RESEARCH ARTICLE

Evaluation of Chitosan Films Doped with Niosomal Sage Nanoparticles (NIS-Sag NPs) Role in Food Packaging Technology

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ABSTRACT

Natural extracts have antibacterial power that supports their use in food applications. In this work, sage extract was encapsulated into niosome nanoparticles. Then, chitosan films were doped with different concentrations (100, 200, and 300 µg) of NIS-Sag NPs. The characteristics of pure chitosan film and chitosan-doped NIS-Sag NPs films were studied using Raman spectroscopy and UV/VIS spectrophotometers. A scanning electron microscope (SEM) was used to study the surface topography and morphology of the films. In addition, mechanical properties and antibiotic sensitivity tests were investigated. The chemical properties of chitosan film doped with NIS-Sag NPs (100-300 µg) were enhanced compared to pure chitosan film, which were confirmed by Raman spectroscopy in addition to the UV absorption measurements. SEM results showed that chitosan-doped NIS-Sag NPs films have a smooth and compacted surface, which is recommended for further applications such as food packaging. The doping process was found to enhance the mechanical properties of the films as it improved the tensile strength and elasticity significantly, especially for concentrations of 300 µg. The antibiotic susceptibility test also confirmed this result. These observed improvements of chitosan film doped with 300 µg NIS-Sag NPs are potentially encouraging to adopt the results in food packaging technology.

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INTRODUCTION

It is extremely important to care for food, as it is considered the source of energy for human beings [1]. Eliminating bacteria is a central method of food preservation. There are several methods that have been used to stop the bacterial growth, such as pulsed electric field [2, 3], magnetic field (as it directly induces cellular free radicals [4, 5] that affect bacteria [6]), and other expensive ways. Recently, there has been an increased focus on researching bio-packaging with chitosan, many of which have indicated that chitosan can be utilized on a large scale in the food industry due to its antimicrobial and antioxidant properties [7]. Furthermore, using it in food packaging has the additional advantage of replacing synthetic materials [8-12]. Chitosan films enhanced with bioactive materials that have an antimicrobial and/or antioxidizing property, such as polyphenols, essential oils, oxide agents, and metal nanoparticles, demonstrate yet stronger antimicrobial and antioxidizing effects. [13-15].

In some cases, these materials may create strong physical bonds with the Chitosan matrix, leading to improved quality of the Chitosan film regarding its mechanical properties, manifested as an improved barrier effect, for example [16-20]. Moreover, tailoring of the properties of chitosan films can be achieved by incorporating nanofillers into the matrix. Examples include binding Chitosan with inversely charged polysaccharides/proteins/lipids, cross-linking with natural aldehydes, or grafting with phenolic acids [21-25]. Multiple nanofillers may be used in combination to accentuate a specific effect of Chitosan films, which broadens the applications of these biodegradable materials when it comes to preserving food quality and safety [26-30]. Amidst the carbohydrates that can be used in biopackaging, chitosan stands out in regards to food packaging films, which is due to its advantageous inherent properties. It has drawn attention to features such as being antimicrobial, antifungal, antioxidant, biocompatible, and biodegradable, in addition to forming a good barrier from the atmosphere and having exceptional film-forming properties [31]. These properties contribute, through multiple methods, to the extension of the shelf-life of packaged food [32, 33].

Food deterioration, caused mainly by microbiological growths and oxidation, results in undesired effects such as repulsive taste, color and texture changes, diminished nutritional value, and even the formation of toxic compounds [34-36]. As such, the search for food-preserving substances is ongoing, and superior compounds, such as chitosan, are desirable, as they are both more effective and natural [37, 38]. Due to their photodegradation and volatile nature, natural plant extracts can gradate or evaporate from films during drying, which decreases their effectiveness in films after drying. The encapsulation of plant extracts could maintain their usefulness for a long time, as the nanoforms control the release of the compounds [39-43].

Risaliti et al., 2019 improved Sage biopharmaceutical properties by Liposomes systems. They succeed in elevating the antioxidant, anti-inflammatory, and antibacterial activities of Sage [44]. The novelty of this work is to encapsulate sage extract in nanocarriers to maintain its usefulness and save its effectiveness for a long time.

