Antioxidant Effect of Thyme Essential Oil on Oxidative Stability of Chicken Nuggets

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***Abstract*—The objectives of the present study were to propose a proper use of thyme essential oil in industrially produced chicken nuggets instead of synthetic antioxidants and investigate its effect on the lipid oxidation; also to evaluate the effects of such intervention on chemical and organoleptic characteristics of freezing stored nuggets. The essential oils of Thymus Daenensis, Thymus vulgaris and Thymus kotschyanus were extracted by using Clevenger apparatus. The essence with greatest antioxidant activity was calculated with IC_{50} and added to chicken nuggets formulation. The antioxidant properties of essential oils were investigated by determination of thiobarbituric acid (TBARS) and peroxide values in prepared chicken nuggets throughout the 6 months storage at -20 ℃. Proximate analysis was determined and organoleptic characteristics were also evaluated in chicken nuggets after 3 and 5 months of storage. Among the four tested essential oils, Zataria multifora essence showed the lowest IC_{50} (1.09 mg/ml) and then highest antioxidant capacity. The antioxidant effect of thyme essential oil was shown by the lower peroxide value and TBARS levels found in thyme-treaded nuggets. Treated nuggets with thyme essential oil had greater acceptability by the panellist than control samples. Thyme essential oil can be successfully used in chicken processing industry as a natural antioxidant substituted for synthetic antioxidants.**

***Index Terms*—chicken nugget, lipid oxidation, thyme, essential oil, peroxide value, thiobarbituric acid**

I. Introduction

Lipid oxidation is one of the deteriorating reactions which occur during cooking and refrigeration period in ready-to-eat meat formulated products. It negatively affects the quality of the product since desirable sensory properties such as color, odor and flavor, as well as nutritional value of food tended to be reduced as a result of oxidative damages. It was shown that unlike meat which can be frozen for several months without any development of rancidity, the oxidized flavor in refrigerated cooked meat may develop within 48 h at 4 ℃ [1]. Oxidative deterioration often determines the shelf life of pre-cooked and refrigerated ready-to-eat products [2]. Meat from spent hens is tough and is preferably used for preparation of comminuted meat products such as kheema, cutlet, nuggets and etc. Fried chicken nuggets are widely consumed all over the world. They are usually coated with bread and batters, packed in polyethylene bags and stored at -18±2 ℃ for 6 months [3]. These preprocessed products are ready-to-fry and serve, and gaining importance in the consumer market. In recent years, due to toxicological concerns associated with the use of synthetic substances in food and increasing awareness about natural foods, great efforts have been made by researches to find safe and potent natural antioxidants from various plant sources in different food formulations to prevent oxidative deterioration of foods [4]. Spices, fruits, nuts, vegetables in fresh or dried form or as essential oils (classified as GRAS), have been extensively investigated for their antioxidant properties in a variety of meats including pork, beef and lamb [5]. Essential oils have a complex composition, from a few dozen to several hundred constituents, especially hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetyls, ethers and esters [6]. Among these essential oils, thyme essence is traditionally used as flavoring agents in meat and meat products and as the harmless sources of antioxidants.

Thyme (Thymus vulgaris L.), an aromatic plant of the Labiateae family, has been long used in foods for culinary purposes [7]. Thyme showed various beneficial effects, e.g., antiseptic, carminative, anti-flatulence, antiviral, anti-cancer, antimicrobial, and anti-oxidative properties [8], [9]. Previous studies showed that a few Thymus species could be proposed as very interesting natural resources with antioxidant activity such as *T. toseviivar. Tosevii (Kicevo), T. toseviivar. Degenii (Kitka), T. toseviivar. Longifrons (Kitka) T. toseviissp.*

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substriatus (Kavadarci), and *T. longidensvar. Lanicaulis (Sonje)* [10]. Several studies have demonstrated Thymus essential oils and extracts are increasingly used in pharmaceutical, cosmetic, and perfume industry, also for flavoring and preservation of several food products as antimicrobial and antioxidant natural ingredient [11], [12]. Eugenol, thymol and carvacrol showed potent antioxidant activity in volatile oil of thyme. The antioxidant activity of thyme is comparable to those of the known antioxidants, α-tocopherol and BHT [5].

Despite the numerous cited studies on the antibacterial and antioxidant activity of herbs and vegetable extracts and subsequent effect on the shelf life of foods, few data exist on the effect of thyme essential oil on the shelf-life of refrigerated industrially prepared nuggets. The objective of the present work was (1) to study the antioxidant activity of four most consumed *thyme essences including Thymus vulgaris, Thymus kotschyanus, Thymus daenensis,* and *Zataria multiflora Boiss* and (2) to evaluate the effectiveness of essential oil in preventing or minimizing lipid oxidation in addition to its effect on chemical and organoleptic characteristics of freezing stored chicken nuggets during 6 months.

