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Original Article

Antioxidant Effects of Chitosan Coating Containing *Thymus fedtschenkoi* Ronneger Essential Oil and Thymol on the Chicken Fillet During Refrigeration

Yaser shahimoridi, Hassan Hassanzadazar* and Majid Aminzare

Department of Food Safety and Hygiene, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran

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***Corresponding author**
Hassanzadazar_h@zums.ac.ir

ABSTRACT

The purpose of this study was to evaluate the antioxidant effect of chitosan coating containing *Thymus fedtschenkoi* Ronniger essential oil (TFEO) and Thymol on chicken fillets under refrigerated conditions. The antioxidant power of prepared coating solutions containing TFEO (1%) and thymol (1%) and their efficacy on the quality of chicken meat during refrigeration (4 ºC) were evaluated using in-vitro techniques (DPPH scavenging and reducing power assays) and in-vivo methods (Determination of Peroxide value (PV), Thiobarbituric acid reactive substance (TBARS), Total carbonyl and Sensorial attributes), respectively. Antioxidant poverty of TFEO was higher than thymol in all concentrations. The results showed that an increasing level of PV, TBARS and carbonyls in the treated samples had a slower trend than in control samples ($P \leq 0.05$). The best antioxidant effect was obtained for chitosan-coated samples containing TFEO 1%. The panelists with sensory attributes were significantly more satisfied with coated samples containing essential oil than the control samples. The chitosan coating containing TFEO 1% could be proposed as a new coating to protect food against oxidative changes.

INTRODUCTION

Poultry meat is consumed as one of the favored foods worldwide and has the desirable nutritional quality for human health. Chicken meat has been preferred over other poultry meats because of its low cost, less fat, easy and fast cooking, portability, ease of digestion and the possibility of producing more and easier than other meats Lipid and protein oxidation are the main reasons for chemical spoilage of chicken meat resulted to the decline of shelflife [1]. Nowadays, one of the main challenges of the food industry is the prevention of chemical spoilage and retarding the lipid and protein oxidation of chicken meat. Several methods have been used to increase the shelf life of foods including adding different food preservatives directly to food or as active packaging to protect food quality [2]. Nowadays, the tendency to use natural preservatives have been increased [3]. Essential oils, plant extracts, bacteriocins, and organic acids are examples applied in food industries as natural preservatives [1-5]. The increasing demand of consumers for fresh, minimally processed, without additives and safe foods has forced food industries to produce food products with minimum nutritional and sensory quality impairment using many non-thermal food processing technologies such as active packaging [1-5]. Food packaging acts as a barrier that improves food quality by protecting against undesirable chemical and microbial agents [6]. Active packaging is a system of packaging that contains components (antimicrobials and antioxidants) such as essential oils that can be released into the environment surrounding the food (active-releasing systems) [6]. During the last two decades, the tendency to use biodegradable packaging materials has grown due to their non-toxicity, biodegradable and eco-friendly attributes [7]. Several studies have been performed on the development of biocompatible packaging materials provided by biopolymers as edible coatings or films. Polysaccharides, lipids and proteins are common materials to provide biopolymers to obtain packaging [1,8]. Direct addition of essential oils, plant extracts and their active ingredients to foods may accompany

undesirable organoleptic attributes. In this regard, edible films and coatings are considered good carriers to reduce adverse effects of this type of natural preservatives [3]. Chitosan as a unique cationic polysaccharide polymer is produced by the deacetylation of chitin obtained from crustacean shells. The biopreservative ability of chitosan has been demonstrated in several studies [1,2]. Functional and innate properties of this polymer such as high coating and film-forming ability as edible film, antimicrobial, antifungal, and antioxidant properties make it a good candidate for food packaging [9]. Enhancement of Antimicrobial and antioxidant effects of chitosan coatings and films combined with different natural bioactive components have been reported in various foods [1]. Medicinal plants and plant-derived antioxidant compounds are introduced with enormous free radical scavenging potential that can be used in pharmaceutical sciences and the food industry because they are a promising source of bioactive molecules such as polyphenols that make them effective at neutralizing free radicals and reactive oxygen species due to their ability as efficient electron donors or hydrogen atoms, resulting in their antioxidant and other biological functions [10,11]. *T hymus fedtschenkoi Ronniger* is a perennial plant, woody at the basal part, semi-shrubby, very branched with ovate to triangular aromatic leaves which is known as ‘‘Avishane gharebaghi” in Persian [12]. It was known as a medicinal plant in Iran, especially in Kerman province (Iran) due to its pharmacological properties like other thyme species. The antispasmodic, anti-flatulence, anti-rheumatic, anti-sciatic, antiseptic, tonic, carminative, digestive, anti-inflammatory, antitussive, expectorant antifungal, antiviral, antibacterial, antiparasitic, antioxidant effects of *Thymus* genera have been demonstrated in several studies [13]. The pharmacological and biological properties of medicinal plants depend on the chemical composition of their essential oils [13]. The most important compounds in *Thymus* essential oil are thymol and carvacrol. Several essential oils and their main ingredients have been introduced into food packaging to preserve food quality, prolonging the shelf life and retardation of food oxidative reactions [6,9]. However, there are no reports on chitosan coatings impregnated with the essential oil of *Thymus fedtschenkoi* Ronniger (TFEO) in chicken meat during refrigeration. Therefore, this study aimed to evaluate the antioxidant effect of chitosan coating containing *Thymus fedtschenkoi* Ronniger essential oil and thymol on chicken fillets during refrigerated conditions.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). *Thymus fedtschenkoi* Ronniger was collected from the mountains of Baft City (Kerman province –Iran) in April 2020. Voucher samples were identified and deposited at the herbarium of Herbal and Traditional Medicines Research Center (KF 1431), Kerman University of Medical Sciences, Kerman, Iran.

