



## Nano-Coating of Chitosan Incorporated with *Cinnamomum Zeylanicum* Essential Oil: Application on Chicken Sausages for Quality Enhancement and Shelf-Life Extension

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### ABSTRACT

The application of edible coatings containing nanoemulsions of essential oil is a growing area of research for quality enhancement of meat and meat products using natural preservation methods. The present work was done to evaluate the quality and shelf-life of chicken sausages coated with an edible coating of nanoemulsions of chitosan and cinnamon essential oil. A total of four types of edible coatings were prepared, viz. T<sub>1</sub>: 0.3 % chitosan only; T<sub>2</sub>: 0.3% chitosan and 0.3% cinnamon essential oil; T<sub>3</sub>: nanoemulsion of 0.3% chitosan; T<sub>4</sub>: nanoemulsion of 0.3% chitosan incorporated with 0.3% cinnamon essential oil. The edible coatings were characterized and chicken sausages were coated with the developed edible coatings while determining the quality and shelf-life at regular intervals during refrigeration storage.: UV-Vis spectrophotometry, particle size analysis, and HR-TEM analysis revealed the spherical-shaped nanoparticles in the 50-200 nm range. Edible coatings significantly (P<0.05) improved the microbiological and physicochemical quality parameters and oxidative changes in T<sub>4</sub> than other treatments throughout the refrigeration storage (4±1°C). Hunter color values were significantly (P<0.05) improved in T<sub>4</sub> compared to other treatments during storage. No sensory discrimination was observed upon applying nano-emulsions over chicken sausages throughout the storage period. The application of the nanoemulsions coating extended the shelf-life by 10-15 days in T<sub>4</sub> than control during refrigeration storage.

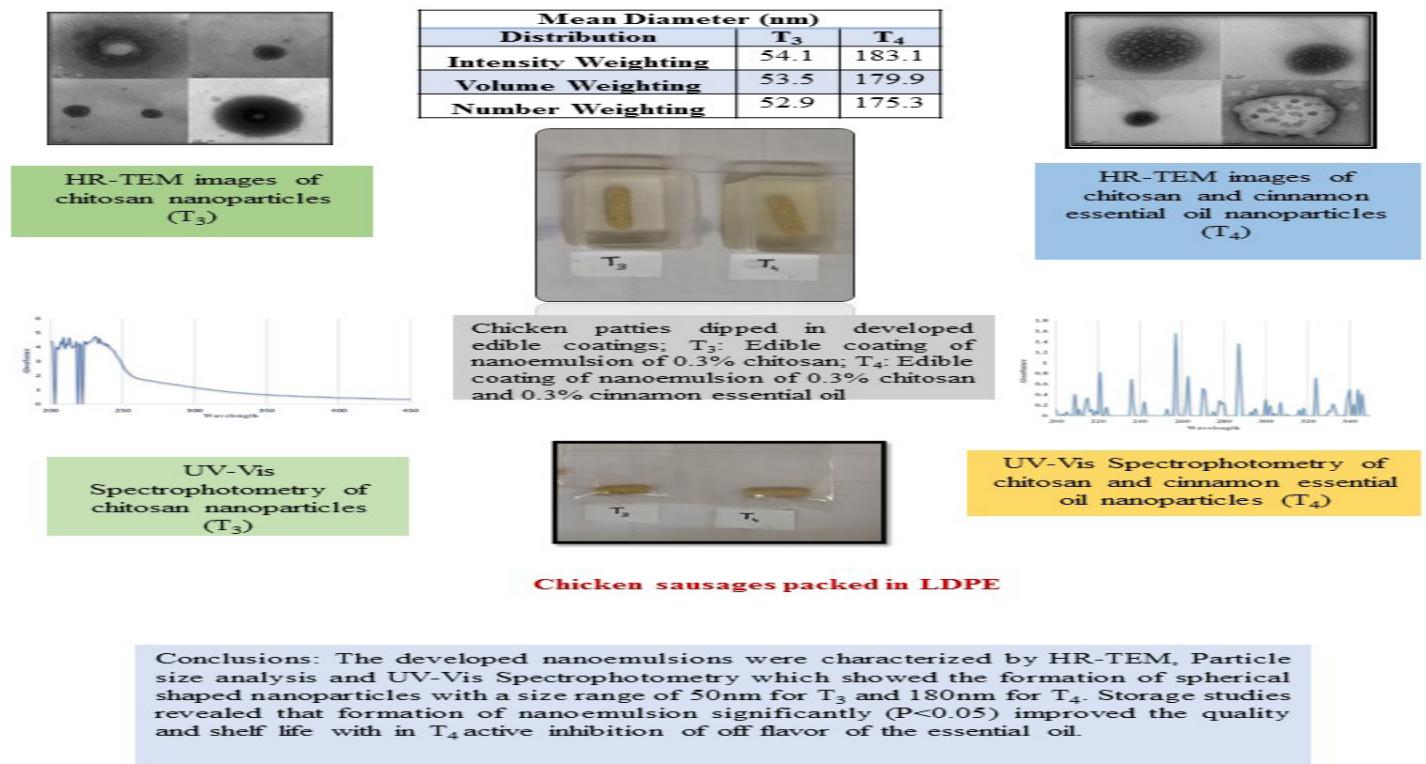
**Keywords:** Chitosan, Cinnamon essential oil, Nanoemulsion, Edible coating, Natural preservation, Shelf-life extension

### Introduction

The demand for convenient packaged processed meat products is growing due to increased urbanization, changing food habits, and shifts in demographic patterns. Consumers are demanding ready-to-eat processed products free from synthetic preservatives, which is often referred to as green consumerism. The present research provides practical solutions to meet these demands.

Packaging is critical for food quality and safety, as well as for increasing product marketability, and is one such area that can tackle this rapidly growing problem of increased synthetic preservatives in meat and meat products. Active packaging, which is actively involved with one or more active functions with added benefits to enhance food quality and safety, could be used to preserve the meat and meat products with added natural preservatives. The inclusion of antimicrobials into packaging material will lead to the in the steady release of

## Graphical Abstract



active compounds, that could be an important approach to prevent microbial growth (Noshirvani et al. 2018), thus preservation of meat and meat products with reduced synthetic preservatives.

Edible coatings and films are prepared from natural polymers such as chitosan, starch, and carrageenan, etc. and combining them with antioxidants, and antimicrobial compounds can provide an added advantage (Zhang et al. 2020). Chitosan coatings are significant for food preservation due to their antimicrobial and antioxidant properties as well as their moisture barrier, gas permeability, biodegradability, and film-forming ability (Saberi et al. 2024a). Chitosan has strong chelating properties for important nutrients and metal cations, thus inhibiting microbial growth by starvation. Moreover, it interacts with the negative phosphate groups present over the genetic material, inhibiting their synthesis and terminating the microbial growth (Saberi et al. 2023a).