II. Materials and Methods

A. Isolation of Essential Oils

Four kinds of thyme including *Thymus vulgaris, Thymus kotschyanus, Thymus daenensis,* and *Zataria multiflora Boiss* (a *thyme*-like plant) were purchased from Iranian markets. Thymus species used in this study were approved by the Research Center of Agriculture in Isfahan. Dried leaves of plants (100g) were subjected to hydrodistillation for 4h using a Clevenger apparatus. The acquired essential oils from different thyme samples were decanted and dried over anhydrous sodium sulfate, and then were stored in brown colored vial at 4 ℃.

B. Antioxidant Activity of Essential Oils

Radical scavenging capacity of the essential oils were measured by DPPH test (2,2-diphenylpicrylhydrazyl) and sample concentration giving 50% inhibition (IC_{50}) was calculated by plotting inhibition percentage against concentrations of the sample. DPPH is the best, easiest and widely used method for testing preliminary free-radical scavenging activity of a compound or a plant extract [13]. The thyme essential oil (TEO) which possessed maximum antioxidant activity was selected for addition to chicken nuggets.

C. DPPH Radical Scavenging Activity Assay

Chicken nuggets were manufactured according to an industrial formula and separated into two lots; one was left as a control and the second was treated with 0.05, 0.1, 0.2ml/100g of TEO. Essential oil was added to meat of nuggets formulation and homogenate carefully. All samples stored at -20 ℃ for 6 months [14]. Sampling was done every month until the end of the storage period and each sample was subjected to TBA and peroxide value analysis. Moisture, protein content, ash and total fat were determined according to the AOAC (1995) method [15]. The free radical scavenging activity of essential oils was determined by Sharififar *et al.* and Choi *et al.* [13], [16] methods. Briefly, 4ml of DPPH methanolic solution (0.1 mM) was added to 1ml of sample solutions in methanol at different concentrations (4.16, 2.08, 1.04, 0.52, 0.26, 0.13, 0.06, 0.03, 0.015, 0.007mg/ml). After 30 min, the absorbance was measured at 517nm. Inhibition of free radical was calculated by the following formula [16]:

DPPH scavenging effect % = A blank - A sample/A blank * 100

where a blank is the absorbance of the control reaction mixture (containing all ingredients except the test compound) and a sample is the absorbance of the tested sample.

D. Thiobarbituric Acid (TBARS)

Briefly, homogenized samples (10g) were mixed with 50ml of 10% (w/w) trichloroacetic acid. Then, the mixture was transferred to a measuring flask and adjusted to 50ml with distilled water. After filtration, 5ml of supernatant were added to 5ml of 2-thiobarbituric acid (0.01M in acetic acid 90%) and heated in a boiling water bath for 10 min to develop a pink color resulting from the reaction between malondialdehyde and 2-thiobarbituric acid. After cooling under tap water, the absorbance was measured at 532 nm using a UV-VIS spectrophotometer (Hitachi U-1100, San Jose, CA, USA). A standard curve was prepared using 1,1,3,3-tetraethoxypropane (TEP) at a concentration ranging from 8-50 nmol and TBARS values were calculated as mg of malondialdehyde (MDA) equivalent/kg sample [17].

E. Peroxide Value

The primary products of lipid oxidation are hydroperoxides which were determined by Peroxide Value (PV) analysis to assess the level of lipid oxidation in chicken nuggets during storage at -20 ℃. Peroxide Value (PV) analysis was performed according to International Dairy Federation method as described by Shantha and Decker (1994) with minor modifications. Total lipid was extracted from the meat samples by the process of Shantha and Decker (1994) using chloroform/methanol at a ratio of 7:3. An aliquot (9.8 ml) of the lipid extract in chloroform–methanol mixture was transferred to a test tube and combined with 50ml of ammonium thiocyanate solution (30%, w/v), 50ml of iron (II) chloride solution [(0.4 g barium chloride dihydrate dissolved in 50ml H_2O), 50ml $FeSO_4$ solution (0.5g $FeSO_4.7H_2O$ dissolved in 50ml H_2O) and 2ml 10N HCl. After shaking, the precipitate of barium sulfate was filtered off to produce a clear solution. The mixture was kept at room temperature for 5 min by subdued lighting. The absorbance of the sample was measured at 500nm against a blank. Results were expressed as meq of peroxide/kg fat [14].

