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ENGINEERED CHITOSAN NANOPARTICLES FOR ENCAPSULATION OF THYMOL

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ABSTRACT
This study successfully obtained chitosan thymol nanoparticles using an electrohydrodynamic technique, which is a simple one-step procedure. The morphological and physical characterization, antioxidant, and antimicrobial activity assessments of electrosprayed thymol-loaded chitosan nanoparticles (CTNPs) were carried out. The ABTS assay and the agar well diffusion test were used to determine the antioxidant and antimicrobial activities of the CTNP samples, respectively. The results showed that CTNPs possessed efficient antimicrobial capacity against *B. cereus, S. aureus, E. coli*, and *S.* typhimurium. CTNPs indicated a radical scavenging activity of 90% regarding the ABTS assay. CTNPs with biological activities could be an effective alternative for practical food safety and health applications. In this study, the use of electrohydrodynamic atomization technique to produce biopolymer nanoparticles present a novel approach for encapsulating thymol-like volatile active agents.
Keywords: Chitosan, thymol, electrohydrodynamic technique, nanoparticles, food safety, nutrition

TİMOL ENKAPSÜLASYONU İÇİN TASARLANMIŞ KİTOSAN NANOPARTİKÜLLER

ÖZ
Bu çalışmada, kitosan timol nanopartikülleri elektrohidrodinamik teknik kullanılarak tek adımlı, basit bir prosedür ile başarı ile elde edilmiştir. Elektrosprey timol yüklü kitosan nanopartiküllerin (KTNP'ler) morfolojik ve fiziksel karakterizasyonu, antioksidan ve antimikrobiyal aktivite değerlendirmeleri gerçekleştirilmiştir. KTNP örneklerinin antioksidan ve antimikrobiyal aktivitelerini belirlemek için sırasıyla ABTS yöntemi ve agar well difüzyon testi kullanılmıştır. Analiz sonuçları, KTNP'lerin *B. cereus, S. aureus, E. coli* ve *S.* typhimurium'a karşı etkili antimikrobiyal aktiviteye sahip olduğunu göstermiştir. KTNP'ler ABTS yöntemine göre % 90 radikal süpürme aktiviteye sahip oldukları görülmüştür. Biyolojik aktiviteye sahip KTNP'lerin gıda güveliği ve sağlık alanındaki pratik uygulamalarda etkili bir alternatif olabileceği görülmüştür. Bu çalışmada, biyopolimer nanopartiküller üretmek için elektrohidrodinamik atomizasyon tekniğinin kullanılması, timol benzeri uçucu aktif bileşenlerin enkapsülasyonu için yeni bir yaklaşım sunmaktadır.
Anahtar kelimeler: Kitosan, timol, elektrohidrodinamik teknik, nanopartikül, gıda güvenliği, beslenme

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INTRODUCTION

Thymol (2-isopropyl-5-methylphenol) is a natural monoterpenoid phenol and the principal component of the thyme (*Thymus vulgaris*) essential oil (Pirbalouti et al., 2014; da Rosa et al., 2015; Escobar et al., 2020). Thyme oil and thymol are categorized by the U.S Food and Drug Administration as generally regarded as safe (GRAS) (Cohen et al., 2021; U.S. Food and Drug Administration, 2024). The investigations on thymol's potential as a food preservative and neutraceutical are continuously increasing due to the numerous functions of thymol, including antifungal (Zhao et al., 2023), antibacterial (Echazú et al., 2017), antioxidant (Doost et al., 2019), antiviral (Nandi and Khanna, 2022), anti-inflamatuar (Sheorain et al., 2019) and anticancer (Qoorchi Moheb Seraj et al., 2022) effects. For instance, volatile organic compounds such as thymol can provide safer natural alternatives to synthetic ones to the preservation of food products (Viacava et al., 2018; Sepahvand et al., 2022). However, its hydrophobic nature, oxidation susceptibility, high volatility, poor dispersion, unpleasant smell, and insolubility of thymol restrict its ability for industrial applications (Pan et al., 2022; Sharma et al., 2023; Zhao et al., 2023). The chemical instability of thymol is the main challenge for long-term applications of it as a preservative in food systems (da Rosa et al., 2015). However, innovative encapsulation strategies may overcome these limitations. Micro-/nano- particle encapsulation is an emerging approach for protecting the various bioactive compounds from harsh environmental conditions and increasing their bioavailability (Bazana et al., 2019; Gao et al. 2021). To enhance the bioavalibity and functional characteristics of thymol, it can be encapsulated within a variety of carrier matrices through different methodologies, including, nanoencapsulation with sodium casein via high shear homogenization (Pan et al., 2014), cyclodextrin inclusion complex via electrospinning (Aytac et al., 2017), loading nanoparticulated form in chitosan-quinoa films (Medina et al., 2019), nanoparticle formation with ethylcellulose/methylcellulose (Wattanasatcha et al., 2012), encasing in caseinate-stabilized nanosuspensions (Zhou et al., 2021), inclusion of γ-cyclodextrin metal-organic framework (MOF)(Pan et al., 2022), integration of starch-based inclusion complexes (Zhou and Kong, 2023), loading in nanoemulsions (Saatkamp et al., 2023), entrapment in chitosan-Aloe vera films (Sharma et al., 2023), encapsulating in zein-gum arabic stabilized Pickering emulsions (Li et al., 2018), nanoliposome and solid lipid nanoparticles (Zabihi et al., 2023), administration in nanoemulsion and nanostructured lipid carriers in alginate-based edible films (Talesh et al., 2024).