Essential oils are used in edible coatings as antimicrobials, promoting food quality by inhibiting microbial growth and serving as antioxidants (Zhang et al. 2021). The cinnamon essential oil contains active compounds such as cinnamaldehyde, cinnamic acid, cinnamyl acetate, eugenol, etc., which are known to have potent antimicrobial, antiulcer, antidiabetic, anti-inflammatory, and antioxidant properties and have been classified as GRAS (Generally Recognized as Safe) (Niu et al. 2018).

The enhanced antimicrobial and antioxidant properties of chitosan nanoparticles could be ascribed to their increased

surface area and higher charge density, thus increased the interference with the negatively charged bacterial surface and enhanced antimicrobial activity (Saberi et al. 2024b). Moreover, chitosan nanoparticles also cause the gradual release of active principles, thus ensuring a protracted outcome while preventing the degradation of active ingredients, thus ensuring the longer stability by formation of nanoparticles (Saberi et al. 2024b). Therefore, the present study was done to evaluate the quality and shelf life of chicken sausage coated with nanoemulsions of chitosan and cinnamon essential oil during refrigeration storage.

## Materials And Methods

### Preparation of nano-coatings

To prepare the edible coating of plain chitosan, 0.3 % chitosan ( $T_1$ ) and 0.3 gm chitosan were mixed in 100 ml of 1 % v/v aqueous acetic acid solution and dissolved using a magnetic stirrer at 1500 rpm for overnight at room temperature. For the preparation of edible coating of 0.3% chitosan and 0.3% cinnamon essential oil ( $T_2$ ), the above steps were performed, followed by the addition of 0.3ml cinnamon essential oil (CEO) and stirring with a magnetic stirrer for 30 minutes at 1500 rpm overnight.

To prepare the edible coatings of nanoemulsions, the method of Mohammadi et al. (2015) was followed with slight modifications. For the preparation of nanoemulsion of

0.3% chitosan and 0.3 % cinnamon essential oil ( $T_4$ ), 0.3 gm chitosan was mixed in 100 ml of 1% v/v aqueous acetic acid solution and dissolved using a magnetic stirrer at 1500 rpm for overnight at room temperature. Then, tween 80 (0.25%) was added, and the whole mixture was homogenized for 45 minutes at 2000 rpm, followed by stirring at 1500 rpm for 1.5 hours with a magnetic stirrer. After that, 0.3ml cinnamon essential oil was gradually added and stirred for about 30 minutes. After that, the dropwise addition of sodium tripolyphosphate solution (0.3 % w/v, 40ml) into emulsion at constant stirring for 30 minutes at 1500 rpm, followed by homogenization for 15 minutes at 10,000 rpm and 15 minutes at 20,000 rpm led to the formation of nanoemulsion of 0.3% chitosan and 0.3 % cinnamon essential oil ( $T_4$ ). Similar steps were followed, except the addition of cinnamon essential oil for the preparation of edible coating of nanoparticles of chitosan ( $T_3$ ).

#### Characterisation of nanoparticle

Developed nanoparticles were characterized using a UV-Vis spectrophotometer by measuring the absorbance at wavelengths ranging from 200 to 450 nanometres to check for the incorporation of essential oil. The particle size was analysed using Particle sizing systems (Inc. Santa Barbara, Calif., USA). Structural investigation of nanocoatings was performed using High-resolution transmission electron microscopy (200 kV HR-TEM JEOL, Japan, Model no. JEM2100F)

#### Experimental setup

Chicken sausages were prepared by making meat emulsion and for conducting storage studies, a total five treatments were made, i.e., C: control sample without any edible coating,  $T_1$ : Sausage coated with an edible coating containing 0.3% chitosan;  $T_2$ : Sausage coated with an edible coating of 0.3% chitosan mixed with 0.3% v/v cinnamon essential oil ;  $T_3$ : Sausage coated with an nanoemulsion of 0.3% chitosan;  $T_4$ : Sausage coated with an nanoemulsion of 0.3% chitosan mixed with 0.3% v/v cinnamon essential oil. Coatings were applied over chicken sausages by dipping them in developed coatings for 10 minutes and then excess coating was drained by keeping over metal rack for 10 minutes under sterile conditions. The quality and shelf-life of the packaged sausages (50  $\mu$  LDPE) was estimated for 30 days at an interval of 5 days at refrigeration temperature.

#### Physicochemical parameters

The pH was recorded as per the method of (Trout et al. 1992). Tyrosine value was determined as per the method of (Strange et al. 1977). Thiobarbituric acid reacting substance (TBARS) was evaluated as per the method of (Witte et al. 1970). DPPH free radical scavenging activity as per the method of (Tepe et al. 2005) to determine the antioxidant properties..

#### Microbiological analysis

Total plate count (TPC), *Staphylococcus aureus* count, Coliform, yeast and mold count, and psychrophilic count were determined as per APHA (2001).

#### Hunter color measurement

The color measurements ( $L^*$ ,  $a^*$ ,  $b^*$  CIELAB values) were carried out using an Ultra Scan VIS spectrophotometer (Hunter Lab, Reston, VA, USA) as per (Ledesma et al. 2016).

#### Sensory evaluation

The sensory evaluation was performed to estimate the shelf life of chicken sausages as per (Miller, 2017), using an 8-point descriptive scale.

#### Statistical analysis

All the experiments were repeated three times in duplicates ( $n=6$ ) and the data generated was analysed using Statistical Package for Social Sciences (SPSS, Version 26.0).

## Results And Discussions

#### Characterisation of developed nanoparticles

The UV-vis spectrophotometry results revealed absorbance maxima of about 232nm in  $T_3$ , while  $T_4$  showed an absorbance maximum of 257 and 287nm. Particle size analysis revealed the size range of 50-200 nm for nanoparticles in  $T_3$  and  $T_4$ . HR-TEM analysis revealed spherical-shaped nanoparticles with a mean diameter of 50nm for chitosan nanoparticles ( $T_3$ ) and 100nm for chitosan nanoparticles incorporated with CEO ( $T_4$ ) (Plate 1 and 2). With respect to the UV-Vis spectrophotometry, similar results were reported by (Ghahfaroki et al. 2017), who found absorption maxima of 285 nm in cinnamon essential oil-incorporated chitosan nanoparticles which confirmed the successful loading of essential oils in chitosan and the formation of nanoparticles. With respect to the particle size analysis, similar reports were also found by (Sonar et al. 2013), where chitosan encapsulated with oregano essential oil had a size range of 25 to 600 nm. In consideration with HR-TEM analysis, (Ghahfaroki et al. 2017) also discovered the spherical shaped cinnamon essential oil-incorporated chitosan nanoparticles corroborating with the present findings.