This study aims to prepare niosome-encapsulated Sage extract and doped chitosan films with different concentrations of Sage extract loaded in niosomes to enhance their physicochemical properties. The presented films containing anti-oxidant Sage in nanoformulation could improve chitosan films as food packaging materials.

MATERIALS AND METHODS

Materials

Tween 80, cholesterol (M.W. 386.65), chitosan medium molecular weight, and ethanol were

bought from Sigma-Aldrich (Chemie, Steinheim, Germany). Acetic acid (purity ≥ 99%), phosphate buffer saline pH 7.4 at 25 °C, and were bought from Bio Shop (Mainway, Burlington, ON L7L 6A4, Canada).

Preparation methods

Preparation of niosaomal Sage nanoparticles

Niosaomal Sage nanoparticles were prepared by the thin-film hydration method. In a round bottom flask, tween 80 and cholesterol were dissolved in the ratio (2:1) in ethanol. Then ethanol evaporated under decreased pressure at 63 °C using a rotary evaporator at 50 rpm to generate a dry, thin coating. Followed by the thin film hydration with PBS (pH 7.4) with 2 mg of Sage extract (the extract was obtained from the Biochemistry Department, Faculty of Applied Medical Sciences, October 6 University, Giza, Egypt) until a final mass concentration of 0.04 mg/mL was reached. The obtained multilamellar niosomes were sonicated for 10 minutes, forming tiny vesicles. Then, a high-speed (11000 rpm x 30 min) cooling centrifuge (VS-18000M, Korea, 220 V/50 Hz) was used, and niosomes were re-suspended in PBS [45].

Characterization of niosaomal Sage nanoparticles

Transmission electron microscope (TEM)

The niosome morphology was characterized by TEM (JEOL JEM.1230, Japan). The accelerating voltage is 100 kV. Niosomes encapsulating Sage extract were negatively stained by phosphotungstic acid and incubated for five minutes on a carbon-coated grid before analysis.

Preparation of chitosan- doped NIS-Sag

Chitosan films were prepared by dissolving an adequate amount of chitosan powder in an acetic acid solution (1% v/v). The chitosan solution was placed on a glass plate to be dried as a control film. The chitosan solution doped with three different concentrations of NIS-Sag NPs (100, 200, and 300 µg) was poured into three glass plates and left to dry at room temperature [45].

Samples characterization

Raman spectroscopy

Raman spectra for both control and doped chitosan films with different concentrations of NIS-Sag NPs (100-300 µg) were obtained using a Thermo Scientific DXRxi Raman spectrometer with an optical microscope.

Scanning Electron Microscope (SEM)

Both control and doped chitosan films with nanoparticles were examined under a field emission scanning electron microscope (JEOL JSM-5510, Tokyo, Japan) with an acceleration voltage of 30 kV.

Light transmission

The light transmission properties of control and doped chitosan films with different concentrations of NIS-Sag NPs were recorded using a UV/VIS spectrophotometer (Shimadzu, Japan).

The mechanical properties

The mechanical properties of control and doped chitosan films with various concentrations of NIS-Sag NPs were assessed using a tensile testing instrument (Z010, Zwick Roell, Germany). The tensile strength (TS), elongation at break (E%), and elastic modulus (EM) of each film were measured in triplicate.

Antibiotic Susceptibility Test

The bacterial strain used in this study is *E. coli* ATCC 25922, which was obtained from Cairo Mercin, Faculty of Agriculture, Ain Shams University. Bacterial suspensions were spread on Müller-Hinton agar plates. Both the control and doped chitosan films with different concentrations of niosomal Sage nanoparticles (100-300 µg) were inoculated in Mueller-Hinton agar plates and then incubated at 37 °C for 24 hrs. The mean diameter of each inhibition zone for each concentration was measured and compared to the control chitosan film zone in each plate. The susceptibility of *E. coli* to the films was determined using the disc diffusion method, which was carried out according to the procedure outlined by the National Committee for Clinical Laboratory Standards

(NCCLS). Three replicate agar plates were used, according to the Clinical and Laboratory Standard Institute (CLSI) guidelines [46].