Chitosan is an abundant, commercially available, unique cationic natural linear polysaccharide obtained from the crustacean exoskeletons, insects, and algae, and it can also be extracted from the cell walls of fungi (Kumar et al., 2004; Mourya and Inamdar 2008; Aranaz et al., 2021). Chitosan is compatible with living organisms and does not cause adverse responses in human cells (Kumar et al., 2004). Chitosan is commonly used in different fields as a dietary supplement (Moraru et al., 2018), food packaging (Amaregouda et al., 2023), and biomedicine (Wang et al., 2020). The carrier matrix is essential for developing the encapsulation systems (Pan et al. , 2022; Cheng et al., 2023). Previous studies have reported the effectiveness of chitosan as a carrier for thymol in different delivering systems such as thymol chitosan nanoemulsions (Liu and Liu 2020), thymol chitosan hydrogels (Echazú et al., 2017), chitosan thymol nanoparticles by ionic crosslinking method (Zhao et al. 2023), ionic gelation method (Medina et al. 2019; Çakır et al., 2020), chitosan encapsulated thymol nano gels (Piri-Gharaghie et al., 2022), thymol loaded chitosan nanoparticles by emulsification (Guo et al., 2022), formation of a chitosan-gelatin copolymer matrix for the nanoencapsulation of thymol (Ojeda-Piedra et al., 2023) and delivering of thymol in chitosan-aloe vera films (Sharma et al., 2023).

Engineered nanoparticles are targeted specific carriers for food additives without disturbing the physicochemical capabilities of delivered compounds (Arserim-Uçar, 2020; Arserim-Uçar and Çabuk, 2020; Sahani and Sharma, 2021).

Spray drying, emusification, evaporation, and coacervation are the most common encapsulation techniques used to produce bioactive agent-loaded micro/nanoparticles. These techniques require harsh chemicals and heating that are not suitable for heat sensitive compounds. However, electrospraying technique does not require any extreme temperature, pressure, and organic solvent for manufacturing functional micro/nano particles (Niu et al. 2020).

Currently, electrohydrodynamic techniques like electrospray have become promising methods for producing micro/nanoparticles in a single step at ambient temperatures (Chakraborty et al., 2009; Gómez-Mascaraque et al., 2017; Arserim-Uçar, 2021; Arserim-Uçar, 2022). Chitosan nanoparticles fabricated through electrospraying have been reported previously. The researchers produced ampicillin-loaded chitosan micro/nanoparticles with a particle diameter of 520 nm, prepared using 90 % acetic acid (v/v) and 2% chitosan (w/v) (Arya et al., 2008). The chitosan particles varied in size, reaching approximately 124 nm when 30% acetic acid (v/v), 10% chitosan (w/v), and 30% etanol (v/v) were used (Zhang and Kawakami 2010).

A recent study aiming to find the smallest particle size for chitosan nanoparticles used different concentrations (0.1, 0.2, and 0.35% w/v) of high molecular weight chitosan and dissolved in 50% acetic acid and resulted in desirable nanoparticles with the particle size varied from 105 to 170 nm (Abyadeh et al., 2017). Although various studies are reporting electrospray chitosan nanoparticles carrying several ingredients, there has been a lack of research addressing the development of electrosprayed chitosan nanoparticles encapsulating thymol. The present study aims to develop a green, one-step method for encapsulating thymol in chitosan polymer via electrospraying technique and subsequently characterizing the developed CTNPs. The antioxidant and antimicrobial properties of the obtained nanoparticles will be assessed to determine their potential use for nutrition, food safety, and biomedical purposes.

MATERIALS AND METHODS

Materials

Low molecular weight chitosan, thymol, and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Acetic acid, ethanol, and hydrochloric acid (%37) were supplied from Isolab (Wertheim, Germany).