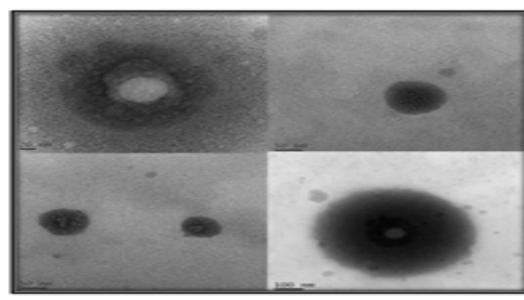
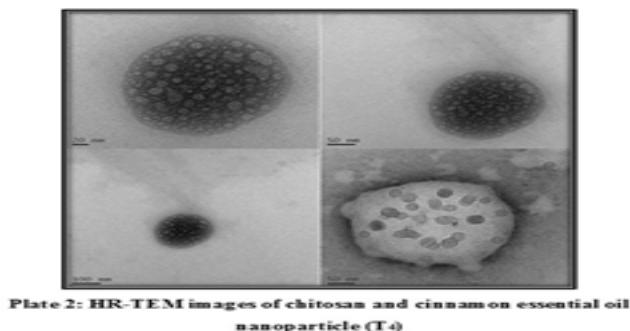


Plate 1: HR-TEM images of chitosan nanoparticles ( $T_3$ )



### Physicochemical parameters

The pH values had an increasing trend with the progression of the storage period in all the treatments (Table 1). The TBARS values in treatments were significantly lower ( $P<0.05$ ) in treatments than control throughout the storage which could be attributed to the decreased availability of oxygen and chelating impact of chitosan on metal ions and the antioxidant activity of essential oils (Table 1). The tyrosine values increased significantly ( $P<0.05$ ) during refrigeration storage for all the treatments (Table 1). The DPPH activity in the current study followed a decreasing

trend of  $T_4 > T_3 > T_2 > T_1 >$  control, which showed a reduction in the DPPH activity with the progression of time (Table 1). Among all the treatments,  $T_4$  had the lowest pH value throughout the storage period, possibly due to inhibition of microbial activity, which prevented protein degradation. The nanoparticles might have enhanced the antimicrobial activity due to the slow release of essential oil and increased surface area, as reported by (Li et al. 2019). With respect to the TBARS value, similar results were also observed by (Yadav et al. 2022) in chicken patties coated edible film of chitosan during storage period. With respect to the tyrosine value, higher tyrosine values in control than treatments during storage might be due to the antimicrobial properties of nanocoatings. The results corroborated with the findings of (Osaili et al. 2019), who observed that chitosan nanoparticles and phytochemicals of cinnamon essential oil inhibited the microbial cells via diverse mechanisms, enhancing the antimicrobial property. The significantly higher ( $P<0.05$ ) DPPH activity in treatments could be due to the improved antioxidative activity of nanoparticles attributing to the improved free radical scavenging activity than plain chitosan (Saberi et al. 2024b).

**Table 1.** Effect of edible coatings on Physico-chemical parameters and antioxidant activity of chicken sausages at refrigeration storage ( $4\pm1^\circ\text{C}$ ) (Mean $\pm$ S.E.)\*

Treatments	Refrigerated storage period (days)						
	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
<i>pH</i>							
Control	6.05 $\pm$ 0.08 <sup>1b</sup>	6.21 $\pm$ 0.03 <sup>2c</sup>	6.33 $\pm$ 0.05 <sup>3d</sup>	6.43 $\pm$ 0.12 <sup>4d</sup>	NE	NE	NE
$T_1$	6.03 $\pm$ 0.16 <sup>1b</sup>	6.21 $\pm$ 0.10 <sup>2c</sup>	6.27 $\pm$ 0.13 <sup>3c</sup>	6.32 $\pm$ 0.18 <sup>4c</sup>	6.50 $\pm$ 0.09 <sup>5d</sup>	NE	NE
$T_2$	6.02 $\pm$ 0.08 <sup>1a</sup>	6.14 $\pm$ 0.06 <sup>2b</sup>	6.19 $\pm$ 0.12 <sup>3b</sup>	6.32 $\pm$ 0.17 <sup>4c</sup>	6.40 $\pm$ 0.18 <sup>5c</sup>	6.45 $\pm$ 0.13 <sup>6c</sup>	6.49 $\pm$ 0.09 <sup>7c</sup>
$T_3$	6.02 $\pm$ 0.06 <sup>1a</sup>	6.13 $\pm$ 0.04 <sup>2b</sup>	6.18 $\pm$ 0.10 <sup>3b</sup>	6.24 $\pm$ 0.09 <sup>4b</sup>	6.28 $\pm$ 0.11 <sup>5b</sup>	6.34 $\pm$ 0.16 <sup>6b</sup>	6.43 $\pm$ 0.13 <sup>7b</sup>
$T_4$	6.02 $\pm$ 0.05 <sup>1a</sup>	6.11 $\pm$ 0.06 <sup>2a</sup>	6.16 $\pm$ 0.07 <sup>3a</sup>	6.18 $\pm$ 0.14 <sup>4a</sup>	6.25 $\pm$ 0.08 <sup>5a</sup>	6.31 $\pm$ 0.11 <sup>6a</sup>	6.38 $\pm$ 0.12 <sup>7a</sup>
<i>TBARS (mg malonaldehyde/kg of meat)</i>							
Control	0.18 $\pm$ 0.02 <sup>1c</sup>	0.38 $\pm$ 0.006 <sup>2d</sup>	0.59 $\pm$ 0.002 <sup>3d</sup>	0.89 $\pm$ 0.004 <sup>4d</sup>	NE	NE	NE
$T_1$	0.18 $\pm$ 0.002 <sup>1c</sup>	0.28 $\pm$ 0.006 <sup>2c</sup>	0.47 $\pm$ 0.004 <sup>3c</sup>	0.75 $\pm$ 0.004 <sup>4c</sup>	1.00 $\pm$ 0.005 <sup>5d</sup>	NE	NE
$T_2$	0.17 $\pm$ 0.002 <sup>1b</sup>	0.20 $\pm$ 0.004 <sup>2ab</sup>	0.35 $\pm$ 0.009 <sup>3b</sup>	0.41 $\pm$ 0.004 <sup>4b</sup>	0.48 $\pm$ 0.003 <sup>5c</sup>	0.75 $\pm$ 0.005 <sup>6b</sup>	0.96 $\pm$ 0.004 <sup>7c</sup>
$T_3$	0.15 $\pm$ 0.002 <sup>1a</sup>	0.22 $\pm$ 0.01 <sup>2b</sup>	0.35 $\pm$ 0.007 <sup>3b</sup>	0.40 $\pm$ 0.003 <sup>4ab</sup>	0.45 $\pm$ 0.004 <sup>4b</sup>	0.64 $\pm$ 0.003 <sup>6a</sup>	0.86 $\pm$ 0.003 <sup>7b</sup>
$T_4$	0.15 $\pm$ 0.003 <sup>1a</sup>	0.18 $\pm$ 0.006 <sup>2a</sup>	0.30 $\pm$ 0.007 <sup>3a</sup>	0.39 $\pm$ 0.004 <sup>4a</sup>	0.44 $\pm$ 0.003 <sup>5a</sup>	0.63 $\pm$ 0.003 <sup>6a</sup>	0.84 $\pm$ 0.005 <sup>7a</sup>
<i>Tyrosine (mg/100g)</i>							
Control	12.78 $\pm$ 0.18 <sup>1d</sup>	20.01 $\pm$ 0.02 <sup>2d</sup>	32.28 $\pm$ 0.31 <sup>3e</sup>	40.30 $\pm$ 0.24 <sup>4d</sup>	NE	NE	NE
$T_1$	11.48 $\pm$ 0.13 <sup>1c</sup>	17.21 $\pm$ 0.18 <sup>2c</sup>	25.58 $\pm$ 0.45 <sup>3d</sup>	34.66 $\pm$ 0.35 <sup>4c</sup>	39.53 $\pm$ 0.58 <sup>5d</sup>	NE	NE
$T_2$	10.41 $\pm$ 0.06 <sup>1b</sup>	15.50 $\pm$ 0.35 <sup>2b</sup>	20.50 $\pm$ 0.06 <sup>3c</sup>	24.40 $\pm$ 0.17 <sup>4b</sup>	29.10 $\pm$ 0.18 <sup>5c</sup>	35.0 $\pm$ 0.48 <sup>6c</sup>	39.20 $\pm$ 0.13 <sup>7c</sup>
$T_3$	9.68 $\pm$ 0.04 <sup>1a</sup>	13.41 $\pm$ 0.16 <sup>2a</sup>	18.20 $\pm$ 0.16 <sup>3b</sup>	24.50 $\pm$ 0.14 <sup>4b</sup>	31.02 $\pm$ 0.38 <sup>5b</sup>	36.70 $\pm$ 0.04 <sup>6b</sup>	38.20 $\pm$ 0.17 <sup>7b</sup>
$T_4$	9.43 $\pm$ 0.03 <sup>1a</sup>	13.30 $\pm$ 0.06 <sup>2a</sup>	16.10 $\pm$ 0.07 <sup>3a</sup>	23.0 $\pm$ 0.09 <sup>4a</sup>	27.30 $\pm$ 0.12 <sup>5a</sup>	31.30 $\pm$ 0.12 <sup>6a</sup>	34.60 $\pm$ 0.24 <sup>7a</sup>
<i>DPPH (%)</i>							
Control	23.59 $\pm$ 0.13 <sup>1e</sup>	22.26 $\pm$ 0.15 <sup>2e</sup>	21.43 $\pm$ 0.12 <sup>2d</sup>	20.97 $\pm$ 0.21 <sup>2e</sup>	NE	NE	NE
$T_1$	25.41 $\pm$ 0.33 <sup>1d</sup>	25.77 $\pm$ 0.12 <sup>1d</sup>	24.11 $\pm$ 0.32 <sup>2c</sup>	22.90 $\pm$ 0.41 <sup>3d</sup>	20.91 $\pm$ 0.19 <sup>4d</sup>	NE	NE
$T_2$	28.79 $\pm$ 0.14 <sup>1c</sup>	32.39 $\pm$ 0.18 <sup>2c</sup>	32.16 $\pm$ 0.16 <sup>2b</sup>	31.94 $\pm$ 0.15 <sup>2c</sup>	30.82 $\pm$ 0.26 <sup>3c</sup>	30.31 $\pm$ 0.21 <sup>34c</sup>	29.85 $\pm$ 0.22 <sup>4c</sup>
$T_3$	32.43 $\pm$ 0.11 <sup>1b</sup>	34.99 $\pm$ 0.12 <sup>2b</sup>	34.94 $\pm$ 0.18 <sup>2a</sup>	33.96 $\pm$ 0.23 <sup>3b</sup>	33.48 $\pm$ 0.12 <sup>3b</sup>	32.76 $\pm$ 0.19 <sup>1b</sup>	30.91 $\pm$ 0.17 <sup>4b</sup>