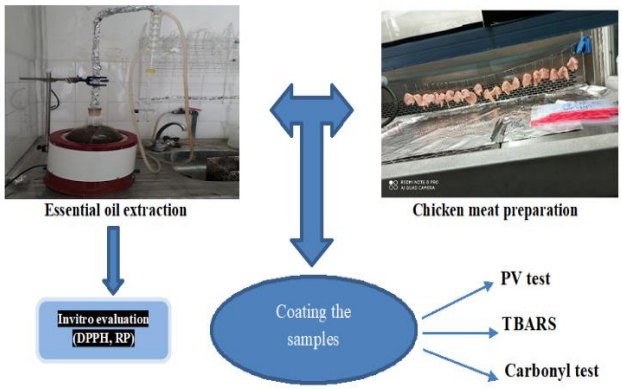

Fig. 1 The stages of the conducted experiments

Essential Oil Extraction

The collected plant was dried in the shade at room temperature. The dry material of *T. fedtschenkoi Ronniger* (200 g) was immersed in water (1000 ml) and subjected to hydro-distillation using a Clevenger-type apparatus for 3 hours (Fig 1). This process was repeated several times to obtain enough volume of essential oil (EO). The obtained EO was filtered through 0.22 micron filters and after dehydration with sodium sulfate was kept in covered glass tubes and stored at 4 °C for further uses [1,3].

GC-MS Analysis

The composition of TFEO was analyzed using a gas chromatographic-mass spectrometric (GC/MS) apparatus (AGILENT 6890; Agilent Technologies, Santa Clara, USA) with an HP-5MS column (30 m × 0.22 mm, 0.25 m film thickness). The temperature of the injector and detector was 250 °C and 265 °C, respectively. The temperature program of the oven was set at first at 50 °C and raised to 265 °C with a gradual increase of 2.5 °C per minute and held isothermal for 30 minutes. The carrier gas was

helium with 1 cm^3 flow rate per minute. For mass spectra recording, the electron ionization voltage was 70 eV in a range of 40- 450 m/z.

Data processing was performed based on comparing acquired retention indices with standard mass spectra using MSD chem Station software (revision E01.01.335; Agilent Technologies) combined with the stored Wiley 7 n.1 Mass Computer Library (Wiley-VCH 2001 data software, Weinheim, Germany) and valid NIST MS Search (ver. 11.0). Relative amount of each component was reported based on peak area in chromatogram as percentages [14].

Assessment of Antioxidant Activity

The antioxidant ability of TFEO and thymol was evaluated in-vitro based on their potential in DPPH (2,2 Diphenyl-2-picrylhydrazyl) radical scavenging and reduction power of Ferric to ferrous. After that antioxidant power of prepared coating solutions containing TFEO and thymol and their efficacy on the quality of chicken meat during refrigeration (4ºC) were evaluated using in vitro techniques (DPPH scavenging and reducing power assays) and in-vivo methods (Determination of Peroxide value (PV), Thiobarbituric acid reactive substance (TBARS), Total carbonyl and Sensorial attributes), respectively (Fig. 1).

Preparation of Chitosan Coating

The coating solution was made ready based on the described method by Yousefizadeh *et al*. (2022) [15]. Briefly, for each treatment, 2 g chitosan powder was solved in 100 ml acetic acid (1%) with stirring for 3 h. at room temperature. Glycerol was added as a plasticizer (1.5 ml) to the chitosan solution and was stirred for 10 minutes on a magnetic hotplate. Before addition, different concentrations of TFEO (0.06, 0.125, 0.25, 0.5, 1, 1.5 1nd 2%) and thymol (0.06, 0.125, 0.25, 0.5, 1, 1.5 and 2%), the mixtures were filtered by Whatman filter papers (No. 2).