Preparation of Electrospraying Solution

Electrosprayed chitosan nanoparticles were obtained using the slightly modified method previously reported by Zhang and Kawakami (2010) and Gómez-Mascaraque et al. (2016). Briefly, 2.5 % (w/v) chitosan was dissolved in a 30 % acetic acid solution (v/v) and stirred overnight for complete dissolution. Thymol was dissolved in ethanol and added to the chitosan solutions at the concentrations of 0.25, 0.5, and 1 % (v/w), a sample without thymol used as control (Table 1), and final ethanol concentration in all chitosan formulations was 30 % (v/v).

Table 1. Electrosprayed chitosan nanoparticles

Samples	Coded
Chitosan solution with 0.25 % (v/w) thymol	CH-TH1
Chitosan solution with 0.5 % (v/w) thymol	CH-TH2
Chitosan solution with 1 % (v/w) thymol	CH-TH3
Chitosan solution without thymol	CH-C

Electrospraying

The electrospraying process was achieved with electrospinning equipment (OptoSense, Tekno-TIP, Türkiye) containing a syringe pump and a high-voltage power supply to spray a chitosan solution for spraying through the drum collector. Based on the serial preliminary experiments, electrospraying parameters were set as follows.

The applied voltage was 18 kV, the flow rate ranged from 0.2 to 0.3 ml/h, and the distance between the syringe and the collector was 12 cm.

Characterizations of Nanoparticles

Morphology and the structure of CTNPs were investigated by scanning electron microscopy (SEM) (Fei Quanta 250 Feg, USA). Samples were placed on conductive two-sided carbon tapes and coated with gold. Thermogravimetric analysis (TGA) and differential termal analysis (DTA) was performed using a Simultaneous Thermal Analyzer (STA 600, Perkin Elmer). Thermograms of the nanoparticles were obtained in the 30-700 °C range using a heating rate of 10 °C min^{-1} and nitrogen gas flow rate of 20 mL min^{-1}. Fourier transform infrared (FTIR) characterization of nanoparticles was carried out with a PerkinElmer Spectrum 400 FTIR spectrometer (Waltham, Massachusetts, USA). The spectrum was recorded within the wavenumber range from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1} at the scan of 64.

Encapsulation Efficiency (EE%) of Nanoparticles

The encapsulation efficiency of the CTNPs was determined according to the slightly modified previous method (Çakır et al., 2020; Zhao et al., 2023). Briefly, 20 mg CTNPs were dissolved in 5 ml of HCI solution (1 M) and heated at 75 °C for 30 min. followed 1 ml ethanol added in the mixture. The mixture was centrifuged at 4500 rpm for 10 min (Hettich, UNIVERSAL 320, Germany) to obtain the CTNPs supernatant, which was then used to determine the encapsulation efficiency. To achieve this, the amount of thymol in the CTNPs was estimated by using a calibration curve of pure thymol in HCI: Ethanol mixture at a wavelength of 275 nm with R^2 of 0.99 (y=0.0016x-0.052) via the Beer-Lambert law. The encapsulation efficiency (%) was calculated from Eqs(1) (Çakır et al., 2020; Pan et al., 2022; Zhao et al., 2023).

$$EE\ (\%) = Weight\ of\ encapsulated\ Thmol / Total\ concentration\ of\ Thymol\ X\ 100 \quad \text{Eq(1)}$$

Antioxidant Activity of Nanoparticles

ABTS (2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical-scavenging activity test was employed to measure the antioxidant capacity of the obtained CTNPs. Briefly, CTNP samples (0.1 to 0.6 mg) were dissolved in HCI solution (1 M) and heated at 75 °C for 30 min. This was followed by adding ethanol to the mixture, and pH was adjusted to the range of ABTS solution. After vortexing, the mixture was centrifuged at 4500 rpm for 10 min (Hettich, UNIVERSAL 320, Germany) to obtain CTNPs supernatant which was then used for antioxidant activity. This CTNP supernatant was used for antioxidant activity. The $ABTS^{\cdot +}$ solution was prepared by mixing 7 mM ABTS radical solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) for 16 h in the dark (Re et al., 1999; Ojeda-Piedra et al., 2023). Absorbance was measured at 734 nm. ABTS scavenging activities were calculated using Eq(2).

$$Antioxidant\ activity\ (\%) = (A\ Control - A\ Sample)/A\ Control\ X\ 100 \quad \text{Eq(2)}$$

Antibacterial Activity of Nanoparticles

The antibacterial activity of CTNPs was tested against *Bacillus cereus* NRRL B-3711, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 using agar well-diffusion assay, described by da Rosa et al. (2015) with slight modifications. Freshly prepared microbial culture of each bacterial strain with a load of McFarland 0.5 (approximately 10^8 CFU/mL) was spread on the Mueller-Hinton agar (MHA) (Oxoid, UK). The solution containing 100 μL of dissolved chitosan thymol-loaded powdered nanoparticles was added into the 8 mm diameter wells. Chitosan nanoparticles without thymol were used as a control. Petris dishes were incubated at 37 °C for 24 h. The antibacterial activity was performed using three replicates by measuring the diameter of the zone of inhibition (mm) around the wells.