T <sub>4</sub>	35.17±0.11 <sup>1a</sup>	35.83±0.12 <sup>2a</sup>	34.99±0.18 <sup>1a</sup>	34.69±0.17 <sup>3a</sup>	34.49±0.13 <sup>3a</sup>	33.96±0.19 <sup>4a</sup>	32.63±0.10 <sup>5a</sup>
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\*n=4, Mean±S.E. with different superscripts row-wise (numerals) and column-wise (alphabet) differ significantly (P <0.05); NE: not examined since the product has spoiled. Control: Control sample without any edible coating; T<sub>1</sub>: Chicken sausage coated with edible coating of 0.3 % chitosan; T<sub>2</sub>: Chicken sausage coated with edible coating of 0.3% chitosan and 0.3 % cinnamon essential oil; T<sub>3</sub>: Chicken sausage coated with nanoemulsion of 0.3% chitosan; T<sub>4</sub>: Chicken sausage coated with nanoemulsion of 0.3% chitosan and 0.3% cinnamon essential oil.

### Microbiological analysis

The TPC showed an increasing trend during storage; however, the treatments had lower values than the control throughout the storage period (Fig. 1). The coliforms were absent throughout the storage period. Yeast and mold were not detected in any sample on the initial day of refrigeration storage, which increased significantly (P<0.05) afterwards (Fig. 1).

Psychrophiles were not detected till the 10<sup>th</sup> day of storage in control, while they were absent till the 15<sup>th</sup> day in T<sub>1</sub> and T<sub>2</sub>, till the 20<sup>th</sup> day in T<sub>3</sub> and 25<sup>th</sup> day in T<sub>4</sub>, while *Staphylococcus aureus* was absent throughout the storage period in all the treatments. In the present study, the control sample exceeded the permissible limit of 4 log<sub>10</sub>cfu/g (FSSAI 2018) during the 10<sup>th</sup>-15<sup>th</sup> days of refrigeration storage, while T<sub>1</sub> crossed the limit during the 15<sup>th</sup>-20<sup>th</sup>, T<sub>2</sub> between 20-25<sup>th</sup> while T<sub>3</sub> & T<sub>4</sub> crossed the permissible limit between 25-30<sup>th</sup> day of storage. These results agreed with the findings of (Saberi et al. 2024a), who reported that the encapsulation of essential oils could reduce their evaporation and efficiently deliver it to the bacterial cell wall. Further, various studies have suggested that chitosan nanocomposites in the form of edible coatings incorporated with different natural antimicrobials improved the quality and shelf-life of food products than normal coatings (Saberi et al. 2023b), thus augmenting the results in the present studies.

The absence of coliforms during storage could be due to their destruction during high-temperature cooking, which is substantially higher than their death point of 57°C. The chitosan could have also prevented the growth of coliform bacteria in chicken sausages, which is in agreement with the results of (Kanatt et al. 2013). The significantly lower (P<0.05) psychrophilic counts in T<sub>4</sub> than T<sub>3</sub> could be due to the antimicrobial effect of cinnamon essential oil. The absence of psychrophiles in all the treatments during the initial storage period could be due to the inhibitory effect of cooking and the slower growth rate of psychrophiles. The absence of *Staphylococcus aureus* in the present study agreed with the results of (Zhang et al. 2015) who reported that cinnamon essential oil has strong antibacterial effects against *Staphylococcus aureus* probably due to the cinnamaldehyde, impairing the cell membrane, causing to bacterial lysis. The lower yeast and mold count in treatments may be due to the antimicrobial effect of chitosan and essential oil. The major component of cinnamon essential oil, i.e., trans-cinnamaldehyde, is associated with an

inhibitory effect on different enzymes, such as fungal cell walls synthesizing enzyme and perturbation of bacterial cell membranes. Further, the nanoparticles, with their expansive surface areas, facilitated the absorption of bioactive agents, the cinnamon essential oil and their nanoscale dimensions significantly enhanced the penetration of bioactive agents inside the cell membrane, thereby reducing the microbial load in T<sub>4</sub> more effectively than other treatments (Saberi et al. 2024b).