Statistical analysis

Data comparisons were performed using one-way analysis of variance (ANOVA) and the Duncan test using SPSS 17.0 software ($p \leq 0.05$). The mean ± standard deviation (SD) was applied for all data representations, knowing that any measurements were done in triplicate for all samples [15].

RESULTS AND DISCUSSION

TEM

The image obtained by TEM showed nearly homogeneous and spherical NIS-Sag NPs with an average particle size of 24.39 nm (Figure 1).

Raman spectroscopy

Raman spectroscopy was used to verify the interactions among the NIS-Sag NPs and chitosan films. Figure 2 shows both control and NIS-Sag DPs-doped chitosan films with Raman spectra in the region of 200–3200 cm^{-1}. The characteristic peaks of chitosan are the band at about 3050 cm^{-1} assigned to -O-H stretching hydrogen bonds; the peak at 2887 corresponds to -C-H asymmetric and symmetric stretching vibrations; and the peak at 2365 cm^{-1} represents -N-H stretching vibrations [47,48]. The depression behavior in the band 3050 cm^{-1} might be connected to the decrease in water content in the NIS-Sag NPs-doped chitosan films due to the higher concentration of NIS-Sag NPs (200 and 300 μg) [49]. Furthermore, the intensity of the peak at 2887 cm^{-1}, which is due to CH_2 stretching in chitosan and Sage, decreased with a shift to lower wavenumbers for the highest level of NIS-Sag NPs (300 μg), as the stronger strength of CH_2 stretching bond leads to its shift to a higher frequency, which proves the existence of interactions among the film components.

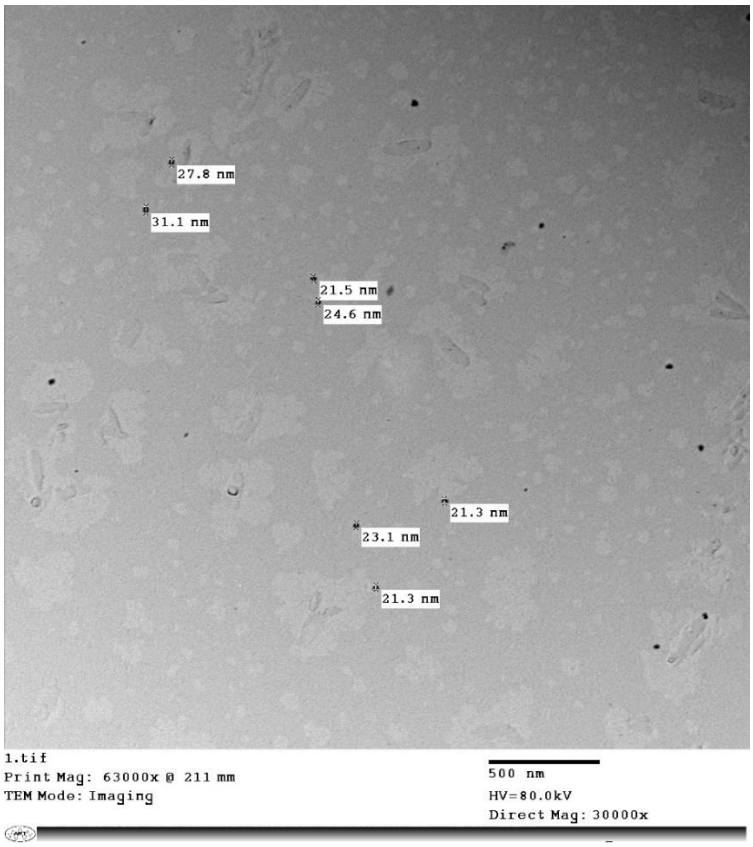

Figure 1.TEM image of the NIS-Sag NPs.

