

## Antimicrobial and Antioxidant Effects of *Thymus daenensis* and *Camellia sinensis* Ethanolic Extracts of Chicken Meat During Frozen Storage

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### Abstract

*Thymus daenensis* Celak. And *Camellia sinensis* (L.) Kuntze are two important medicinal plants with boost antimicrobial and antioxidant activities. The current research was carried out to evaluate the antimicrobial, and antioxidant activity of *T. daenensis* and *C. sinensis* extracts on chicken meat during frozen storage. The extraction was isolated from the aerial parts of *T. daenensis* and *C. sinensis*. Chicken samples were treated into different concentrations of *C. sinensis* and *T. daenensis*. Chemical, microbial and sensory properties of treated chicken samples were analyzed through 60 days storage at -18 °C. Samples treated with *C. sinensis* (0.5%) and *T. daenensis* (0.5%) had the lowest pH ( $6.25 \pm 0.19$ ), total volatile nitrogen ( $33.68 \pm 0.64$  mg/100 g chicken meat) and thiobarbituric acid ( $1.01 \pm 0.00$  mg malonaldehyde/kg oil). Furthermore, chicken samples treated with *C. sinensis* (0.5%) and *T. daenensis* (0.5%) had the lowest loads of total bacteria ( $10.91 \pm 0.81$  log CFU/g), *L. monocytogenes* ( $5.82 \pm 0.82$  log CFU/g) and psychrophilic bacteria ( $6.16 \pm 0.22$  log CFU/g) and the best sensory properties. *C. sinensis* and *T. daenensis* extracts are efficient candidate as natural preservatives for chicken meat.

**Keywords:** *Thymus daenensis*, *Camellia sinensis*, Antimicrobial activity, Antioxidant activity, Chicken meat properties, *Listeria monocytogenes*.

### Introduction

In recent years, consumption of chicken meat and its products has been increasing. Meat and its products can easily be contaminated with different microorganisms. If their transport and maintenance conditions are not appropriate, they will lead to the growth of pathogenic microorganism and finally, the quality is reduced and public health is at risk [1,2]. In addition, increasing reports of food-borne diseases and, in particular, secondary contamination of food products during post-processing phases have led to consumer concerns

as well as producers and other factors involved in food industry [3].

Recently, the use of extracts and essential oils has been considered as antimicrobial and antioxidant preservatives [4-7]. A convenient plant with antioxidant, antimicrobial, and antifungal properties is *T. daenensis*. It is a member of Lamiaceae family and is also applied in the traditional medicine of Iran especially for its medicinal advantageous such as antitussive, expectorant, antispasmodic, antibroncholytic, carminative, anthelmintic, and unfluctuating diuretic possessions. Therapeutic and perfumed characters of *Thymus* genus have ended it popular herbs amongst individuals of many places of the world. The issued findings revealed

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that main instable ingredients gotten from the aerial part of the herb are  $\gamma$ -terpineol, geranial, carvacrol, linalool, trans-thujan and thymol [8,9]. This herb is native of Iran and contains phenolic compounds, especially thymol. Antimicrobial effects against human pathogens and its antioxidant properties have been shown in previous studies [8,9]. *C. sinensis* is one of the most widely used herbs in the world as a beverage. *C. sinensis* is a recurrent herb in the family of Theaceae cultivated in tropical climate area [10,11]. *C. sinensis* contains phenolic compounds, especially catechins, which have high antioxidant and antibacterial activity [10,11]. In previous studies, the effects of essential oils and extracts of eucalyptus, cumin, and *T. daenensis* on increase chicken meat shelf life in refrigerator temperatures have been conveyed [12,13].

The advent of multidrug-resistant bacteria is a singularity of apprehension to the practitioners, and is a main reason of disappointment in treatment of infectious diseases. *Listeria monocytogenes* (*L. monocytogenes*) is a gram-positive, motile, facultative anaerobic, and intracellular bacterium with an emergence of antibiotic resistance [14,15]. This bacterium can be found universally in the food systems with advanced importance through consumption of contaminated foodstuffs. Febrile gastroenteritis, infection of the central nervous system and meningitis are the main routinely recognized clinical signs of the listeriosis [14, 15]. Abortion may occur due to the occurrence of listeriosis in pregnant women [14,15]. A literature survey showed that *L. monocytogenes* and other food-borne bacterial strains isolated from human clinical infections and also those of food samples harbored high prevalence of antibiotic resistance against commonly used antibacterial agents including penicillins, tetracyclines, aminoglycosides, macrolides, lincosamides, folate inhibitors, fluoroquinolones, and phenicols [16,17]. Therefore, it is important to find novel antibacterial substitutions for treatments of cases of human and animal listeriosis. Considering the consumer's approach to consuming food without chemical preservatives and the use of natural ingredients replacing chemical compounds in order to increase the shelf-life of meat products, this study aimed to investigate the combined and individual effects of *T. daenensis* and *C. sinensis* extract on chicken meat quality during frozen storage.