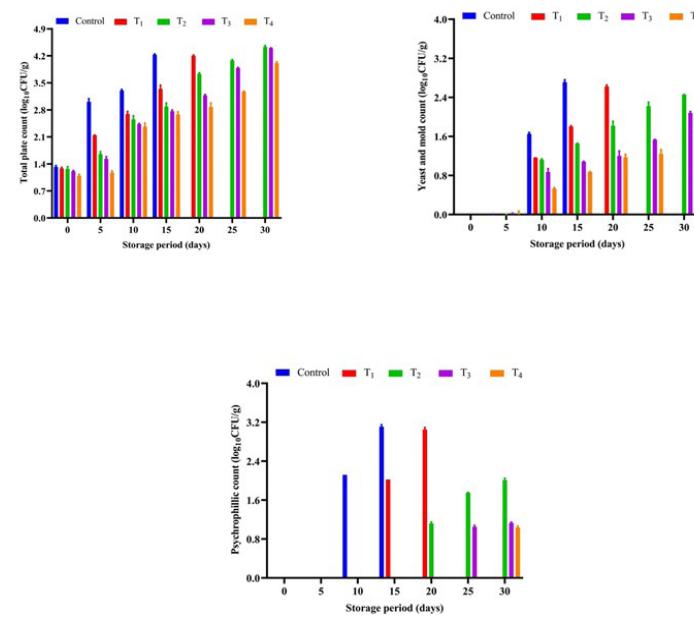


Figure 1: Effect of edible coatings on microbiological quality (log<sub>10</sub>CFU/g) of chicken sausage during refrigeration storage (4±1°C)

### Hunter color analysis

The lightness (L\*), Redness (a\*), and yellowness (b\*) values decreased significantly (P<0.05) in all the treatments throughout the storage period. The chroma value reported a significant increase during storage period while hue did not follow any significant trend during storage in all the treatments. The browning index and total colour change followed an increasing trend in all the treatments with the progression of storage period (Table 2). The improved hunter color values in T<sub>4</sub> during storage period might be due to the anti-oxidative effect of nanoparticles of chitosan and cinnamon essential oil. Yaghoubi et al. (2021) reported improved hunter color values in chicken samples coated with 1% chitosan and 1500 ppm *Artemisia fragrance* essential oil than control during storage.

**Table 2.** Effect of edible coating on Hunter colour analysis of chicken sausages at refrigeration storage (4±1°C) (Mean±S.E.)\*