Statistical Analysis

The statistical analysis of this research result was conducted using a one-way analysis of variance (ANOVA) using Tukey's comparison test using the Minitab 17 software version. A significance level of $p<0.05$ was used. The data were presented with mean ± standard deviation.

RESULTS AND DISCUSSION

Characterizations of Nanoparticles

Scanning Electron Microscopy Analysis

SEM was utilized to examine the morphology of developed nanoparticles using the electrospray method. SEM images provide information about the morphology, structure, and size of the obtained nanoparticles. Figure 1 illustrates the chitosan electrosprayed nanoparticles with thymol that appeared as a sphere with granular structures and smoother surfaces, although less homogeneous in size**.** Thymol-free chitosan nanoparticles (CH-C) exhibited a spherical shape with an average diameter of 154.88±106.44 nm. Diameters of the obtained CTNPs with thymol content of 0.25, 0.5, and 1 % (v/w) were 121.26±111.07 nm, 118.54±76.87 nm, and 139.66±94.69 nm, respectively. A recent study presented electrosprayed thymol-loaded alginate microparticles in spherical and ellipsoidal shape with an average particle diameter of 597 μm (Ahmady et al., 2023). As reported in the study of Gómez-Mascaraque et al. (2016) (-)-epigallocatechin gallate-loaded chitosan particles were not homogeneous in size. Our results are consistent with the findings of the relevant literature. Notably, the concentration of the chitosan and operating parameters significantly affected the particle shape, size, surface characteristics of particles, encapsulation efficiency, and the release of the active agent (Zhang and Kawakami, 2010; Gómez-Mascaraque et al., 2016; Abyadeh et al., 2017) .

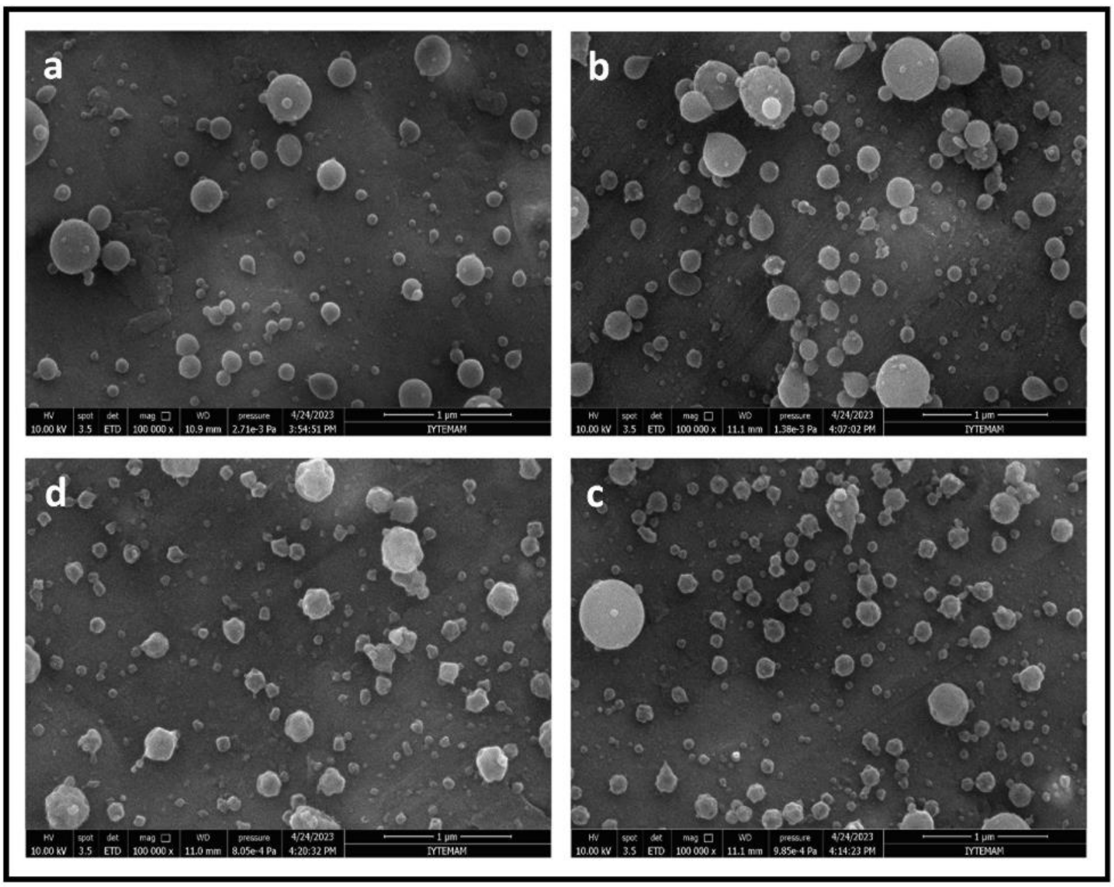

Figure 1. SEM images of (a) Thymol free chitosan nanoparticles (CH-C), thymol-loaded chitosan nanoparticles; (b) CH-TH1, (c) CH-TH2, (d) CH-TH3