After addition TFEO and thymol concentrations separately, final solutions were homogenized at 10000 rpm for 2 minutes under sterile condition using a homogenizer (KMA, Germany). A chitosan coating solution without TFEO and thymol concentrations was set as a control treatment.

In Vitro Assessment of Antioxidant Activity

DPPH Radical Scavenging Assay

Antioxidant potential and power of scavenging free DPPH radicals of chitosan coatings containing TFEO (0.06, 0.125, 0.25, 0.5, 1, 1.5 1nd 2%) and thymol (0.06, 0.125, 0.25, 0.5, 1, 1.5 1nd 2%) were evaluated in-vitro. For this purpose, 0.1 ml of each chitosan solution was added to a methanolic solution containing 0.5 mM DPPH (3.9 ml) and 99.5 % methanol (2.4 ml). The mixtures were homogenized (IKA T10, Staufen, Germany) for 1 min., and placed in a dark room for 1 h. at room temperature. The absorbance of solutions was measured spectrophotometrically (Milton Roy Company, Warminster, USA) at 517 nm wavelength. The discoloration rate of solutions shows the scavenging ability rate of DPPH free radicals. A chitosan coating solution containing butylated hydroxytoluene 1% (BHT 1%) is mentioned as a positive control [15]. The final scavenging rate of each treatment was calculated by the following formula:

DPPH scavenging ability (%)=(blank absorbance-Test absorbance)/(blank absorbance) ×100.

Reducing Power Assay

Reducing power or the electron-donating ability of chitosan coatings containing different concentrations of TFEO (0.06, 0.125, 0.25, 0.5, 1, 1.5 1nd 2%) and thymol (0.06, 0.125, 0.25, 0.5, 1, 1.5 1nd 2%) was evaluated in-vitro [15]. Briefly, 400 µL of each treatment was added to a mixture solution containing 1ml sodium phosphate buffer (pH=6.6) and 1ml potassium ferricyanide 1%. After homogenization (IKA T10 basic, Staufen, Germany) for 2 minutes, the final mixtures were placed in an incubator at 55 °C for 20 minutes. In the following, 1 ml trichloroacetic acid (10% w/v) was added to the test tubes. The solutions were centrifuged at 2500 rpm for 10 minutes. 1 ml of the supernatant solution was mixed with 1 ml of distilled water and 200 µL of 0.1% (w/v) ferric chloride. After 10 minutes, the absorbance of solutions was measured using a spectrophotometer (Milton Roy Company, Warminster, USA) at 700 nm wavelength. A chitosan coating solution containing butylated hydroxytoluene 1% (BHT 1%) is mentioned as the positive control. A higher absorbance rate indicates a higher reducing power of the reaction solutions of TFEO and thymol.

In vivo Assessment of Antioxidant Activity (food model)

Preparation of Chicken Fillets

Fresh chicken carcasses were provided from the Zanjan meat center market and transported to the

laboratory under cold conditions. All chickens were washed and filleted into sterile pieces of equal size weighing about 25 g fillets and kept at 4 °C.

Coating Chicken Fillets

According to the obtained results of the Invitro assessment, the best biological concentration of TFEO (1%) and thymol (1%) was chosen to prepare chitosan solutions for coating the fillets. Four groups of samples coated with chitosan solutions containing TFEO (1%) and thyme (1%), separately including uncoated fillets, fillets coated with chitosan (2%) without active ingredients, coated samples with chitosan solution containing TFEO (1%) and coated fillets with chitosan solution containing thymol (1%). All samples were drained and dried under the sterile condition for 30 minutes and were packed in sterile LDPE plastic bags, labeled and stored in the refrigerator (4±1 °C) for 12 days. Chemical and sensory qualities were evaluated on days 0, 3, 6, 9 and 12.

Evaluation of Peroxide Value (PV)

The peroxide value of treated chicken samples was conducted according to the described method by Arfat *et al*. (2015) with some modifications based on lipid extraction [16]. Methanol and chloroform mixture in distilled water (DW) was used as a solvent for lipid extraction [17]. Briefly, 1 gram of extracted lipid sample was dissolved in 30 ml of chloroform acetic acid (2:3 V/V) solution. Then, 0.5 ml of saturated potassium iodide solution was added and the mixture was kept in the dark room for 1 min. After the addition of 30 ml of DW, 0.5 ml of starch solution (1% w/v) was added as an indicator. Liberated iodine from potassium iodide was titrated with a standardized solution of 0.01 N sodium thiosulfate. Titration was continued to change the solution color to milky white. The results were expressed as milliequivalents of free iodine per kilogram of lipid and were calculated using the following formula [16]:

$$PV = \frac{1000 \times (V1 - V0) \times N}{W}$$

V_1 = Used volume of sodium thiosulfate for each sample (ml); V_0 = Used volume of sodium thiosulfate for blank solution (ml); N= Normality of Sodium thiosulfate; W= Sample weight (g)