Treatments	Refrigerated storage period (days)						
	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
<i>Lightness (L*)</i>							
Control	55.85±0.16 <sup>1d</sup>	54.62±0.18 <sup>2d</sup>	53.37±0.06 <sup>3d</sup>	52.87±0.06 <sup>4e</sup>	NE	NE	NE
T <sub>1</sub>	57.09±0.15 <sup>1c</sup>	56.65±0.28 <sup>2c</sup>	56.34±0.05 <sup>3c</sup>	55.79±0.004 <sup>4d</sup>	55.25±0.01 <sup>5d</sup>	NE	NE
T <sub>2</sub>	58.14±0.18 <sup>1b</sup>	58.02±0.11 <sup>2b</sup>	57.75±0.008 <sup>3b</sup>	57.39±0.02 <sup>4c</sup>	56.67±0.01 <sup>5c</sup>	55.73±0.02 <sup>6c</sup>	55.22±0.07 <sup>7c</sup>
T <sub>3</sub>	59.56±0.02 <sup>1a</sup>	59.21±0.02 <sup>2a</sup>	58.79±0.01 <sup>3a</sup>	58.53±0.01 <sup>4b</sup>	58.16±0.01 <sup>5b</sup>	57.77±0.06 <sup>6b</sup>	56.83±0.05 <sup>7b</sup>
T <sub>4</sub>	59.57±0.02 <sup>1a</sup>	59.31±0.02 <sup>2a</sup>	58.83±0.01 <sup>3a</sup>	58.69±0.01 <sup>4a</sup>	58.48±0.01 <sup>5a</sup>	57.27±0.08 <sup>6a</sup>	57.12±0.04 <sup>7a</sup>
<i>Redness (a*)</i>							
Control	8.32±0.03 <sup>1c</sup>	7.89±0.08 <sup>2d</sup>	7.54±0.02 <sup>3e</sup>	6.35±0.01 <sup>4e</sup>	NE	NE	NE
T <sub>1</sub>	8.51±0.01 <sup>1b</sup>	8.24±0.13 <sup>2c</sup>	7.83±0.17 <sup>3d</sup>	7.56±0.02 <sup>4d</sup>	6.78±0.01 <sup>5d</sup>	NE	NE
T <sub>2</sub>	8.63±0.07 <sup>1a</sup>	8.40±0.06 <sup>2b</sup>	8.21±0.07 <sup>3c</sup>	7.89±0.01 <sup>4c</sup>	7.65±0.01 <sup>5c</sup>	7.32±0.05 <sup>6c</sup>	6.71±0.04 <sup>7c</sup>
T <sub>3</sub>	8.67±0.06 <sup>1a</sup>	8.49±0.01 <sup>2ab</sup>	8.31±0.04 <sup>3b</sup>	8.24±0.09 <sup>4b</sup>	7.93±0.01 <sup>5b</sup>	7.52±0.05 <sup>6b</sup>	7.22±0.03 <sup>7b</sup>
T <sub>4</sub>	8.71±0.05 <sup>1a</sup>	8.54±0.01 <sup>2a</sup>	8.47±0.04 <sup>2a</sup>	8.35±0.01 <sup>3a</sup>	8.16±0.01 <sup>4a</sup>	7.83±0.06 <sup>5a</sup>	7.64±0.06 <sup>6a</sup>
<i>Yellowness (b*)</i>							
Control	22.46±0.33 <sup>1d</sup>	21.63±0.03 <sup>2d</sup>	19.25±0.03 <sup>3e</sup>	17.14±0.02 <sup>4d</sup>	NE	NE	NE
T <sub>1</sub>	23.20±0.27 <sup>1c</sup>	21.86±0.04 <sup>2c</sup>	20.25±0.03 <sup>3d</sup>	20.11±0.01 <sup>4c</sup>	19.85±0.01 <sup>5d</sup>	NE	NE
T <sub>2</sub>	23.93±0.08 <sup>1b</sup>	22.06±0.03 <sup>2b</sup>	21.34±0.12 <sup>3c</sup>	20.21±0.08 <sup>4b</sup>	20.05±0.01 <sup>5c</sup>	19.62±0.02 <sup>6c</sup>	18.57±0.07 <sup>7c</sup>
T <sub>3</sub>	24.09±0.04 <sup>1a</sup>	22.26±0.01 <sup>2a</sup>	21.91±0.02 <sup>3b</sup>	21.30±0.07 <sup>4a</sup>	21.02±0.01 <sup>5b</sup>	20.21±0.02 <sup>6b</sup>	19.89±0.02 <sup>7b</sup>
T <sub>4</sub>	24.18±0.04 <sup>1a</sup>	22.29±0.01 <sup>2a</sup>	22.01±0.06 <sup>3a</sup>	21.35±0.02 <sup>4a</sup>	21.15±0.01 <sup>5a</sup>	20.71±0.07 <sup>6a</sup>	20.16±0.06 <sup>7a</sup>
<i>Chroma</i>							
Control	23.95±0.33 <sup>1e</sup>	23.03±0.04 <sup>2d</sup>	20.68±0.02 <sup>3e</sup>	18.28±0.02 <sup>4e</sup>	NE	NE	NE
T <sub>1</sub>	24.71±0.27 <sup>1d</sup>	23.36±0.03 <sup>2c</sup>	21.71±0.05 <sup>3d</sup>	21.49±0.01 <sup>4d</sup>	20.98±0.01 <sup>5d</sup>	NE	NE
T <sub>2</sub>	25.44±0.02 <sup>1c</sup>	23.61±0.02 <sup>2b</sup>	22.87±0.12 <sup>3c</sup>	21.70±0.08 <sup>4c</sup>	21.46±0.01 <sup>5c</sup>	20.94±0.02 <sup>6c</sup>	19.74±0.07 <sup>7c</sup>
T <sub>3</sub>	25.60±0.04 <sup>1b</sup>	23.83±0.01 <sup>2a</sup>	23.43±0.02 <sup>3b</sup>	22.84±0.008 <sup>4b</sup>	22.46±0.01 <sup>5b</sup>	21.56±0.02 <sup>6b</sup>	21.16±0.02 <sup>7b</sup>
T <sub>4</sub>	25.70±0.04 <sup>1a</sup>	23.87±0.01 <sup>2a</sup>	23.58±0.06 <sup>3a</sup>	22.92±0.02 <sup>4a</sup>	22.67±0.01 <sup>5a</sup>	22.14±0.07 <sup>6a</sup>	21.56±0.06 <sup>7a</sup>
<i>Hue</i>							
Control	1.21±0.001 <sup>1b</sup>	1.22±0.002 <sup>1c</sup>	1.19±0.009 <sup>2c</sup>	1.21±0.01 <sup>1c</sup>	NE	NE	NE
T <sub>1</sub>	1.21±0.001 <sup>1b</sup>	1.21±0.005 <sup>1b</sup>	1.20±0.008 <sup>2b</sup>	1.22±0.001 <sup>3b</sup>	1.24±0.007 <sup>4d</sup>	NE	NE
T <sub>2</sub>	1.22±0.006 <sup>1a</sup>	1.20±0.003 <sup>2ab</sup>	1.20±0.003 <sup>2b</sup>	1.19±0.001 <sup>3a</sup>	1.20±0.009 <sup>2c</sup>	1.21±0.003 <sup>4b</sup>	1.22±0.009 <sup>1b</sup>
T <sub>3</sub>	1.22±0.006 <sup>1a</sup>	1.20±0.005 <sup>2ab</sup>	1.20±0.001 <sup>2b</sup>	1.20±0.003 <sup>2a</sup>	1.21±0.005 <sup>3b</sup>	1.21±0.004 <sup>3b</sup>	1.22±0.002 <sup>3b</sup>
T <sub>4</sub>	1.22±0.005 <sup>1a</sup>	1.20±0.004 <sup>2a</sup>	1.20±0.006 <sup>2a</sup>	1.19±0.006 <sup>3a</sup>	1.20±0.008 <sup>4a</sup>	1.20±0.006 <sup>4a</sup>	1.20±0.003 <sup>4a</sup>
<i>Browning Index</i>							
Control	61.41±0.72 <sup>1b</sup>	60.11±0.14 <sup>2d</sup>	54.37±0.06 <sup>3e</sup>	47.37±0.07 <sup>4d</sup>	NE	NE	NE
T <sub>1</sub>	62.21±0.61 <sup>1d</sup>	61.27±0.31 <sup>2c</sup>	54.01±0.23 <sup>3d</sup>	52.76±0.05 <sup>4c</sup>	51.46±0.03 <sup>5b</sup>	NE	NE
T <sub>2</sub>	62.90±0.05 <sup>1c</sup>	57.64±0.13 <sup>2b</sup>	55.78±0.32 <sup>3c</sup>	52.80±0.22 <sup>4b</sup>	52.85±0.45 <sup>4b</sup>	52.32±0.05 <sup>5c</sup>	49.23±0.14 <sup>6c</sup>
T <sub>3</sub>	61.53±0.11 <sup>1b</sup>	56.87±0.05 <sup>2a</sup>	56.20±0.08 <sup>3b</sup>	54.81±0.03 <sup>4a</sup>	54.10±0.02 <sup>5a</sup>	51.89±0.05 <sup>6b</sup>	51.69±0.05 <sup>6b</sup>
T <sub>4</sub>	61.80±0.12 <sup>1a</sup>	56.87±0.04 <sup>2a</sup>	56.62±0.19 <sup>2a</sup>	54.91±0.01 <sup>3a</sup>	54.37±0.04 <sup>4a</sup>	54.17±0.19 <sup>4a</sup>	52.63±0.01 <sup>5a</sup>
<i>Total colour change value</i>							
Control	1.20±0.14 <sup>1d</sup>	1.53±0.22 <sup>1d</sup>	3.66±0.10 <sup>2e</sup>	2.90±0.03 <sup>3e</sup>	NE	NE	NE
T <sub>1</sub>	62.08±0.16 <sup>1c</sup>	58.56±0.11 <sup>2c</sup>	54.01±0.10 <sup>3d</sup>	53.91±0.15 <sup>3d</sup>	52.76±0.01 <sup>4d</sup>	NE	NE
T <sub>2</sub>	63.46±0.06 <sup>1b</sup>	62.64±0.13 <sup>2b</sup>	62.11±0.15 <sup>3c</sup>	61.35±0.06 <sup>4c</sup>	60.60±0.02 <sup>5c</sup>	59.53±0.03 <sup>6c</sup>	58.65±0.11 <sup>7c</sup>
T <sub>3</sub>	64.83±0.01 <sup>1a</sup>	63.83±0.02 <sup>2a</sup>	63.29±0.02 <sup>3b</sup>	62.83±0.01 <sup>4b</sup>	62.34±0.01 <sup>5b</sup>	61.67±0.02 <sup>6b</sup>	60.64±0.01 <sup>7b</sup>
T <sub>4</sub>	64.87±0.04 <sup>1a</sup>	63.93±0.03 <sup>2a</sup>	63.38±0.02 <sup>3a</sup>	63.04 <sup>4a</sup>	62.72±0.03 <sup>5a</sup>	61.41±0.04 <sup>6a</sup>	61.05±0.05 <sup>7a</sup>

\*n=4, Mean±S.E. with different superscripts row-wise (numerals) and column-wise (alphabet) differ significantly (P <0.05); NE: not examined since the product has spoiled. Control: Control sample without any edible coating; T<sub>1</sub>: Chicken sausage coated with edible

coating of 0.3 % chitosan; T<sub>2</sub>: Chicken sausage coated with edible coating of 0.3% chitosan and 0.3 % cinnamon essential oil; T<sub>3</sub>: Chicken sausage coated with nanoemulsion of 0.3% chitosan; T<sub>4</sub>: Chicken sausage coated with nanoemulsion of 0.3% chitosan and 0.3% cinnamon essential oil.