Thermogravimetric Analysis (TGA)

TGA and DTA were used to determine the weight loss of CTNP samples based on temperature. TGA and DTA curves of different CTNPs, pure thymol, chitosan, and chitosan control samples, were shown in Fig.2. Pure chitosan (CH) had higher thermal stability than the electrosprayed chitosan sample without thymol (CH-C). Electrosprayed chitosan nanoparticles containing thymol (CH-TH1, CH-TH2, CH-TH3) exhibited a greater decomposition temperature than chitosan samples. The thermal stability of thymol improved by encapsulating it in chitosan using the electrospraying method, as observed in the TGA/DTA analysis. TGA curves of the samples revealed the successful encapsulation of thymol into the electrosprayed chitosan nanoparticles. These results are in agreement with the findings of the Wang et al. (2022) and Baldassarre et al. (2023).

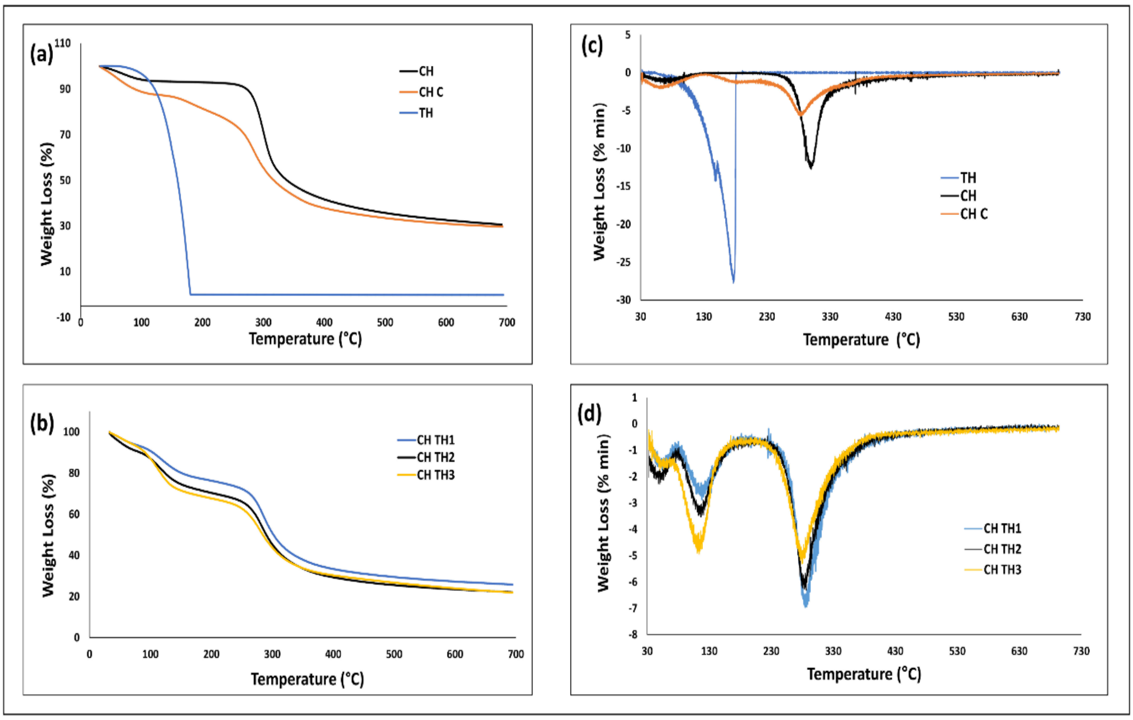

Figure 2. TGA (a) Thymol(TH) , pure chitosan(CH), chitosan nanoparticles(CH-C), (b) thymol-loaded chitosan nanoparticles(CH-TH1, CH-TH2, CH-TH3) and DTA thermograms of (c) thymol(TH), pure chitosan(CH), chitosan nanoparticles(CH-C), (d) thymol-loaded chitosan nanoparticles(CH-TH1, CH-TH2, CH-TH3)

The initial weight loss between 30 and 120 °C was the evaporation of the residual solvents, also known as the vaporization process (Zhu et al., 2024). Thymol is a volatile chemical, and pure thymol is evaporated and degraded before 200 °C (da Rosa et al., 2015; Baldassarre et al., 2023; Zhang et al., 2023). The second weight loss of approximately 30% was observed in the temperature range 225-400 °C (Fig.2) due to the thymol degradation (Xu et al., 2023) and breaking of the molecular chain of chitosan (Gu et al., 2019; Wang et al., 2022).