Determination of Thiobarbituric Acid Reactive Substance (TBARS)

The TBARS value was measured according to the described method by Hassanzadazar *et al*. (2018) calorimetrically with some modifications [18]. Briefly, 1 g of each sample was added to the mixture of acetic acid (5%) and BHT solutions (5 ml+5 ml). The homogenized mixture was centrifuged at rmp3000 rpm. for 10 minutes. The upper phase was discarded and 2.5 ml of the lower phase was mixed in another tube with 1.5 ml of the prepared BHT solution and homogenized. Then, the mixture was placed in a water bath (75 °C) for 30 minutes to complete the reaction. After cooling, the absorbance of solutions was read spectrophotometerically (Milton Roy Company, Warminster, USA) at 532 nm wavelength. A standard curve was used to calculate the amount of TBA based on mg of malondialdehyde per kg of sample. 1,1,3,3-Tetra-methoxypropane (TMP) was used to provide the standard curve.

Determination of Protein Oxidation

Protein oxidation was evaluated by the carbonyl method as described by Tripaldi *et al*. (2020) with some modifications [19]. Briefly for carbonyl quantification, 1 g of meat sample was homogenized in 10 ml of potassium chloride solution (0.15 M) using a homogenizer (IKA T10 basic, Ultra turax, Germany) for 60 S. In an Eppendorf vial, 100 microliter of homogenate was added to 1 ml of 10% trichloroacetic acid and centrifuged for 5 min at 6000 rpm. Then the supernatant was discarded and 1 ml of 2 M HCl with 0.2% 2,4-dinitrophenyl hydrazine (DNPH) was added to the tubes. After incubation at room temperature for 1 h and shaking every 15 min., 1 ml of 10% trichloroacetic acid was added to the mixture and again vortexed and centrifuged for 5 min at 6000 rpm. The supernatant was discarded carefully with the Pasteur pipet without damaging the precipitate. The precipitate was washed with 1 ml of ethanol/ethyl acetate solution (1:1), vortexed, and centrifuged for 5 min at 8000 rpm. The washing and centrifuging procedure was repeated two to three times. Then, the precipitate was completely dried. After this, The precipitate was dissolved in the mixture solution containing 1.5 ml of sodium phosphate buffer (20 mM) with guanidine hydrochloride (6 M) with a final pH=6.5. It was shaken and centrifuged for 2 min at 6000 rpm. Carbonyl concentration was measured

spectrophotometrically at 370 nm. Carbonyl concentration (nano-molar) was calculated as:

[Abs 370nm/21.0 /mM/cm) ×1000]

21.0 /Mm/cm is the molar extinction coefficient of carbonyls.

Sensory Analysis

The effect of chitosan coating impregnated with TFEO and thymol on organoleptic attributes of chicken fillets was evaluated by 10 panelists selected and trained from staff and students of the School of Public Health, Zanjan University of Medical Sciences, Iran. The meat samples were cut into little cubes and cooked in a microwave oven for 10 min. A complete block design was performed and randomly served. Taste, color, odor and overall acceptability were analyzed using 5 5-point hedonic scale (1: really dislike, 5: really like). The average scores of taste, color and odor were considered to obtain overall acceptability [1,2].

Statistical Analysis

All experiments were conducted in three repetitions at 5 time periods for 4 treatments and analyzed using SPSS software version 19 (SPSS, Inc., Chicago, IL). One-way ANOVA and Tukey's test were used for statistical analysis of the data and determination of the significant difference between the samples ($P < 0.05$). All results were expressed as mean values ± standard deviation in tables and figures.

RESULTS AND DISCUSSION

GC–MS Analysis of *Thymus Fedtschenkoi* Essential Oil

The chemical composition of TFEO is presented in Table (1). The dried PF plant yielded 1.3 % (v/w) of EO. Twenty-thirty various components, representing 99.9% of total TFEO. The main compounds of TFEO were thymol (56.30%), Glycidyl Oleate (5.81%), Oleate (5.33%), Beta-Simon (4.16%) and gamma-terpinene (3.66%). Most of the volatiles recorded in TFEO belong to the monoterpene group, and thymol is one of the most important compounds of oxygen monoterpene with antioxidant, antibacterial and antifungal properties [20]. In agreement with this study, Hasani (2013) found that thymol (62.15%) was the main compound of *Thymus fedtschenkoi* essential oil followed by carvacrol (4.82%) and p-cymene (12.03%), respectively [21]. But in contrary to the present study, Ghelichnia (2018) reported that the main component of TFEO was Carvacrol (41.84%) [12]. Such variations in the chemical composition of essential oils can caused by the harvesting time and stage of maturity, conditions of EO extraction, plant organ used for extraction, soil composition, plant cultivars and genetics [1].