### Sensory evaluation

The appearance and color scores decreased faster in treatments than in control with the advancement of the storage period (Table 3). Among all the treatments, flavor scores were significantly improved in T<sub>4</sub>, possibly due the nanoencapsulation of cinnamon essential oil, thus, limiting the off flavour.

The texture score decreased in all the treatments as the storage period progressed, while the aftertaste scores were significantly prominent in T<sub>2</sub>, possibly due to free cinnamon essential oil. All the treatments were equally acceptable on the first day of storage, showing no significant difference among themselves. However, overall acceptability scores was significantly (P<0.05) maintained in T<sub>3</sub> and T<sub>4</sub> than other

treatments throughout the storage period. With respect to the appearance and color, T<sub>4</sub> had an acceptable score till the end of storage, which might be due to nanoparticles with active compounds in the coating matrix forming a semipermeable barrier to protect the bioactive compound against oxygen and humidity. The encapsulation of cinnamon essential oil reduced its prominent off-flavor as observed in T<sub>4</sub> and also reported by (Vital et al. 2021). The overall acceptability scores were significantly improved (P<0.05) in T<sub>3</sub> and T<sub>4</sub> throughout the storage period. However, decrease in overall acceptability scores might be due to the reduction in the scores of other attributes with the advancement of the storage period.

**Table 3.** Effect of edible coatings on sensory characteristics of chicken sausages at refrigeration storage (4±1°C) (Mean±S.E.) \*

Treatments	Refrigerated storage period (days)						
	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
<i>Appearance and colour</i>							
Control	7.80±0.13 <sup>1b</sup>	7.24±0.26 <sup>2c</sup>	6.19±0.12 <sup>3e</sup>	5.12±0.25 <sup>4c</sup>	NE	NE	NE
T <sub>1</sub>	7.78±0.12 <sup>1ab</sup>	7.28±0.11 <sup>2bc</sup>	6.28±0.12 <sup>3d</sup>	5.32±0.16 <sup>4b</sup>	5.20±0.12 <sup>4c</sup>	NE	NE
T <sub>2</sub>	7.75±0.12 <sup>1a</sup>	7.33±0.11 <sup>2b</sup>	6.35±0.12 <sup>3c</sup>	5.33±0.16 <sup>4b</sup>	5.28±0.14 <sup>4b</sup>	5.00±0.14 <sup>5b</sup>	4.28±0.16 <sup>6c</sup>
T <sub>3</sub>	7.76±0.32 <sup>1ab</sup>	7.43±0.42 <sup>2a</sup>	7.03±0.45 <sup>3b</sup>	6.36±0.27 <sup>4a</sup>	6.37±0.12 <sup>4a</sup>	5.75±0.17 <sup>5a</sup>	5.18±0.17 <sup>6b</sup>
T <sub>4</sub>	7.75±0.11 <sup>1a</sup>	7.48±0.12 <sup>2a</sup>	7.14±0.17 <sup>3a</sup>	6.46±0.18 <sup>4a</sup>	6.39±0.09 <sup>5a</sup>	5.82±0.17 <sup>6a</sup>	5.24±0.10 <sup>7a</sup>
<i>Flavour</i>							
Control	7.78±0.13 <sup>1a</sup>	7.00±0.15 <sup>2e</sup>	5.98±0.21 <sup>3d</sup>	4.97±0.11 <sup>4d</sup>	NE	NE	NE
T <sub>1</sub>	7.76±0.32 <sup>1a</sup>	7.29±0.14 <sup>2d</sup>	6.69±0.36 <sup>3c</sup>	5.55±0.14 <sup>4c</sup>	5.13±0.18 <sup>5c</sup>	NE	NE
T <sub>2</sub>	7.69±0.13 <sup>1b</sup>	7.20±0.43 <sup>2c</sup>	6.56±0.27 <sup>3b</sup>	5.49±0.14 <sup>4b</sup>	5.05±0.15 <sup>5b</sup>	4.98±0.11 <sup>6b</sup>	4.70±0.12 <sup>7b</sup>
T <sub>3</sub>	7.77±0.21 <sup>1a</sup>	7.66±0.23 <sup>2b</sup>	7.11±0.41 <sup>3a</sup>	6.49±0.11 <sup>4a</sup>	6.39±0.22 <sup>5a</sup>	5.90±0.29 <sup>6a</sup>	5.23±0.26 <sup>7a</sup>
T <sub>4</sub>	7.76±0.13 <sup>1a</sup>	7.61±0.16 <sup>2a</sup>	7.09±0.17 <sup>3a</sup>	6.46±0.19 <sup>4a</sup>	6.37±0.21 <sup>5a</sup>	5.87±0.22 <sup>6a</sup>	5.19±0.20 <sup>7a</sup>
<i>Texture</i>							
Control	7.55±0.14 <sup>1a</sup>	7.17±0.13 <sup>2c</sup>	6.10±0.11 <sup>3c</sup>	5.14±0.18 <sup>4c</sup>	NE	NE	NE
T <sub>1</sub>	7.52±0.13 <sup>1a</sup>	7.25±0.22 <sup>2b</sup>	6.45±0.16 <sup>3b</sup>	5.70±0.12 <sup>4b</sup>	4.87±0.12 <sup>5b</sup>	NE	NE
T <sub>2</sub>	7.50±0.13 <sup>1a</sup>	7.29±0.14 <sup>2b</sup>	6.49±0.16 <sup>3b</sup>	5.74±0.12 <sup>4b</sup>	4.93±0.12 <sup>5b</sup>	4.40±0.15 <sup>6b</sup>	4.22±0.26 <sup>6b</sup>
T <sub>3</sub>	7.52±0.12 <sup>1a</sup>	7.40±0.18 <sup>2a</sup>	7.10±0.12 <sup>3a</sup>	6.38±0.12 <sup>4a</sup>	6.04±0.16 <sup>5a</sup>	5.80±0.10 <sup>6a</sup>	5.09±0.12 <sup>7a</sup>
T <sub>4</sub>	7.54±0.22 <sup>1a</sup>	7.43±0.23 <sup>2a</sup>	7.18±0.11 <sup>3a</sup>	6.45±0.18 <sup>4a</sup>	6.15±0.19 <sup>5a</sup>	5.81±0.24 <sup>6a</sup>	5.12±0.27 <sup>7a</sup>
<i>After taste</i>							
Control	7.52±0.13 <sup>1a</sup>	7.26±0.14 <sup>2c</sup>	6.17±0.22 <sup>3b</sup>	5.11±0.17 <sup>4d</sup>	NE	NE	NE
T <sub>1</sub>	7.54±0.22 <sup>1a</sup>	7.30±0.22 <sup>2ac</sup>	6.20±0.11 <sup>3b</sup>	5.50±0.15 <sup>4b</sup>	5.32±0.19 <sup>4b</sup>	NE	NE
T <sub>2</sub>	7.49±0.13 <sup>1b</sup>	7.27±0.17 <sup>2b</sup>	7.19±0.11 <sup>3c</sup>	5.05±0.15 <sup>4c</sup>	6.41±0.10 <sup>5c</sup>	5.83±0.16 <sup>6c</sup>	5.65±0.23 <sup>7c</sup>
T <sub>3</sub>	7.53±0.21 <sup>1a</sup>	7.31±0.19 <sup>2ac</sup>	6.22±0.12 <sup>3b</sup>	5.56±0.16 <sup>4b</sup>	5.30±0.17 <sup>5b</sup>	5.25±0.21 <sup>6b</sup>	5.11±0.11 <sup>7b</sup>
T <sub>4</sub>	7.55±0.18 <sup>1a</sup>	7.37±0.24 <sup>2ab</sup>	7.14±0.19 <sup>3a</sup>	6.44±0.19 <sup>4a</sup>	6.28±0.23 <sup>5a</sup>	5.60±0.12 <sup>6a</sup>	5.25±0.20 <sup>7a</sup>
<i>Meat flavour intensity</i>							
Control	7.41±0.14 <sup>1a</sup>	7.18±0.17 <sup>2c</sup>	6.17±0.12 <sup>3d</sup>	4.98±0.16 <sup>4c</sup>	NE	NE	NE
T <sub>1</sub>	7.39±0.13 <sup>1a</sup>	7.24±0.14 <sup>1bc</sup>	6.25±0.19 <sup>2c</sup>	5.64±0.14 <sup>3b</sup>	4.87±0.17 <sup>4c</sup>	NE	NE
T <sub>2</sub>	7.35±0.12 <sup>1a</sup>	7.29±0.13 <sup>1b</sup>	6.32±0.16 <sup>2b</sup>	5.68±0.18 <sup>3b</sup>	5.19±0.16 <sup>4b</sup>	4.45±0.16 <sup>5c</sup>	4.28±0.14 <sup>6b</sup>