Fourier Transform Infrared Spectroscopy (FT-IR)

The spectra of electrosprayed thymol-loaded chitosan nanoparticles (CH-TH1, CH-TH2, CH-TH3), chitosan control nanoparticles (CH-C), pure thymol (TH) and chitosan powder (CH) are shown in Fig.3. These samples exhibited the characteristic FT-IR spectra of chitosan absorption bands at 3300 and 3400 cm^{-1} (O-H and N-H stretching), 3000 and 2800 cm^{-1} (C-H stretching) (Sutharsan et al., 2023), 1638-1655 cm^{-1} amide I and 1542-1560 cm^{-1} (N-H bending from amide group), 1558 cm^{-1} amide II (N-H bending), 1405 cm^{-1} ($-CH_2$ bending), 1378-1380 cm^{-1} ($-CH_3$ symmetrical deformation), 1382 cm^{-1} amide III (C-N stretching), 1150-1040 cm^{-1} (C-O-C stretching) in glycosidic linkages and 1021-1024 cm^{-1} (skeletal vibration of C-O stretching) (Mucha and Pawlak, 2002; Lawrie et al., 2007; Songsurang et al., 2011; Leceta et al.,2013). The characteristic spectra of thymol existing in the 1250-1750 cm^{-1} region, peaks in this region attributed to the phenolic groups of thymol C=C stretching, -OH bending, and C-O stretching (Celebioglu et al., 2018), peaks at 3080 cm^{-1} attributed to the phenolic hydroxyl, and peaks around 2800-3200 cm^{-1} represent the methyl absorption (Zhao et al., 2023). In the spectrum of the thymol, the phenol ring is responsible for the peaks within the range of 1621 and 1459 cm^{-1} (Milovanovic et al., 2016). All the electrosprayed CTNPs had the characteristic absorption peaks of chitosan and thymol (Fig. 3). FT-IR spectroscopy confirmed the interaction between the chitosan polymer and thymol. These findings revealed a successful encapsulation of thymol into chitosan electrosprayed nanoparticles.

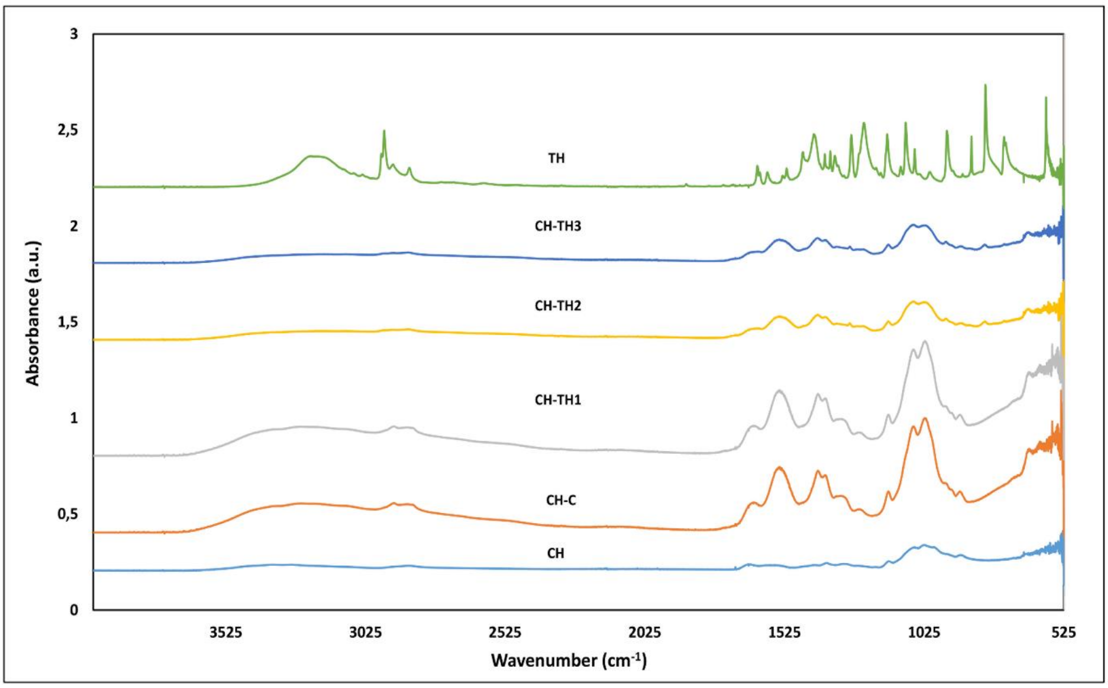

Figure 3. FTIR spectra of thymol(TH), pure chitosan(CH), chitosan nanoparticles(CH-C), and thymol-loaded chitosan nanoparticles(CH-TH1, CH-TH2, CH-TH3)