In vitro Antioxidant Ability

The antioxidant power of the free form of TFEO and thymol and chitosan coating containing TFEO and thymol based on DPPH scavenging ability and reducing power are shown in Table 2 and Table 3, respectively. The results showed that the antioxidant activity in all samples was dose-dependent ($P \leq 0.05$) and higher concentrations of TFEO (2%) and thymol (2%) showed higher antioxidant properties after BHT as a synthetic standard antioxidant. Antioxidant poverty of PFEO was higher than thymol in all concentrations which might be due to synergetic effects of trace components present in TFEO [22]. Comparing of same concentrations of antioxidant properties of TFEO and thymol in free and coating forms showed expected decreasing antioxidant ability in coating form. Dadashpour *et al*. (2011) showed higher DPPH scavenging and reducing power of *Thymus daenens*is than thymol which is in agreement with the results of the present study [23].

Effect of Chitosan Coating containing TFEO and thymol on Peroxide value

Producing free radicals from fatty acids due to active agents like the presence of oxygen, ambient temperature, light and chemical oxidants leads to lipid oxidation in chicken meat and results in changes in sensory attributes such as discoloration and off-flavor plus with reduction in nutritional quality. The first sign of lipid oxidation is the production of hydroperoxides [24]. The primary oxidation in food was measurable by the determination of peroxide value (PV). The content in mEq oxygen/kg of oils extracted from meat samples is stated as PV. The proposed maximum level of hydroperoxides is 10 meq peroxide/kg meat fat [24]. PV changes in each treatment during the 12-day storage period are shown in Figure 2. The initial PVs of filet samples were in the range of 0.34 to 0.46 meq/kg of lipid and increased during the stages of storage to maximum levels at control (9.65 ± 0.48 meq/kg of lipid) and lowest amount in coated treatments with chitosan containing thymes 1% (3.7 ± 0.32 meq/kg of lipid) at the last day of refrigeration.

Table 1 Chemical composition of *Thymus fedtschenkoi* essential oil.

NO	General name	Retention Time (min.)	Kovats indices	Area(%)
1	α-Thujene	9.199	1010	0.67
2	α-Pinene	9.402	1015	0.75
3	Camphene	9.765	1021	0.73
4	3-Octanone	10.350	1041	1.5
5	*β*-myrcene	10.465	1055	1.25
6	4-Carene	11.139	1098	1.29
7	β-Cymene	11.330	1131	4.16
8	Cineole	11.501	1155	0.89
9	Linalool	12.786	1167	1.42
10	Camphol	14.374	1288	3.35
11	4-Terpineol	14.536	1366	1.89
12	Thymol methyl ether	15.458	1399	0.92
13	4-Isopropylanisole	15.655	1407	2.82
14	Ascaridol	16.317	1499	1.14
15	Thymol	16.819	1550	56.30
16	Caryophyllene	19.383	1600	3.02
17	γ-Terpinene	12.029	1188	3.66
18	Oleate	30.012	1650	5.33
19	Acilitia	30.062	1680	0.69
20	Glycidyl Palmitate	31.207	1700	0.62
21	Glycidyl oleate	32.893	1760	5.81
22	AC1NSKA3	33.453	1810	0.77
23	17-Pentatriacontene	33.561	1835	0.9
Total	-	-	-	99.9

Table 2 Antioxidant power of different concentrations of free TFEO and thymol using DPPH scavenging and reducing power assays

DPPH scavenging assay							
Concentration (mg/ml)	20	15	10	5	2.5	1.25	0.06
T. fedtschenkoi	84.92 ± 2.8 aA	81.02 ± 4.9 aA	78.24 ± 2.2 aA	65.23 ± 2.1 bA	50.70 ± 3.9 cA	35.99 ± 2.3 dA	26.30 ± 4.1 eA
Thymol	80.06 ± 4.5 aA	74.23 ± 7.8 aA	71.03 ± 0.6 aB	56.46 ± 3.0 bB	41.55 ± 3.7 cB	26.06 ± 6.2 dB	21.38 ± 1.9 dA
BHT	98.15 ± 0.2 aB	97.19 ± 1.7 aB	95.78 ± 1/6 bC	85.99 ± 1.2 cC	74.26 ± 3.5 dC	63.99 ± 3.9 eC	57.02 ± 8.6 eB
Reducing power assay							
T. fedtschenkoi	1.28 ± 0.01 aA	1.21 ± 0.01 BA	1.18 ± 0.07 bA	1.02 ± 0.08 bA	0.81 ± 0.08 cA	0.60 ± 0.05 dA	0.44 ± 0.12 eA
Thymol	1.19 ± 0.01 aA	1.11 ± 0.02 bA	1.03 ± 0.05 bA	0.87 ± 0.1 cB	0.64 ± 0.05 dB	0.52 ± 0.02 eB	0.25 ± 0.1 fB
BHT	1.32 ± 0.03 aA	1.20 ± 0.01 bA	1.12 ± 0.01 bA	1.08 ± 0.01 cA	0.95 ± 0.07 dC	0.70 ± 0.1 eC	0.48 ± 0.1 fA