T <sub>3</sub>	7.40±0.21 <sup>1a</sup>	7.39±0.22 <sup>1a</sup>	7.10±0.19 <sup>2a</sup>	6.39±0.17 <sup>3a</sup>	6.33±0.01 <sup>3a</sup>	5.72±0.11 <sup>4b</sup>	5.03±0.13 <sup>5a</sup>
T <sub>4</sub>	7.37±0.22 <sup>1a</sup>	7.42±0.16 <sup>1a</sup>	7.16±0.11 <sup>2a</sup>	6.43±0.11 <sup>3a</sup>	6.35±0.11 <sup>3a</sup>	5.78±0.12 <sup>4a</sup>	5.08±0.14 <sup>5a</sup>
<i>Overall acceptability</i>							
Control	7.66±0.13 <sup>1a</sup>	7.47±0.12 <sup>2d</sup>	6.17±0.11 <sup>3c</sup>	5.24±0.11 <sup>4c</sup>	NE	NE	NE
T <sub>1</sub>	7.62±0.22 <sup>1a</sup>	7.50±0.11 <sup>2cd</sup>	6.20±0.21 <sup>3bc</sup>	5.30±0.13 <sup>4bc</sup>	5.17±0.16 <sup>5d</sup>	NE	NE
T <sub>2</sub>	7.60±0.12 <sup>1a</sup>	7.55±0.21 <sup>2bc</sup>	6.24±0.21 <sup>2b</sup>	5.34±0.24 <sup>3b</sup>	5.23±0.18 <sup>3c</sup>	4.78±0.11 <sup>4b</sup>	4.09±0.17 <sup>5c</sup>
T <sub>3</sub>	7.65±0.11 <sup>1a</sup>	7.59±0.11 <sup>2ab</sup>	7.02±0.12 <sup>3a</sup>	6.40±0.11 <sup>4a</sup>	6.32±0.21 <sup>5b</sup>	5.83±0.19 <sup>6a</sup>	5.06±0.10 <sup>7b</sup>
T <sub>4</sub>	7.65±0.11 <sup>1a</sup>	7.64±0.13 <sup>1a</sup>	7.06±0.08 <sup>2a</sup>	6.44±0.11 <sup>3a</sup>	6.39±0.07 <sup>3a</sup>	5.89±0.05 <sup>4a</sup>	5.26±0.09 <sup>5a</sup>

\*n=4, Mean±S.E. with different superscripts row-wise (numerals) and column-wise (alphabet) differ significantly (P <0.05); NE: not examined since the product has spoiled. Control: Control sample without any edible coating; T<sub>1</sub>: Chicken sausage coated with edible coating of 0.3 % chitosan; T<sub>2</sub>: Chicken sausage coated with edible coating of 0.3% chitosan and 0.3 % cinnamon essential oil; T<sub>3</sub>: Chicken sausage coated with nanoemulsion of 0.3% chitosan; T<sub>4</sub>: Chicken sausage coated with nanoemulsion of 0.3% chitosan and 0.3% cinnamon essential oil.

## Conclusions

Nanoparticles of chitosan were successfully prepared and applied as a coating to enhance chicken sausage quality and shelf life. The UV-vis spectrometry confirmed the presence of chitosan nanoparticles in T<sub>3</sub> and the successful loading of cinnamon essential oil in chitosan nanoparticles in T<sub>4</sub>. The particle size analysis and HR-TEM results revealed the successful formation of nanoparticles in T<sub>3</sub> and T<sub>4</sub> in the 50-200 nm size range and spherical-shaped nanoparticles with essential oil encapsulation. Edible coating of nanoemulsion of chitosan alone and cinnamon essential oil on sausages showed significantly improved physicochemical, microbiological, sensory, and hunter color values until the 30<sup>th</sup> day of refrigeration storage. Sensory scores were significantly improved in treatments compared to the control group throughout the storage period. Among all the treatments, T<sub>4</sub> had significantly higher sensory scores with the progression of the storage period. Nanoencapsulation of essential oil significantly reduced the sharp off flavor of cinnamon essential oil in T<sub>4</sub>, as revealed by increased sensory scores. There was a significant improvement in the shelf life of T<sub>3</sub> and T<sub>4</sub> for about 10 -15 days than control.

## Competing Interests

The authors do not have any competing interests among themselves or others related to this research work.

## Ethics Statement

Not applicable

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