## Material and Methods

### Moral Analysis

The investigation was accepted by the Moral Assembly of Research of the Faculty of Veterinary Medicine. Corroboration of the current research and the certificates related to sample collection were permitted by the professor of the above mentioned institute.

### Herb Materials and Chicken Meat Samples

*T. daenensis* and *C. sinensis* were prepared from the local market of Yasouj city, Iran. *T. daenensis* and *C. sinensis* as medicinal herbs were identified by an expert professor of the Department of Medicinal Herbs, Faculty of Agriculture, Islamic Azad University, Shahrekord, Iran and stored at the herbarium of this center (No. IAUSh 12 and 13). The dried aerial parts of *T. daenensis* and *C. sinensis* medicinal herbs were powdered by the mill (IKA model A10). Then, the powders were subjected to extract by ethanol (70%) as a solvent. Briefly, 25 g of *C. sinensis* and *T. daenensis* powders were placed in ethanol (70%) for 72 h. The extracts were then plated out of what man No. 1 filter paper and finally the solution was dried at laboratory situation (25 °C) [18].

The fresh chicken meat was prepared from the Sepid Morgh Ehsan Company of Kohgiluyeh and Boyer-Ahmad province, Iran. Chicken meat samples were sliced to 0.5 × 4 × 5 cm under a laminar hood and sterile conditions. Then, samples were dipped into *C. sinensis* and *T. daenensis* extracts for 2 minutes. Treatments included control (chicken meat samples without extracts (T0)), T1 (chicken meat samples treated with 0.5% *C. sinensis* extract), T2 (chicken meat samples treated with 0.5% *T. daenensis* extract), and T3 (chicken meat samples treated with 0.5% *C. sinensis* and 0.5% *T. daenensis* extracts) groups. The samples were then placed in sterile plastic bags and stored at -18 °C for 60 days. Chicken meat samples were evaluated for contents of pH, total volatile nitrogen (TVN), Thiobarbituric acid reactive substances (TBARS), total bacterial count (TBC), psychrophilic bacterial count, *L. monocytogenes* count and sensory properties [19].

### pH Measurement

Chicken meat samples were homogenized by a homogenizer and then 5 g of each sample were added to 45 ml of distilled water. Samples pH was

finally measured using the pH meter (HI 9219, Hanna Instruments; Woonsocket, RI, USA). Briefly, 5 g of each sample was blended with distilled water (45 mL) and then disintegrated (twofold) in a mincer and carefully blended to promise acceptable homogeneity. Then, pH was measured [20].

#### Total volatile Nitrogen (TVN) Value

Based on previously described method TVN was evaluated (FAO, 1986). Minced samples (10 g) were homogenized with distilled water (100 mL) using blender. Sample was washed with water and then was blended with magnesium oxide (2 g) and also antifoaming agent (2 drops). Achieved blending was boiled (10 min) and distilled (25 min) in boric acid solution (2%, 25 mL), and partitioned methyl red indicator (few drops). Contents of the previous stage and also the blank solution (boric acid (2%, 25 mL)) were titrated using 0.1 N H<sub>2</sub>SO<sub>4</sub> (titer). TVN (mg N/100 g flesh) was finally measured using the subsequent formula [21]:

$$\text{TVN} = 14 (\text{titer} - \text{blank}).$$

#### Thiobarbituric Acid Reactive Substances (TBARS) Analysis

TBARS was determined [22] by blending samples (10 g) with trichloroacetic acid (20%, 25 mL) and then homogenized for about 30 s. The achieved solution was filtered. The achieved solution (2 mL) was blended with aqueous TBA (2 mL, 0.02%). After that, it incubated in a dark place at laboratory situation for 20 h. Absorbance of the achieved

solution was measured at 532 nm using spectrophotometer (Shimadzu, Japan). The calibration curve was constructed in the concentration range of malondialdehyde (MDA) (Fig. 1). TBARS was presented as mg MDA/kg sample [23].

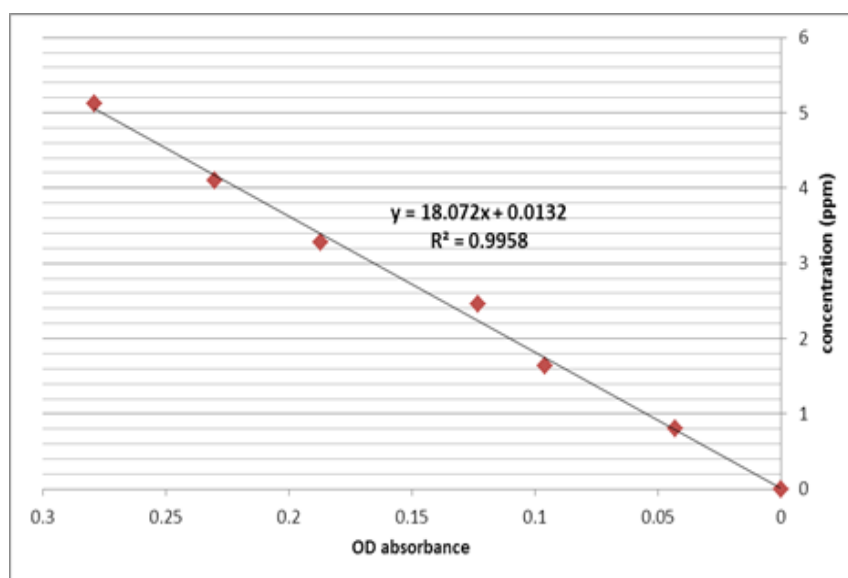
#### Total Bacterial and psychrophilic counts