Different capital letters in each column show significant difference among treatments ($P \leq .05$)
Different small letters in each row show significant difference among treatments ($P \leq .05$).

Table 3 Antioxidant power of chitosan coating containing three concentrations of TFEO and Thymol

DPPH scavenging assay							
Concentration (mg/ml)	20	15	10	5	2.5	1.25	0.06
T. fedtschenkoi	78.02 ± 2.1 aA	69.56 ± 7.03 bA	64.07 ± 3.05 bA	49.08 ± 2.25 cA	41.59 ± 5.6 cA	30.59 ± 0.99 dA	22.19 ± 2.95 eA
Thymol	76.23± 1.65 aA	66.49 ± 5.2 bA	61.83 ± 3.54 bA	46.87 ± 2.17 cA	35.42 ± 4.5 dA	22.86 ± 4.96 eB	19.71 ± 2.5 eA
BHT	90.55 ± 1.23 aB	87.19 ± 8.17 aB	82.01 ± 1.08 bB	75.36 ± 6.3 bB	65.02±4.05 bB	57.88 ± 5.31 bC	45.96 ± 6.87 cB
Reducing power assay							
T. fedtschenkoi	1.26 ± 0.09 aA	1.18 ± 0.07 aA	1.15 ± 0.06 aA	0.98 ± 0.1 bA	0.81 ± 0.08 cA	0.44 ± 0.09 dA	0.3 ± 0.1 eA
Thymol	1.10 ± 0.01 aB	1.07 ± 0.06 aA	0.97 ± 0.17 aB	0.83 ± 0.12 bB	0.53 ± 0.25 cB	0.46 ± 0.06 dA	0.23 ± 0.11 eB
BHT	1.30 ± 0.1 aA	1.19 ± 0.01 bA	1.13 ± 0.15 bC	0.91 ± 0.1 cA	0.84 ± 0.16 cC	0.66 ± 011 dB	0.47 ± 0.15 eC

Different capital letters in each column show significant difference among treatments ($P \leq .05$)
Different small letters in each row show significant difference among treatments ($P \leq .05$).

Raising Trend of PV in treated samples with chitosan+PFEO 1% was lower than samples coated with chitosan+thymol 1%, but no significant difference was seen between the coated treatments containing PFEO and thymol on the first and last days of storage ($P \geq 0.05$). The obtained results showed that the increasing level of PV in the treated samples had a slower trend than control samples ($P \leq 0.05$). The oxidative reactions were inhibited because of the release of the phenolic compounds from chitosan coatings containing PFEO and thyme. It has been proven that phenolic compounds react with oxygen during the autoxidation process. This phenomenon delays the beginning of the oxidative process in lipids [25]. This result is completely in line with the reported results by Giatrakou *et al.* (2010) and Bazargani-Gilani, Aliakbarlu, & Tajik. (2015) conducted on ready-to-cook poultry products and chicken meat [26].

Effect of Chitosan Coating Containing TFEO and Thymol on TBARS Value

TBARS method was used to evaluate lipid oxidation and the thiobarbituric acid index shows the progression degree of secondary metabolites of lipid oxidation. The results of TBARS changes in poultry filet samples during refrigeration are demonstrated in Figure 3. The initial content of TBARS value was 0.34-0.46 mg MDA/kg in the fillets. An increasing trend was observed in all the sample groups during storage time, but TBARS values in the samples containing TFEO and thymol were significantly lower than those in the control sample ($P \leq 0.05$). The best antioxidant effect was obtained for coated samples containing TFEO 1% which attained its maximum value with 1.1 ± 0.09 mg MDA/kg filet at the end of the storage time. According to previous studies, TBARS value higher than 1 mg MDA/ kg tissue shows initiating spoilage of meat samples [26]. Jouki *et al*. (2020) reported that 3 mg MDA/kg tissue is the maximum limit of TBARS value showing good quality of the meat [24]. The main reason for the lower content of TBARS value in the treated samples with TFEO and thymol is the presence of phenolic compounds in the TFEO that can interact with free radicals and prevent the initiating of the radicals chain, decompose produced peroxides and bind with metal ions [24].