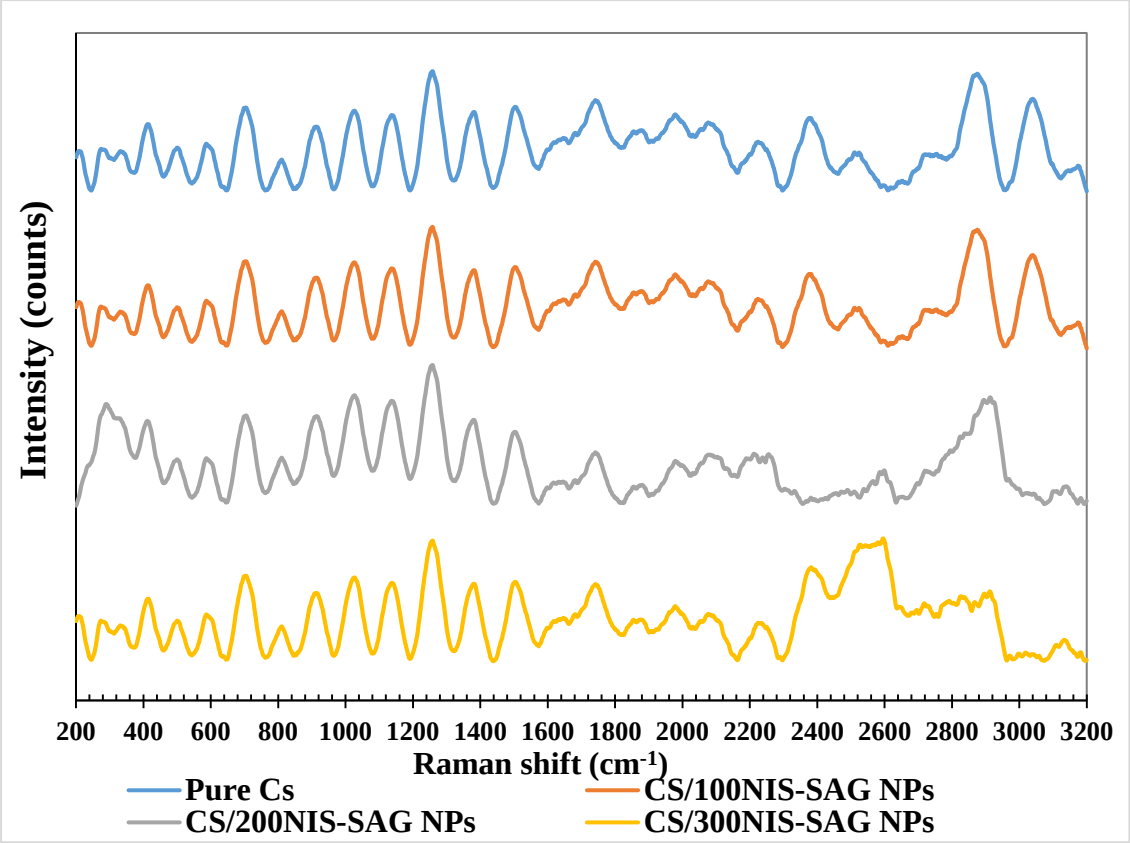

Figure 2. Raman spectra (a) of control CS, CS/100NIS-Sag NPs, CS/200NIS-Sag NPs, and CS/300NIS-Sag NPs films in 200–3200 cm^{-1}. The shaded parts indicate bands with changes.

Scanning Electron Microscope (SEM)

Figure 3 shows the surface characteristics of both control and doped NIS-Sag NPs chitosan films. It has been observed that the surface morphology of pure chitosan is smooth and compact, without any cracks or pores. Also there are tiny particles on the surface. This agrees with the results of other similar studies [50, 51]. Nevertheless, the doped films exhibited nearly the same forms, with heterogeneous forms for the CS/200NIS-Sag NPs film. The nearly similar surface shapes revealed from SEM micrographs indicate the incorporation of NIS-Sag NPs into chitosan films [52]. The optical microscopy images showed a difference between the films, as with increasing Sage concentration, the shape of the film changed, and the difference was significant in the CS/300NIS-Sag NPs film. Optical microscope images also reflect a large scale, which provides a complete picture of the films. While SEM probes at the nanoscale, it only detects minor changes.