Ten grams of sample were mixed with 90 ml of distilled water and then transferred to a stomacher bag and homogenized by a Stomacher (Stomacher 400, UK) for about two min. Total bacterial count was done using pour plate method on plate count agar media (PCA, Merck, Germany). All media were then incubated at 37 °C for 48 h. Psychrophilic bacteria were counted using the PCA media. All media were incubated at 7 °C for 10 days [24].

#### *L. monocytogenes* Growth Conditions

Standard strain of *L. monocytogenes* (PTCC 1163) was purchased from Iranian Scientific and Industrial Research Organization (Tehran, Iran). The bacteria were transferred to Tryptic Soy Broth (TSB, Merck, Germany) and incubated at 30 °C for 48 h.

Then, the culture medium containing bacteria were centrifuged for 5 min at 2000 rpm and upper liquid layer was replaced with Ringer solution. For complete isolation of the culture medium from bacteria, the solution was again centrifuged for 5 min.



**Fig. 1** A linear regression curve of standard concentration with a correlation coefficient of 0.9958 and regression equation of  $y = 18.072x + 0.0132$ . Each point in the regression represents the replicate measurement ( $n=3$ ).

The number of bacteria in the underlying fluid was analyzed by turbidimetry at 570 nm, so that the absorption of light from 0.08 to 0.1 was approximately equal to  $10^8$  bacteria per mL. In order to confirm the results, bacterial counting performed on Muller Hinton Agar (MHA, Merck, Germany). Then, chicken meat samples were inoculated with *L. monocytogenes* ( $1 \times 10^4$  CFU/g). *L. monocytogenes* were counted every 10 day intervals. In this experiment, CHROM agar TM Listeria (Chromogenic culture medium, France) and its CHROM agar *L. Monocytogenes* supplement were used to count the *L. monocytogenes* bacteria. For this purpose, 5 g of chicken meat samples were added to 45 ml of physiologic serum and serial dilutions (1:10) were done after homogenization. Then, 0.1 ml of diluted sample was placed on CHROM agar TM Listeria medium and at that point incubated for 24 h at 37 °C. After incubation, blue colonies with white halo were counted according to previously described method [25].

#### Sensory Characteristics

Sensory analysis was performed afterward heating of 60 g of samples in NaCl solution (0.6%) in as light boiling (one muscle/two solution) at 80 °C internal temperature of. Then, chicken meat samples (control and treatments) were randomly encoded and evaluated by a sensory group of 10 trained expert people. Meat samples were evaluated in terms of flavor, texture and overall acceptance based on the 5-point hedonic scale (one= the least desirable, 5= the most desirable). Before evaluation, meat pieces were cut into standard sizes and placed in a non-penetrating plastic container to reach the equilibrium temperature for 2 h. The panelists used distilled water at ambient temperature for oral washing between samples. Sensory evaluation of the treatments was also performed after 15, 30 and 60 days [21].

#### Statistical Analysis

**Table 1** Effect of *Thymus daenensis* Celak. and *Camellia sinensis* (L.) Kuntze extracts on pH changes in chicken meat samples during the frozen storage period.

| Treatment      | pH contents in days of storage |                |                |                |                |                 |                |
|----------------|--------------------------------|----------------|----------------|----------------|----------------|-----------------|----------------|
|                | 0                              | 10             | 20             | 30             | 40             | 50              | 60             |
| T <sub>0</sub> | 5.24 ± 0.02 ag*                | 5.57 ± 0.02 af | 5.70 ± 0.01 ae | 6.10 ± 0.05 ad | 6.21 ± 0.06 ac | 6.33 ± 0.02 ab  | 6.96 ± 0.03 a  |
| T <sub>1</sub> | 5.21 ± 0.04 ag**               | 5.42 ± 0.03 bf | 5.51 ± 0.02 be | 5.81 ± 0.07 bd | 6.02 ± 0.00 bc | 6.19 ± 0.01 b   | 6.68 ± 0.01 ab |
| T <sub>2</sub> | 5.22 ± 0.01 af                 | 5.41 ± 0.01 be | 5.50 ± 0.03 be | 5.75 ± 0.03 bd | 5.95 ± 0.00 bc | 6.10 ± 0.01 b   | 6.61 ± 0.03 ab |
| T <sub>3</sub> | 5.20 ± 0.04 ad                 | 5.29 ± 0.11 cd | 5.33 ± 0.02 cd | 5.49 ± 0.50 cd | 5.78 ± 0.12 bc | 6.00 ± 0.00 abc | 6.25 ± 0.19 ac |

\*Dissimilar small letters in each rows show statistically significant differences about  $P \leq 0.05$ .