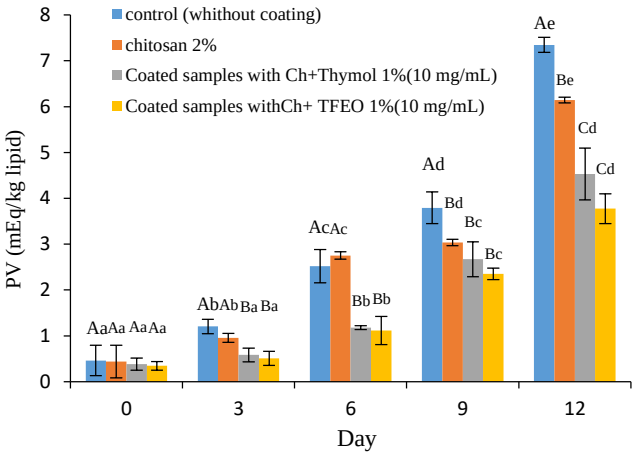

Fig. 2 Changes in peroxide value (meq/kg of lipid) of coated chicken filet samples during 12 days storage at (4 ˚C)

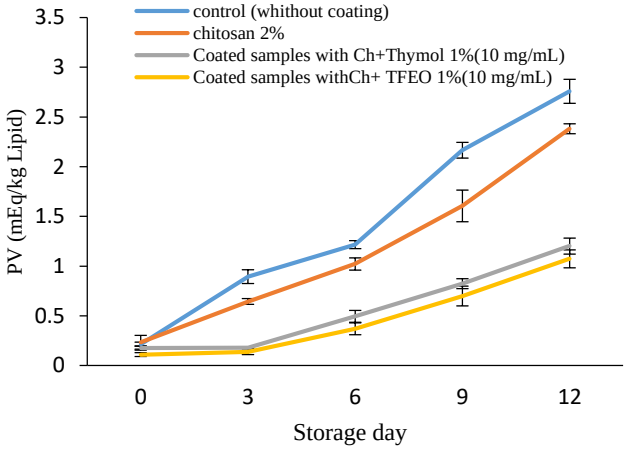

Fig. 3 Changes in TBARS values (mg malonaldehyde/kg sample) of poultry filet couting with chitosan during 12 days storage at refrigeration temperature (Mean ± SD).

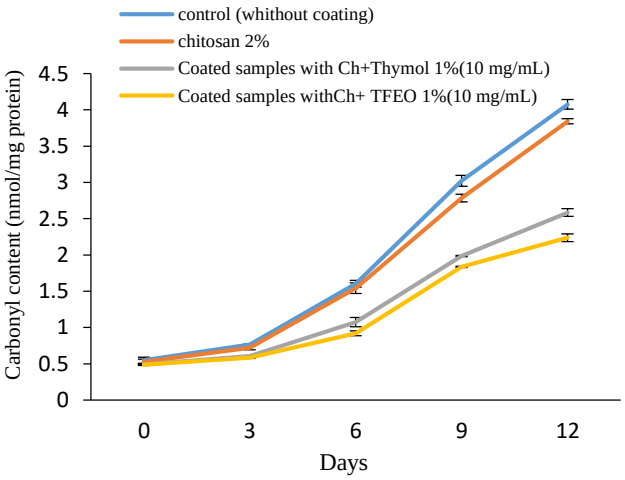

Fig. 4 Changes in carbonyls content of ground beef packaged with starch films during 20 days storage at refrigeration temperature (Mean ± SE).