Encapsulation Efficiency of Thymol-Loaded Nanoparticles

The encapsulation efficiency (EE %) of electrosprayed thymol-loaded chitosan nanoparticles (CTNPs: CH-TH1, CH-TH2, CH-TH3) are shown in Table 2. The EE % of CTNPs ranged from 29.38 to 42.19%. These results are consistent with previous studies. As reported by Wattanasatcha et al. (2012)*,* the loading capacity of thymol in ethylcellulose/methylcellulose nanospheres was 43.53%. Similarly, the encapsulation efficiency was calculated at 41.92% for chitosan thymol nanoparticles produced using an ionic crosslinking approach (Zhao et al., 2023). The low encapsulation efficiency of thymol may be due to the volatile nature of the thymol, besides the presence of thymol on the outer layer of chitosan electrosprayed nanoparticles can cause the evaporation of the thymol (Liu et al. 2021). However, alginate thymol microparticles produced by the electrospraying method achieved a high encapsulation efficiency of 88.9 % (Ahmady et al., 2023). Another study found that employing a coaxial electrospray technique within a core-shell of zein and shellac enhanced the encapsulation efficiency of thymol to 81.34%. Also, a higher concentration of thymol leads to a reduction in the homogeneity of particle size (Liu et al., 2021). Encapsulation efficiency is dependent on the physical interaction between thymol and the functional groups of the carrier polymer and the methodology used to produce nanoparticles (Sheorain et al., 2019; Niu et al. 2020; Wang et al., 2022; Ahmady et al., 2023).

Table 2. Encapsulation efficiency of electrosprayed thymol-loaded chitosan nanoparticles

CTNPs	Encapsulation Efficiency (%)
CH-TH1	29.38± 0.011[c]
CH-TH2	41.58± 0.041[b]
CH-TH3	42.19± 0.046[a]

[a,b,c;] Different letters in the same column show a statistically significant difference ($p<0.05$).
All values are means ± SD, n=3

Antioxidant Activity of Nanoparticles

The results in Fig 4. illustrate the antioxidant activity of the CNTPs determined by ABTS method on the basis of the free radical scavenging activities.

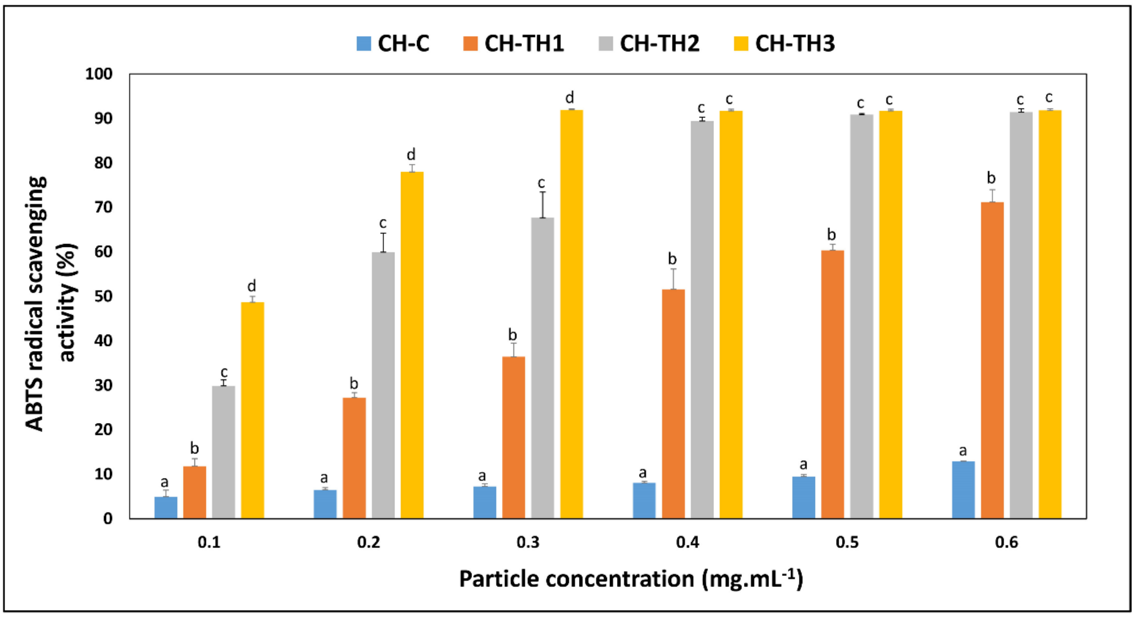

Figure 4. ABTS radical scavenging activity of chitosan nanoparticles (mg. mL^{-1})(CH-C) and thymol-loaded chitosan nanoparticles(CH-TH1, CH-TH2, CH-TH3)

