelation technique, and interaction between the two studied compounds occurred due to the positive charge of chitosan and the negative charge of cinnamaldehyde. The Schiff base reaction was successful and its formation was evidenced by ^{1}H NMR and FT-IR techniques. In general, antimicrobial activity was significantly ($p<0.05$) better for cinnamaldehyde loaded chitosan nanoparticles when compared to chitosan nanoparticles. Nanoparticles from a natural source were obtained, suggesting that elaboration of new materials and compounds for food preservation is possible while proving antimicrobial activity against *E. coli* or *L. monocytogenes*. It is apparent that the obtained nanoparticles are promising as antimicrobial agents due to their biological and physiochemical properties.

5. REFERENCES

1. Choi, A.J.; Kim, C.J.; Cho, Y.J.; Hwang, J. K.; Kim, C. T. Characterization of Capsaicin-Loaded Nanoemulsions Stabilized with Alginate and Chitosan by Self-Assembly. *Food Bioprocess Technol.* **2011**, *4* (6), 1119–1126. https://doi.org/10.1007/s11947-011-0568-9.
2. Neethirajan, S.; Jayas, D. S. Nanotechnology for the Food and Bioprocessing Industries. *Food Bioprocess Technol.* **2011**, *4* (1), 39–47. https://doi.org/10.1007/s11947-010-0328-2.
3. Singh, T.; Shukla, S.; Kumar, P.; Wahla, V.; Bajpai, V. K.; Rather, I. A. Application of Nanotechnology in Food Science: Perception and Overview. *Front. Microbiol.* **2017**, *8*, 1501. https://doi.org/10.3389/fmicb.2017.01501.
4. Pathakoti, K.; Manubolu, M.; Hwang, H.M. Nanostructures: Current Uses and Future Applications in Food Science. *J. Food Drug Anal.* **2017**, *25* (2), 245–253. https://doi.org/10.1016/j.jfda.2017.02.004.
5. Sotelo-Boyás, M. E.; Correa-Pacheco, Z. N.; Bautista-Baños, S.; Corona-Rangel, M. L. Physicochemical Characterization of Chitosan Nanoparticles and Nanocapsules Incorporated with Lime Essential Oil and Their Antibacterial Activity against Food-Borne Pathogens. *LWT* **2017**, *77*, 15–20. https://doi.org/10.1016/j.lwt.2016.11.022.
6. Ezhilarasi, P. N.; Karthik, P.; Chhanwal, N.; Anandharamakrishnan, C. Nanoencapsulation Techniques for Food Bioactive Components: A Review. *Food Bioprocess Technol.* **2013**, *6* (3), 628–647. https://doi.org/10.1007/s11947-012-0944-0.
7. Hasheminejad, N.; Khodaiyan, F.; Safari, M. Improving the Antifungal Activity of Clove Essential Oil Encapsulated by Chitosan Nanoparticles. *Food Chem.* **2019**, *275*, 113–122. https://doi.org/10.1016/j.foodchem.2018.09.085.
8. Cui, H.; Bai, M.; Rashed, M. M. A.; Lin, L. The Antibacterial Activity of Clove Oil/Chitosan Nanoparticles Embedded Gelatin Nanofibers against *Escherichia coli* O157:H7 Biofilms on Cucumber. *Int. J. Food Microbiol.* **2018**, *266*, 69–78. https://doi.org/10.1016/j.ijfoodmicro.2017.11.019.
9. Tian, H.; Lu, Z.; Li, D.; Hu, J. Preparation and Characterization of Citral-Loaded Solid Lipid Nanoparticles. *Food Chem.* **2018**, *248*, 78–85. https://doi.org/10.1016/j.foodchem.2017.11.091.
10. Manivasagan, P.; Oh, J. Marine Polysaccharide-Based Nanomaterials as a Novel Source of Nanobiotechnological Applications. *Int. J. Biol. Macromol.* **2016**, *82*, 315–327. https://doi.org/10.1016/j.ijbiomac.2015.10.081.
11. Soto-Chilaca, G. A.; Ramírez-Corona, N.; Palou, E.; López-Malo, A. Food Antimicrobial Agents Using Phenolic Compounds, Chitosan, and Related Nanoparticles. *J. Food Bioeng. Nanoprocesing* **2016**, *1* (2), 165–181.
12. Ghaderi-Ghahfarokhi, M.; Barzegar, M.; Sahari, M. A.; Azizi, M. H. Nanoencapsulation Approach to Improve Antimicrobial and Antioxidant Activity of Thyme Essential Oil in Beef Burgers During Refrigerated Storage. *Food Bioprocess Technol.* **2016**, *9* (7), 1187–1201. https://doi.org/10.1007/s11947-016-1708-z.
13. Mohammadi, A.; Hashemi, M.; Hosseini, S. M. Chitosan Nanoparticles Loaded with *Cinnamomum zeylanicum* Essential Oil Enhance the Shelf Life of Cucumber during Cold Storage. *Postharvest Biol. Technol.* **2015**, *110*, 203–213. https://doi.org/10.1016/j.postharvbio.2015.08.019.
14. Hu, J.; Wang, X.; Xiao, Z.; Bi, W. Effect of Chitosan Nanoparticles Loaded with Cinnamon Essential Oil on the Quality of Chilled Pork. *LWT - Food Sci. Technol.* **2015**, *63* (1), 519–526. https://doi.org/10.1016/j.lwt.2015.03.049.
15. Campos, E. V. R.; Proença, P. L. F.; Oliveira, J. L.; Pereira, A. E. S.; de Morais Ribeiro, L. N.; Fernandes, F. O.; Gonçalves, K. C.; Polanczyk, R. A.; Pasquoto-Stigliani, T.; Lima, R.; et al. Carvacrol and Linalool Co-Loaded in β-Cyclodextrin-Grafted Chitosan Nanoparticles as Sustainable Biopesticide Aiming Pest Control. *Sci. Rep.* **2018**, *8* (1), 7623. https://doi.org/10.1038/s41598-018-26043-x.
16. Kong, M.; Chen, X. G.; Xing, K.; Park, H. J. Antimicrobial Properties of Chitosan and Mode of Action: A State of the Art Review. *Int. J. Food Microbiol.* **2010**, *144* (1), 51–63. https://doi.org/10.1016/j.ijfoodmicro.2010.09.012.
17. Sahariah, P.; Másson, M. Antimicrobial Chitosan and Chitosan Derivatives: A Review of the Structure–Activity Relationship. *Biomacromolecules* **2017**, *18* (11), 3846–3868. https://doi.org/10.1021/acs.biomac.7b01058.
18. Perinelli, D. R.; Fagioli, L.; Campana, R.; Lam, J. K. W.; Baffone, W.; Palmieri, G. F.; Casettari, L.; Bonacucina, G.