\*\*Dissimilar capital letters in each column show statistically significant differences about  $P \leq 0.05$ .

All experiments were performed in a completely randomized design with three replications. All data were conveyed as mean plus standard deviation. The means were analyzed by SPSS 21 software and two-way analysis of variance (ANOVA) and repeated measures test with a significant level of less than 0.05 was used for statistical analysis.

## Results

#### pH Analysis

Table 1 represents the effects of *T. daenensis* and *C. sinensis* extracts on pH changes in chicken meat samples during the frozen storage period. Contents of pH of all studied samples has been increased in the storage period of 60 days. Increase in the contents of pH in the control group were entirely higher than other treatments ( $P \leq 0.05$ ). However, chicken meat samples of T<sub>3</sub> treatment had the lowest pH contents in the 60<sup>th</sup> day of storage period. Control group had the highest ( $6.96 \pm 0.03$ ) content of pH in the 60<sup>th</sup> days of storage period, while T<sub>3</sub> group had the lowest ( $6.25 \pm 0.19$ ).

#### TVN Changes

The results related to the effects of *T. daenensis* and *C. sinensis* extracts on TVN changes in chicken meat samples during the frozen storage period can be observed in Table 3. Contents of TVN of all studied samples have been increased in the storage period of 60 days.

Increase in the contents of TVN in the control group were entirely higher than other treatments ( $P \leq 0.05$ ). However, chicken meat samples of T<sub>3</sub> treatment had the lowest TVN contents in the 60<sup>th</sup> day of storage period. Control group had the highest ( $54.12 \pm 1.37$  mg/100 g chicken meat) content of TVN in the 60<sup>th</sup> days of storage period, while T<sub>3</sub> group had the lowest ( $33.68 \pm 0.64$  mg/100 g chicken meat).

**Table 2** Effect of *Thymus daenensis* Celak. and *Camellia sinensis* (L.) Kuntze extracts on TVN changes in chicken meat samples during the frozen storage period.

| Treatment      | TVN contents in days of storage (mg/100 gr chicken meat) |                 |                 |                  |                 |                 |                 |
|----------------|--|-----------------|-----------------|------------------|-----------------|-----------------|-----------------|
|                | 0  | 10              | 20              | 30               | 40              | 50              | 60              |
| T <sub>0</sub> | 14.70 ± 0.11 ag*   | 21.86 ± 0.69 af | 29.80 ± 0.96 ae | 35.49 ± 3.83 ad  | 43.34 ± 1.98 ac | 50.36 ± 1.03 ab | 54.12 ± 1.37 a  |
| T <sub>1</sub> | 14.54 ± 0.01 ag**  | 20.16 ± 0.68 bf | 27.25 ± 0.32 be | 28.63 ± 0.74 bd  | 35.15 ± 0.25 bc | 41.24 ± 0.87 b  | 48.09 ± 0.58 ab |
| T <sub>2</sub> | 14.51 ± 0.03 af  | 18.65 ± 2.05 be | 25.08 ± 2.17 bd | 25.15 ± 0.25 bcd | 30.86 ± 0.60 c  | 37.38 ± 0.29 bc | 41.11 ± 0.15 ac |
| T <sub>3</sub> | 14.51 ± 0.38 ae  | 16.37 ± 0.62 ce | 22.22 ± 1.77 cd | 21.40 ± 2.26 cd  | 28.57 ± 0.59 cd | 22.31 ± 0.04 bd | 33.68 ± 0.64 ad |

\*Dissimilar small letters in each rows show statistically significant differences about  $P \leq 0.05$ .

\*\*Dissimilar capital letters in each column show statistically significant differences about  $P \leq 0.05$ .

### TBARS Changes

In Table 3, the results show that the TBARS values of all studied samples have been increased in the storage period of 60 days. Increase in the contents of TBARS in the control group was entirely higher than other treatments ( $P \leq 0.05$ ). However, chicken meat samples of T<sub>3</sub> treatment had the lowest TBARS contents in the 60<sup>th</sup> day of storage period. Control group had the highest ( $1.67 \pm 0.04$  mg malonaldehyde/kg oil) content of TBARS in the 60<sup>th</sup> days of storage period, while T<sub>3</sub> group had the lowest ( $1.01 \pm 0.00$  mg malonaldehyde/kg oil).