The findings revealed that the CTNPs displayed a statistically significant antioxidant activity (*p<0.05*) (Fig 4). As demonstrated by the ABTS experiment, the antioxidant activity of nanoparticles increased with an increasing thymol concentration. Thymol possesses antioxidant activity due to its phenolic structure (Sheorain et al., 2019). Also, as reported in the literature, low molecular weight chitosans (<3 kDa) have antioxidant activity (Tomida et al., 2009). In the study of Sheorain et al. (2019), a DPPH assay was used to predict the radical scavenging activity of thymol-loaded tragacanth gum-chitosan nanoparticles, and antioxidant activity was increased with an increase in thymol content. In another study conducted by Echazú et al. (2017), radical scavenging activity determined by DPPH assay showed that chitosan hydrogels containing

1.25 and 2.5 mg/mL of thymol have antioxidant activity. In addition, in the polymeric delivery systems, thymol antioxidant capacity increased with different delivering systems, such as thymol emulsification with Quillaja Saponin (Doost et al., 2019) and thymol in Tween 80 micelles (Deng et al. 2016).

Antibacterial Activity of Nanoparticles

The antimicrobial efficacy of the electrosprayed nanoparticles against the tested microorganisms has been assessed by measuring the diameter of the inhibition zone (Table 3). The test showed that the loading of the thymol into the chitosan nanoparticles could maintain and improve its antibacterial activity. The antibacterial effectiveness was enhanced by increasing the concentration of thymol in the chitosan nanoparticles, with a statistically significant increase ($p<0.05$). Previous studies have reported the antimicrobial activity of thymol in different delivery systems. In particular, chitosan thymol nanoparticles obtained by ionic crosslinking method exhibited higher antifungal activity against *B. cinerea* than non-encapsulated thymol (Zhao et al., 2023). Additionally, thymol-loaded γ-cyclodextrin metal-organic framework inclusion complexes showed antibacterial activity against *E.coli* and *S.aureus* (Pan et al., 2022). Moreover, chitosan thymol nanoparticles produced through ionic gelation demonstrated antibacterial effects against *S.aureus*, *L.innocua,* and *S.typhimurium* (Medina et al., 2019). The study conducted by Wattanasatcha et al. (2012) found that thymol-encapsulated ethylcellulose/methylcellulose nanospheres displayed high antibacterial activity against *E.coli, S.aureus* and *P.aeruginosa.* In addition, caseinate-stabilized thymol nanosuspensions showed antibacterial activity against *S.aureus*, *L.monocytogenes, E.coli,* and *S.typhimurium* (Zhou et al., 2021). Also, alginate thymol microparticles produced by the electrospraying method showed antibacterial activity against *S. aureus* and *E.coli* (Ahmady et al., 2023). Apart from that, encapsulation systems developed with thymol are also effective in preserving food. Thymol-loaded nanoparticles improve the quality and extend the shelf life of chestnuts (Guo et al., 2022). Chitosan nanoemulsion encapsulated with thymol prolonged the shelf life of fresh pork (Liu and Liu, 2020).

Table 3. Antibacterial effect of electrosprayed chitosan nanoparticles and thymol-loaded chitosan nanoparticles on selected bacteria

Chitosan nanoparticles	Zone of growth inhibition (mm)			
	S. aureus	*B. cereus*	*E. coli*	*S.thyphimirium*
CH-C	-	-	-	-
CH-TH1	10.86±0.06[b]	-	-	-
CH-TH2	11.00±0.10[b]	13.68±0.11[b]	11.63±0.14[b]	10.74±0.20[b]
CH-TH3	12.62±0.18[a]	14.34±0.31[a]	14.30±0.34[a]	11.39±0.26[a]
Gen(10 μg)	24.58±0.19	34.59±0.07	25.70±0.95	23.25±0.27

[a,b]; Different letters in the same column show a statistically significant difference (p<0.05). All values are means ± SD, n=3, -: no inhibition zone.

CONCLUSION

In this study, thymol was successfully encapsulated within chitosan polymer, resulting in nanoparticles of nanoscale dimensions. Regarding the TGA/DTA analyses, chitosan nanoparticles effectively improved the thermal stability of thymol. The obtained CTNPs provide promising results with antioxidant and antimicrobial activity. SEM, FT-IR, and TGA analyses revealed characteristic features of the generated nanoparticles, which can serve as critical knowledge during their application. CTNPs are obtained through a simple one-step procedure using the electrospray technique, providing a new strategy for encapsulating thymol-like volatile compounds. Additionally, CTNPs can be used as preservatives for food safety and nutritional purposes. This work has the potential for the future use of thymol in food safety, nutrition, and medical field applications.

DECLARATION OF CONFLICT OF INTEREST

The author of this article declares no conflict of financial or personal interest.

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