Chitosan-Based Nanosystems and Their Exploited Antimicrobial Activity. *Eur. J. Pharm. Sci.* **2018**, *117*, 8–20. https://doi.org/10.1016/j.ejps.2018.01.046.
19. Ferreira, J. P.; Alves, D.; Neves, O.; Silva, J.; Gibbs, P. A.; Teixeira, P. C. Effects of the Components of Two Antimicrobial Emulsions on Food-Borne Pathogens. *Food Control* **2010**, *21* (3), 227–230. https://doi.org/10.1016/j.foodcont.2009.05.018.
20. Gómez-Estaca, J.; López de Lacey, A.; López-Caballero, M. E.; Gómez-Guillén, M. C.; Montero, P. Biodegradable Gelatin–Chitosan Films Incorporated with Essential Oils as Antimicrobial Agents for Fish Preservation. *Food Microbiol.* **2010**, *27* (7), 889–896. https://doi.org/10.1016/j.fm.2010.05.012.
21. Gutiérrez, L.; Escudero, A.; Batlle, R.; Nerín, C. Effect of Mixed Antimicrobial Agents and Flavors in Active Packaging Films. *J. Agric. Food Chem.* **2009**, *57* (18), 8564–8571. https://doi.org/10.1021/jf901459e.
22. Ben Arfa, A.; Preziosi-Belloy, L.; Chalier, P.; Gontard, N. Antimicrobial Paper Based on a Soy Protein Isolate or Modified Starch Coating Including Carvacrol and Cinnamaldehyde. *J. Agric. Food Chem.* **2007**, *55* (6), 2155–2162. https://doi.org/10.1021/jf0626009.
23. Parris, N.; Cooke, P. H.; Hicks, K. B. Encapsulation of Essential Oils in Zein Nanospherical Particles. *J. Agric. Food Chem.* **2005**, *53* (12), 4788–4792. https://doi.org/10.1021/jf040492p.
24. Ziani, K.; Chang, Y.; McLandsborough, L.; McClements, D. J. Influence of Surfactant Charge on Antimicrobial Efficacy of Surfactant-Stabilized Thyme Oil Nanoemulsions. *J. Agric. Food Chem.* **2011**, *59* (11), 6247–6255. https://doi.org/10.1021/jf200450m.
25. Keawchaoon, L.; Yoksan, R. Preparation, Characterization and in Vitro Release Study of Carvacrol-Loaded Chitosan Nanoparticles. *Colloids Surf. B Biointerfaces* **2011**, *84* (1), 163–171. https://doi.org/10.1016/j.colsurfb.2010.12.031.
26. Du, W.L.; Niu, S.S.; Xu, Y.L.; Xu, Z.R.; Fan, C.L. Antibacterial Activity of Chitosan Tripolyphosphate Nanoparticles Loaded with Various Metal Ions. *Carbohydr. Polym.* **2009**, *75* (3), 385–389. https://doi.org/10.1016/j.carbpol.2008.07.039.
27. Li, Q.; Liu, C.G.; Huang, Z.H.; Xue, F.F. Preparation and Characterization of Nanoparticles Based on Hydrophobic Alginate Derivative as Carriers for Sustained Release of Vitamin D_3. *J. Agric. Food Chem.* **2011**, *59* (5), 1962–1967. https://doi.org/10.1021/jf1020347.
28. Rhim, J.W.; Hong, S.I.; Park, H.M.; Ng, P. K. W. Preparation and Characterization of Chitosan-Based Nanocomposite Films with Antimicrobial Activity. *J. Agric. Food Chem.* **2006**, *54* (16), 5814–5822. https://doi.org/10.1021/jf060658h.
29. McClements, D. J. Edible Nanoemulsions: Fabrication, Properties, and Functional Performance. *Soft Matter* **2011**, *7* (6), 2297–2316. https://doi.org/10.1039/C0SM00549E.
30. McClements, D. J.; Rao, J. Food-Grade Nanoemulsions: Formulation, Fabrication, Properties, Performance, Biological Fate, and Potential Toxicity. *Crit. Rev. Food Sci. Nutr.* **2011**, *51* (4), 285–330. https://doi.org/10.1080/10408398.2011.559558.

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