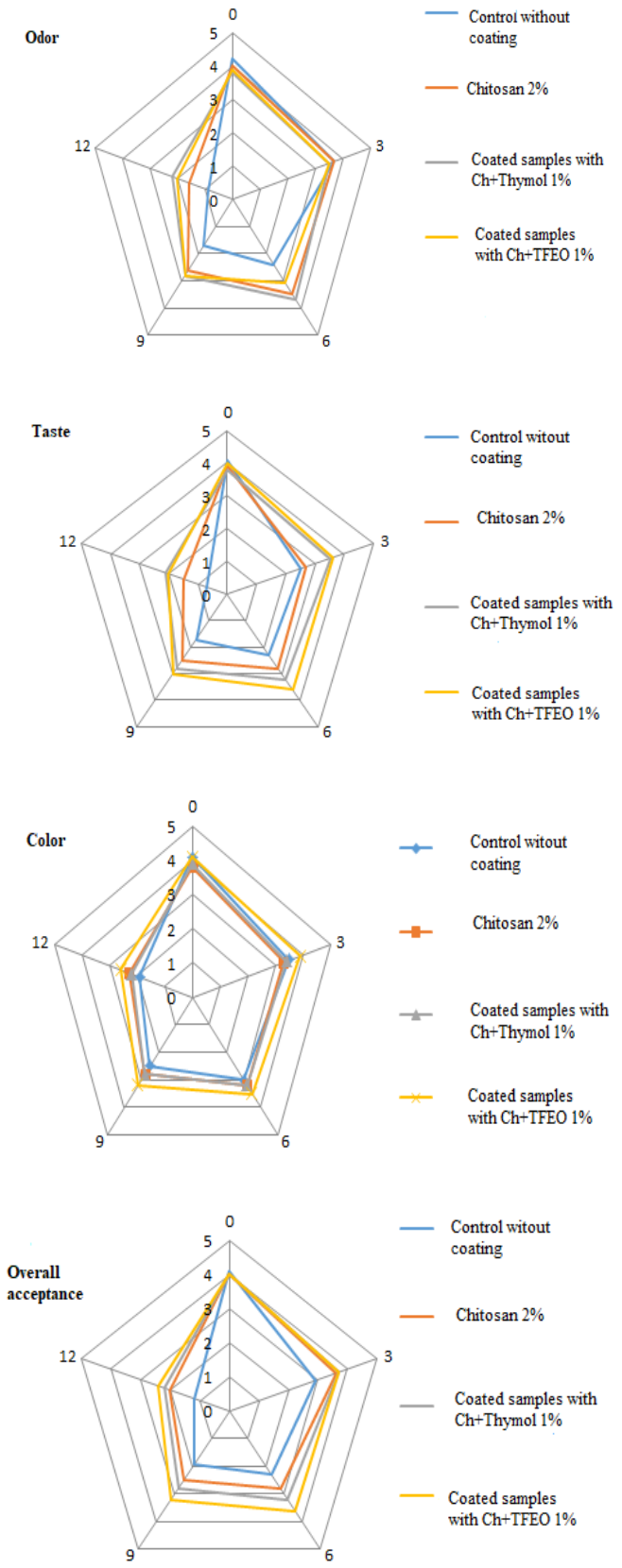

Fig. 5 Changes in sensory properties of poultry filet packaged with coating chitosan during 12 days storage at refrigeration temperature (Mean ± SD).

Protein Oxidation

The protein carbonyl content of the poultry filet samples is shown in Figure 4. The formation of carbonyl compounds is a result of oxidative degradation of some amino acid side chains like histidine, arginine lysine and proline residues [26]. The concentration of protein carbonyls in treated filet samples was lower compared to the control samples during the day's refrigeration storage ($P \leq 0.05$). Similar results were reported by Bazargani- Gilani *et al*. (2015) [26]. It was reported that phenolic constituents with a free hydroxyl group can prevent the sulfhydryl group of proteins (-SH) from more oxidation in the treated samples compared to the control samples [26]. The results showed that the inhibitions against Carbonyl formation were between 56.5 and 63.8 % at the end day of cold storage. The fillet samples coated with Ch +TFEO% had the largest reduction in carbonyl content at the end of the storage period.

Sensory Analysis

Evaluation of sensory attributes is used for assessing the chicken fillets quality and their overall acceptability. The mean sensory scores of poultry are indicated in Figure 5. In this study, the scores of all sensory properties decreased over the 12-day storage. The panelists were significantly more satisfied with coated samples containing essential oil than the control samples ($P \leq 0.05$). Missed scores in taste attribute were the results of off-flavor samples during the storage period. Secondary products of oxidation including aldehydes, ketones, hydrocarbons, alcohols, and esters can lead to taste deterioration [28]. The secondary lipid oxidation was delayed by TFEO and thymol products compared to the untreated samples.

The overall comparison of sensory evaluations indicates the correlation of low scores with high lipid and protein oxidation products such as ammonia that can produce discoloration, off-odor, and off-flavor. The obtained results of the present study are consistence with the results of other studies that improve the sensory attributes of meat samples coated with different coatings containing different essential oils [1,26].

CONCLUSION

The results of the present study indicate that chitosan-based coatings containing TFEO and thymol can prevent undesirable oxidative reactions in chicken fillets during 12 days cold storage (4 ± 1 °C) that lead to extending the shelf life of treated samples. PV, TBARS and carbonyl contents of all treated samples

remained within an acceptable range. The results show that the chitosan coating containing TFEO 1% followed by chitosan coating containing thymol 1 % had the highest antioxidant potential and also can extend the stability of chicken fillets, significantly (P<0.05). The chitosan coating containing TFEO 1% could be proposed as a new coating to protect food against oxidative changes and is a good option for food storage.

Declaration of Competing Interest

The authors declare no conflict of interest.

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