The exponential growth of TBC in all studied samples has been increased in the storage period of 60 days. Chicken meat samples had counts between  $5.00 \pm 0.00$  to  $18.20 \pm 0.21$  log CFU/g. Increase in the TBC in the control group were entirely higher than other treatments ( $P \leq 0.05$ ). However, chicken meat samples of T<sub>3</sub> treatment had the lowest TBC in the 60<sup>th</sup> day of storage period. Control group had the highest ( $18.20 \pm 0.21$  log CFU/g) total bacterial counts in the 60<sup>th</sup> days of storage period, while T<sub>3</sub> group had the lowest ( $10.91 \pm 0.81$  log CFU/g) (Table 4).

### Total Bacterial Count

**Table 3** Effect of *Thymus daenensis* Celak. and *Camellia sinensis*(L.) Kuntze extracts on TBARS changes in chicken meat samples during the frozen storage period.

| Treatment      | TBARS contents in days of storage (mg malonaldehyde/kg oil) |                |                |                |                |                |                |
|----------------|---|----------------|----------------|----------------|----------------|----------------|----------------|
|                | 0   | 10             | 20             | 30             | 40             | 50             | 60             |
| T <sub>0</sub> | 0.15 ± 0.02 ag*   | 0.65 ± 0.02 af | 0.94 ± 0.04 ae | 1.06 ± 0.05 ad | 1.24 ± 0.04 ac | 1.43 ± 0.05 ab | 1.67 ± 0.04 a  |
| T <sub>1</sub> | 0.12 ± 0.02 aF1**   | 0.47 ± 0.04 be | 0.87 ± 0.02 bd | 0.89 ± 0.04 bd | 1.06 ± 0.05 bc | 1.21 ± 0.06 b  | 1.33 ± 0.04 ab |
| T <sub>2</sub> | 0.12 ± 0.02 ag  | 0.41 ± 0.01 cf | 0.79 ± 0.01 ce | 0.84 ± 0.03 bd | 0.99 ± 0.02 c  | 1.09 ± 0.05 bc | 1.20 ± 0.01 ac |
| T <sub>3</sub> | 0.10 ± 0.01 ag  | 0.35 ± 0.04 cf | 0.59 ± 0.01 de | 0.69 ± 0.04 cd | 0.90 ± 0.01 cd | 1.01 ± 0.02 bc | 1.01 ± 0.00 ad |

\*Dissimilar small letters in each rows show statistically significant differences about  $P \leq 0.05$ .

\*\*Dissimilar capital letters in each column show statistically significant differences about  $P \leq 0.05$ .

**Table 4** Effect of *Thymus daenensis* Celak. and *Camellia sinensis* (L.) Kuntze extracts on total bacterial counts in chicken meat samples during the frozen storage period.

| Treatment      | Total bacterial counts in days of storage (log CFU /g) |                |                 |                 |                 |                 |                 |
|----------------|--|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | 0  | 10             | 20              | 30              | 40              | 50              | 60              |
| T <sub>0</sub> | 5.23 ± 0.17 ag*  | 8.51 ± 0.54 af | 11.25 ± 0.91 ae | 13.46 ± 0.44 ad | 14.84 ± 0.23 ac | 16.02 ± 0.64 ab | 18.20 ± 0.21 a  |
| T <sub>1</sub> | 5.13 ± 0.12 ag**                                       | 7.63 ± 0.33 bf | 9.32 ± 0.27 be  | 10.53 ± 0.11 bd | 13.35 ± 0.31 b  | 14.49 ± 0.42 ab | 13.11 ± 0.90 bc |
| T <sub>2</sub> | 5.00 ± 0.00 af   | 7.22 ± 0.16 be | 8.87 ± 0.16 bd  | 9.37 ± 0.32 c   | 12.25 ± 0.30 bc | 13.07 ± 0.03 ac | 12.37 ± 0.93 bc |
| T <sub>3</sub> | 5.05 ± 0.03 ag   | 6.16 ± 0.24 ec | 6.91 ± 0.26 ce  | 8.16 ± 0.21 d   | 9.44 ± 0.59 C   | 11.08 ± 0.29 ad | 10.91 ± 0.81 bd |

\*Dissimilar small letters in each rows show statistically significant differences about  $P \leq 0.05$ .

\*\*Dissimilar capital letters in each column show statistically significant differences about  $P \leq 0.05$ .

**Table 5** Effect of *Thymus daenensis* Celak. and *Camellia sinensis* (L.) Kuntze extracts on *L. monocytogenes* counts in chicken meat samples during the frozen storage period.