UV Spectrophotometry

The optical transmittance percentage of control chitosan and NIS-Sag NPs-doped chitosan films was measured over the visible spectrum range (Figure 4). The addition of NIS-Sag to the chitosan film decreased its transparency. At 700 nm, the transmittance rate was found to be 88.2% for the control chitosan film, 80.2% for the CS/100NIS-Sag NPs film, 80.1% for the CS/200NIS-Sag NPs film, and 76.1% for the CS/300NIS-Sag NPs film. The pigments in sage extract may be the cause of the gradual decrease in the doping film transparency rate. The spectrophotometry analysis reinforces those results of the optical microscope and SEM, which showed a decrease in optical transmittance with increasing concentrations, although the difference between NIS-

Sag 100 and 200 was small, especially at higher wavelengths.

Mechanical properties

Tensile strength, elongation at break, and elastic modulus were measured to determine the mechanical characteristics of the control and chitosan films doped with 100-300 μg NIS-Sag NPs (Table 1). Tensile strength was clearly decreased for CS/100NIS-Sag NPs and CS/200NIS-Sag NPs compared to the control chitosan film, whereas there was a significant increase in the tensile strength for CS/300NIS-Sag NPs film. The elastic modulus for all doped films displayed an increase in elastic modulus when compared with the control one. The CS/100NIS-Sag NPs and CS/300NIS-Sag NPs films showed a more significant increase in elastic modulus. Usually, films with good tensile strength possess poor elongation at break [53]. Chitosan is, without doubt, one of the main biopolymers that constitute the new generation of active food packaging, performing interesting functions in the preservation of foods [54-56]. The elongation at break decreased significantly in the CS/100NIS-Sag NPs film and was elevated for CS/200NIS-Sag NPs and CS/300NIS-Sag NPs compared to the control one.

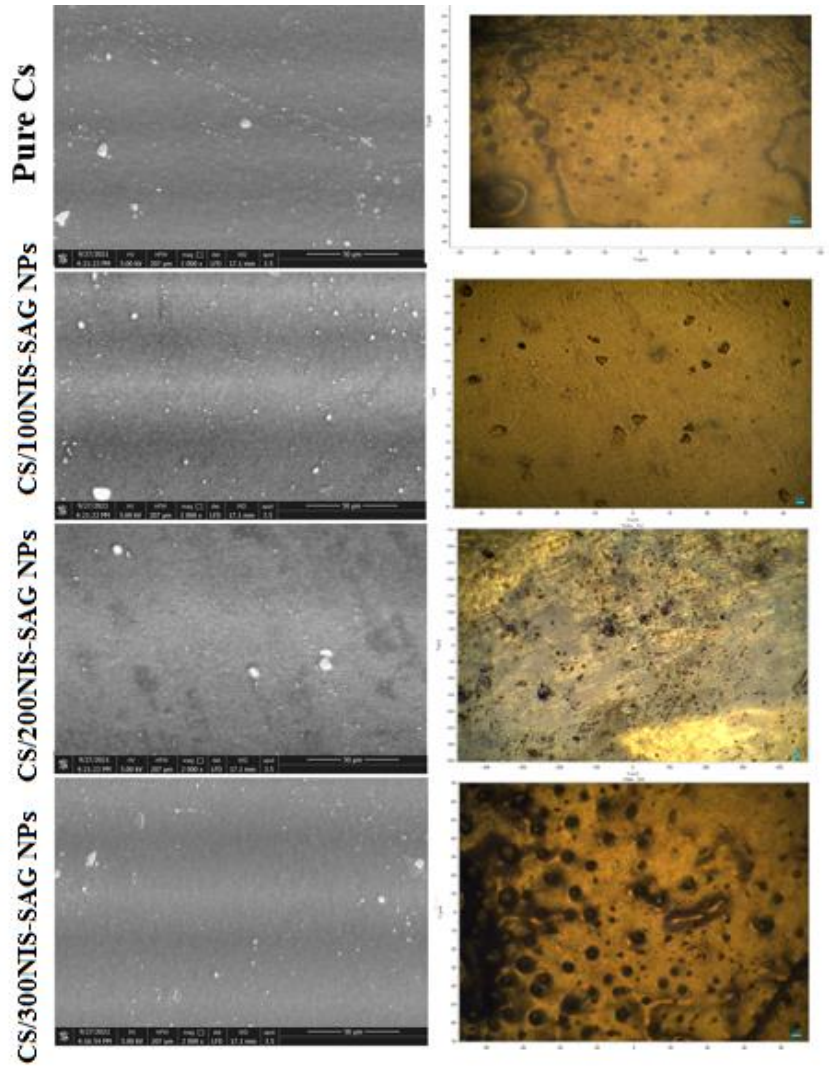

Figure 3. The SEM images (left column) and light microscope photos magnification power × 400 (right column) of: control film and chitosan film doped with NIS-Sag NPs (100 μg) (CS/100NIS-Sag NPs), NIS-Sag NPs (200 μg) (CS/200NIS-Sag NPs) and NIS-Sag NPs (300 μg) (CS/300NIS-SAG).