| Treatment      | <i>L. monocytogenes</i> counts in days of storage (log CFU/g) |                |                |                |                |                |                |
|----------------|---|----------------|----------------|----------------|----------------|----------------|----------------|
|                | 0   | 10             | 20             | 30             | 40             | 50             | 60             |
| T <sub>0</sub> | 4.25 ± 0.06 ag*   | 4.74 ± 0.21 af | 4.49 ± 0.38 ae | 5.19 ± 0.68 ad | 6.49 ± 1.08 ac | 6.94 ± 0.92 aB | 7.01 ± 1.64 aA |
| T <sub>1</sub> | 4.23 ± 0.02 af**  | 4.39 ± 0.08 af | 4.62 ± 0.20 be | 5.05 ± 0.42 ad | 5.68 ± 0.14 bc | 6.49 ± 0.13 bB | 6.15 ± 0.22 bA |
| T <sub>2</sub> | 4.21 ± 0.04 af  | 4.38 ± 0.00 af | 4.67 ± 0.24 be | 5.09 ± 0.28 ad | 5.32 ± 1.47 bc | 6.00 ± 0.80 bB | 6.65 ± 1.64 bA |
| T <sub>3</sub> | 4.18 ± 0.05 af  | 4.33 ± 0.00 cf | 4.54 ± 0.36 be | 4.60 ± 0.48 bd | 4.50 ± 0.47 c  | 5.25 ± 0.34 dB | 5.82 ± 0.82 dA |

\*Dissimilar small letters in each rows show statistically significant differences about  $P \leq 0.05$ .

\*\*Dissimilar capital letters in each column show statistically significant differences about  $P \leq 0.05$ .

**Table 6** Effect of *Thymus daenensis* Celak. and *Camellia sinensis* (L.) Kuntze extracts on psychrophilic bacteria counts in chicken meat samples during the frozen storage period.

| Treatment      | Psychrophilic bacterial counts in days of storage (log CFU/g) |                |                |                |                 |                 |                 |
|----------------|---|----------------|----------------|----------------|-----------------|-----------------|-----------------|
|                | 0   | 10             | 20             | 30             | 40              | 50              | 60              |
| T <sub>0</sub> | 1.50 ± 0.43 ag  | 3.66 ± 0.22 af | 5.81 ± 0.59 ae | 6.58 ± 0.24 ad | 10.02 ± 0.51 ac | 10.90 ± 0.87 ab | 12.53 ± 0.57 a  |
| T <sub>1</sub> | 1.54 ± 0.13 ag  | 2.59 ± 0.19 bf | 4.37 ± 0.31 be | 4.60 ± 0.21 bd | 7.98 ± 1.30 bc  | 8.59 ± 1.08 b   | 10.67 ± 0.98 ab |
| T <sub>2</sub> | 1.36 ± 0.16 ag  | 1.90 ± 0.10 cf | 3.51 ± 0.27 cd | 3.37 ± 0.20 ce | 6.28 ± 0.10 c   | 6.77 ± 0.42 bc  | 9.73 ± 0.59 ab  |
| T <sub>3</sub> | 1.44 ± 0.37 ae  | 1.50 ± 0.49 ce | 1.46 ± 0.43 de | 2.01 ± 0.55 d  | 3.70 ± 0.35 cd  | 5.50 ± 0.31 bd  | 6.16 ± 0.22 ac  |

Dissimilar small letters in each rows show statistically significant differences about  $P \leq 0.05$ .

Dissimilar capital letters in each column show statistically significant differences about  $P \leq 0.05$ .

#### *L. monocytogenes* Counts

In Table 5, the effects of *T. daenensis* and *C. sinensis* extracts on *L. monocytogenes* counts in chicken meat samples at frozen temperatures (-18 °C) are presented. The results showed that there was an increase in *L. monocytogenes* growth up to 60<sup>th</sup> day in the control group equal to  $7.01 \pm 1.64$  Log CFU /g. The counts of all treated groups were significantly less than the control group during the whole storage period ( $P < 0.05$ ). However, chicken meat samples of T<sub>3</sub> treatment had the lowest *L. monocytogenes* counts in the 60<sup>th</sup> day of storage period.

#### Psychrophilic Bacteria Count

The results demonstrated that the count of psychrophilic bacteria in the control group increased up to 60<sup>th</sup> day during the frozen storage period, and it reached to about  $12.53 \pm 0.57$  Log CFU/g (Table 6). Significant differences were observed between the all treated groups and control on day 60 ( $P \leq 0.05$ ). However, chicken meat samples of T<sub>3</sub> treatment had the lowest psychrophilic bacteria counts in the 60<sup>th</sup> day of storage period.