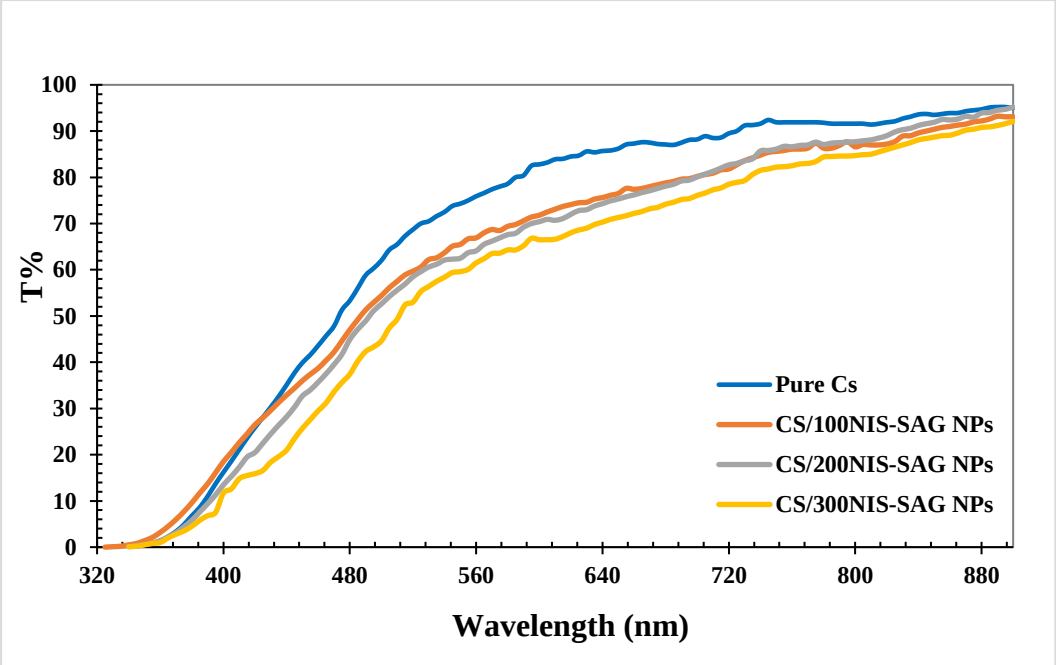

Figure 4. Optical transmittance of pure CS, CS/100NIS-Sag NPs, CS/200NIS-Sag NPs, and CS/300NIS-Sag NPs films in 320–900 nm.

Table 1. Mechanical properties of the chitosan films: TS (tensile strength, MPa), EM (Elastic modulus, MPa), and E% (Elongation at break, %). Values are the mean ± standard deviation (n = 3). The superscript letters (a, b and c) denote significant differences among the films ($p < 0.05$).

Films	TS (MPa)	EM (MPa)	E (%)	Thickness (µm)
Control chitosan	203.465^{a}	97.57^{a}	18.425^{a}	143
CS/100NIS-Sag NPs	88.15^{b}	193.35^{b}	9.1^{b}	134
CS/200NIS-Sag NPs	92.285^{c}	102.91^{c}	20.56^{c}	138
CS/300NIS-Sag NPs	401.885^{d}	217.23^{d}	20.01^{c}	138

Antibiotic Susceptibility Test

A clear inhibition zone is observed around the doped chitosan films with NIS-Sag NPs (100-300 µg), but with variation among the different concentrations, where the CS/300NIS-Sag NPs recorded the highest mean diameter value, as shown in Table 2 and Figure 5 (a, b, and c). The results revealed a significant difference between control and doped chitosan films with NIS-Sag (100 and 200 µg), where P <0.001. The zones of inhibition are 18.4 ± 0.1, 31.3 ± 0.12, and 32 ± 0.15 mm for Sage concentrations of 100, 200, and 300 µg, respectively, although the increase in bacterial inhibiting capacity becomes nearly saturated at Sage concentrations of 300 µg.