#### Sensory Characteristics

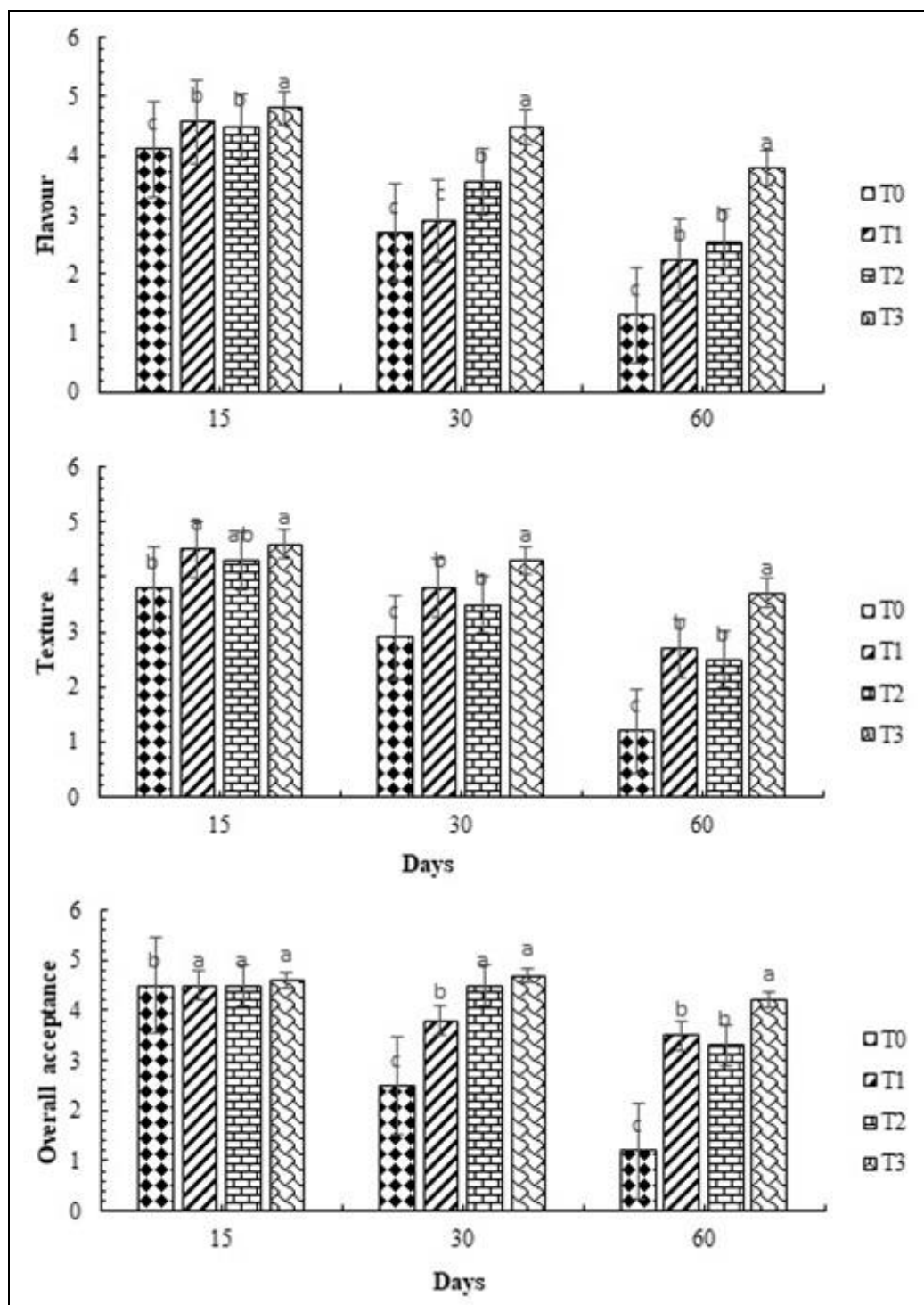
According to our results (Fig. 2), the use of simultaneous *T. daenensis* and *C. sinensis* ethanolic extracts for coating chicken meat increased total

acceptance to a higher level than that of the control sample ( $P \leq 0.05$ ). However, the mean value given to all studied samples have been decreased in the frozen storage period of 60 days. There were not significant differences for total acceptance between the *T. daenensis* group and *C. sinensis* group in the 60<sup>th</sup> days, but the overall acceptability of *T. daenensis* group was significantly higher than *C. sinensis* group in the 30<sup>th</sup> days ( $P \leq 0.05$ ).

#### Discussion

The results of the present study showed that *C. sinensis* and *T. daenensis* ethanolic extracts had some compounds, which were suitable to use as source of antioxidant and antimicrobial agents. Several investigations showed the high antimicrobial and antioxidant activities of *C. sinensis* extract [26,27].

Another part of the present investigation was done to assess the effects of *C. sinensis* and *T. daenensis* ethanolic extracts on shelf life of chicken meat samples. Totally, pH content of chicken meat samples can be related to several factors such as nutrition, temperature, storage conditions and meat buffering capacity.



**Fig. 2** Effect of *Thymus daenensis* Celak. and *Camellia sinensis* (L.) Kuntze extracts on sensory characters (flavor, texture and overall acceptance) of chicken meat samples during the frozen storage period. Dissimilar letters in each column of figures showed statistical significant differences about  $P \leq 0.05$ .

An upsurge in pH may be because of construction of volatile compounds including ammonia and trimethylamine by activity of endogenous enzymes or microbial enzymes [28]. Mohammadzadeh and Rezaei (2012) stated that increase in the pH of immersed meat samples in a solution contain

polyphenol components was less than the control treatment [10]. Therefore, lower pH contents of samples treated with *T. daenensis* and *C. sinensis* ethanolic extracts in comparison with control group can be attributed to the antibacterial and antioxidant properties of ethanolic extracts.

Antibacterial activity of *T. daenensis* and *C. sinensis* extracts is due to the presence of biological compounds such as thymol, carvacrol, cymene, epigallocatechingallate and theobromine [29,30].

A wide range of volatile basic compounds, including ammonia, methylamine, dimethylamine, trimethylamine and other similar compositions produced by microbial activity and endogenous enzymes are considered as TVN. TVN is mainly used as an indicator for meat deterioration especially in chicken meat samples. TVN content of 40 to 50 mg/100 g meat is deterioration criteria used for study the quality of chicken meat samples [31]. Results of the present study showed that TVN contents in the control group were exceeded than the limited standard at 40 days of storage. However, TVN contents in treated samples with *C. sinensis* and *T. daenensis* extracts were not exceeded from the limited standards even at the end of storage period. Soncu and Kolsarici [32] stated that *C. sinensis* extract caused a significant reduction of TVN during the processing of nuggets and chicken burgers. Baydar, Sagdic [33] stated that the lower amounts of TVN in samples treated with herbal extracts. They showed decrease in the TVN contents can be attributed to the antibacterial and antioxidant activities and especially presence of phenolic compounds in studied medicinal herbs. Malonaldehyde is an appropriate indicator for determining the progression of lipid oxidation. The presence of such compounds in chicken meat causes changes in its sensory characteristics including flavor and odor [34]. Increase in the TBARS index can be due to the enhancement of free iron and other pro-oxidant components in meat, as well as the production of aldehydes from secondary products resulting from decomposition of hydroperoxides [35]. The results demonstrated that the amounts of TBARS in chicken meat samples treated with *C. sinensis* and *T. daenensis* extracts were entirely lower than those of control group. This matter is mainly due to the higher antioxidant effects of *C. sinensis* and *T. daenensis* extracts. Haghparast and Kashiri [36] conveyed the lower amounts of lipid oxidation indicators such as TBARS and fatty acids in food samples treated with *C. sinensis* which was similar to our findings. Mohammadzadeh and Rezaei [10] stated that complete immersion of Rainbow Trout into the *C. sinensis* extract caused significant decrease in the TBARS contents compared to the control group.

A wide range of antimicrobial activity of *T. daenensis* and *C. sinensis* extracts were also found in the present study. This matter is mainly due to the presence of polyphenolic compounds in the *T. daenensis* and *C. sinensis* extracts. Polyphenolic compounds can easily penetrate into the microbial cell membrane and cause significant changes in the metabolism of RNA and DNA units with inhibit microbial growth and proliferation [37]. Carvacrol, o-cymene, dodecane,  $\gamma$ -terpinene, pulegone, limonene, cineole and neomenthol were the most important antimicrobial compounds in *T. daenensis* extract. Catechin, epicatechin, epigallocatechin, catechin epigallocatechingallate, that called catechingallates were the most important antimicrobial compounds in *C. sinensis* extract [32, 38]. Similar antimicrobial effects have been conveyed previously [39-41]. Thymol and carvacrol belonging to monoterpenoid phenol are the main constituents in the extract from *T. daenensis*. Carvacrol is an isothymol that inhibited activity of ATPase enzymes; and to cause increasing nonspecific penetrable bacterial cell membrane, so increase microorganism sensitivity to entry extraneous matters [42, 43]. Cis space structure of essential components is another important factor, which increase the antimicrobial effects of medicinal herbs. Cis space structure around the twofold linkage cause hyper antibacterial activity and hyper reaction group in aliphatic alcohols (like linalool) and phenols (like thymol) [42,43]. Additionally, maximum investigations on the mechanism of action of phenolic compositions intensive on their activities on cell membranes such as changing their functions, structures, diameters and permeabilities. Carvacrol and thymol caused increase in the permeability of cell membrane and act inversely toward bacteria [44]. Cymene in combination with carvacrol caused severe changes in the transportation of carvacrol across the membrane. Accordingly, the antimicrobial effects of carvacrol is amplified due to presence of cymene [45].

Using *T. daenensis* and *C. sinensis* extracts were also caused significant changes in the organoleptic properties of chicken meat samples. Otherwise, they caused significant increase in the values given to flavor, texture and overall acceptance of chicken meat samples. This finding is mainly due to the presence of fragrant and tasty components in the *T. daenensis* and *C. sinensis* extracts. Thus, using these two natural extract can



be recommended as suitable preservatives with high antibacterial and antioxidant effects on chicken meat samples.

## Conclusion

To put it in a nutshell, the present study was the first report of using of *T. daenensis* and *C. sinensis* ethanolic extracts as antimicrobial and antioxidant agents to increase the shelf-life of chicken meat samples. Based on results, both *T. daenensis* and *C. sinensis* ethanolic extracts were suitable candidate as natural preservatives with high antimicrobial and antioxidant properties in chicken meat samples. However, further studies are required to found nutritional indices and biological activities of *T. daenensis* and *C. sinensis* extracts on other food models.

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