The chitosan structure has two reactive groups, which are the free amine groups and the hydroxyl groups. Modification of such groups in chitosan enhances its mechanical properties, resulting in an increase in its applications. Chitosan comes out of key interest for the development of films and, in particular, active packaging for foods or pharmaceuticals, due to its intrinsic antimicrobial,

antifungal [57], and antioxidant properties, good biodegradability and biocompatibility, good oxygen and carbon dioxide barrier, and excellent film-forming properties [58–61].

This study revealed that niosome-encapsulated Sage is an important factor in softening the chitosan film surface, enhancing the film's mechanical properties, and decreasing microbial growth.

Table 2. The mean inhibition zone diameter (mm) of chitosan films doped with different concentrations of NIS-Sag (100-300 µg) and control chitosan film on E. coli. The superscript letters (a,b,c,d) denote significant differences among the films ($p < 0.05$).

Films	Mean inhibition zone diameter (mm)
Control chitosan	10 ± 0.1^{a}
CS/100NIS-Sag NPs	18.4 ± 0.1^{b}
CS/200NIS-Sag NPs	31.3 ± 0.12^{c}
CS/300NIS-Sag NPs	$32 \pm 0.15^{c,d}$

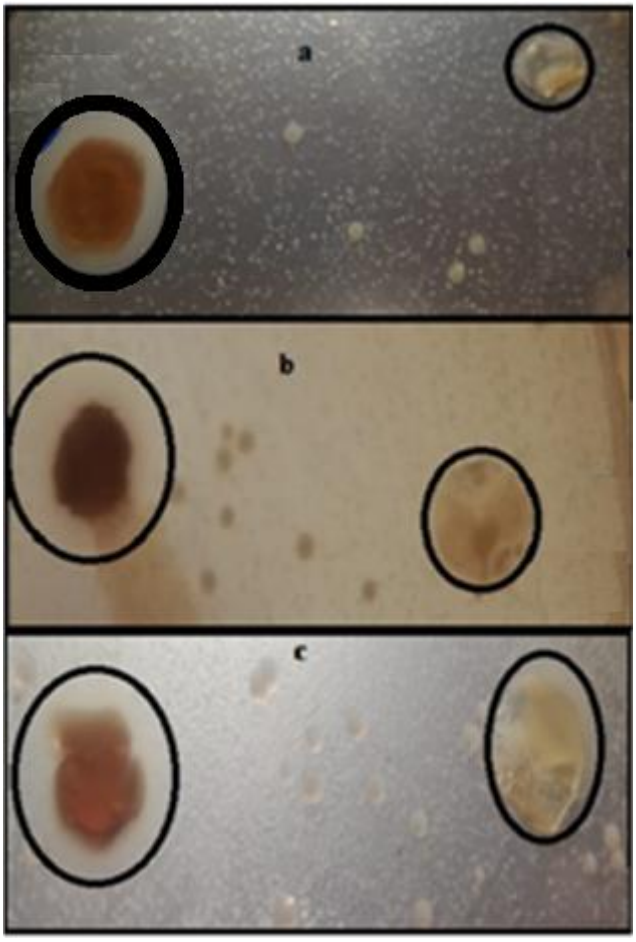

Figure 5. (a, b, and c) shows the inhibition zone of chitosan films doped with different concentrations of CS/100NIS-Sag NPs, CS/200NIS-Sag NPs, and CS/300NIS-Sag NPs films, respectively, on the left side and the control film of chitosan on the right side of each dish.

CONCLUSION

The incorporation of antimicrobial Sage extract into chitosan films was carried out in an effective way to save the extract during the film drying. Niosomes were used to encapsulate the extract. The addition of nisomal sage extract to the chitosan films enhanced their mechanical and antibacterial properties. Our results suggest enhanced niosomal Sage chitosan films for the food packaging process.

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