F. Sensory Evaluation

Sensory assessment was performed using the Hedonic system. Chicken nuggets (100g) were fried for 8 min. Organoleptic properties including odor, hardness, saltiness, fatness and homogeneity perception of chicken

nugget samples were assessed at the third and fifth months by a panel of 30 panelists. Sensory hedonic scheme ranged from 0 (bad) to 1 (good). Therefore, acceptability of nugget samples with essence was determined. All measurements were carried out in triplicate. Statistical comparisons were made between treatments by one-way ANOVA and Tukey's test using SPSS program and the results were presented in terms of p values ($p<0.05$).

III. Results and Discussion

A. DPPH Radical Scavenging Activity of Essential Oils

Fresh and in particular processed poultry meat products are very susceptible to oxidative deterioration [18]. Then, much attention lately has been focused on extracts of herbs and spices which have been used traditionally to improve the sensory characteristics and extend the shelf-life of foods. In this study, DPPH test was performed and IC_{50} of tested essential oils were determined (Fig. 1). Generally, samples with the lowest IC_{50} have greater antioxidant capacity. According to Fig. 1, IC_{50} of essential oils were 1.09mg/ml, 1.56mg/ml, 1.61 mg/ml and 2.03mg/ml for *Zataria multiflora*, *Thymus kotschyanus*, *Thymus d Daenensis* and *Thymus vulgaris* respectively. Therefore, it is expected that *Zataria multiflora* with the lowest IC_{50} and *Thymus vulgaris* with the highest IC_{50} possess the maximum and minimum antioxidant activity, respectively. Then, *Zataria multiflora* was selected for further studies. *Zataria multiflora Boiss* is a thyme-like plant belonging to the *Lamiaceae* family and only grows in the wild in Iran, Pakistan and Afghanistan. Different studies showed antioxidant activities of *Zataria multiflora* essential oil [19]. Aerial parts of this plant is not only a popular condiment plant, but is also used in traditional folk remedies because of its antiseptic, analgesic, carminative, anthelmintic and antidiarrheal properties [20].

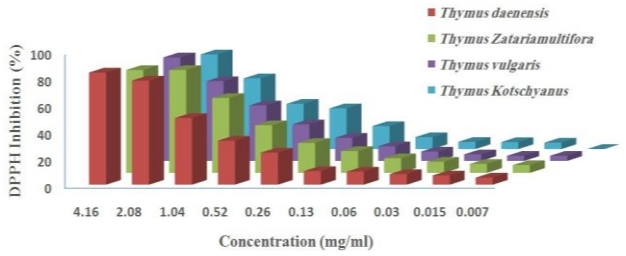

Figure 1. DPPH radical scavenging activity [IC_{50} (mg/ml)] of *Zataria multiflora*, *Thymus kotschyanus*, *Thymus daenensis* and *Thymus vulgaris* essential oils.

B. Chemical Analysis

Moisture, ash, protein and fat content and their changes in samples within 6 months of storage are shown in Table I. According to Tukey's test, no significant differences ($P>0.05$) were found for moisture, ash, protein and lipid content between all samples at first and third months. Then it can be concluded that addition of essential oil had no notable effect on proximate analysis. Such behavior has also been noted for addition of *Zataria multiflora* to rainbow trout filets by Shabanpoor *et al.* [21] and no significantly effect on chemical properties was observed. Dadfar *et al.* [22] reported no changes in beef meat proximate analysis with the addition of savory essential oil. Viuda *et al.* [23] observed that with addition of 0.02% essential oil of *thyme* and oregano, ash content in some samples showed significantly higher rates while protein and fat did not change significantly in any of the samples.

TABLE I. The Effect of Thyme Essential Oil on Moisture, Ash, Protein and Fat of Chicken Nugget Stored at -20 ℃

month		Concentration (ml/100g)			
		0.05	0.1	0.2	Control
0	M	44.37±0.61	44.36±0.61	45.69±0.61	45.68±0.61
1	M	44.33 ±0.57	45.33 ±0.57	45.66 ±0.57	45.66 ±0.57
3	M	44.0 ±1.00	44.00 ±0.00	45.00 ±0.00	45.00 ±0.00
0	A	1.80 ±0.28	2.00 ±0.50	2.16 ±0.28	1.83 ±0.28
1	A	1.83 ±0.28	2.00 ±0.50	2.16 ±0.28	1.83 ±0.28
3	A	2.16 ±0.28	1.83 ±0.28	2.33 ±0.28	1.83 ±0.28
0	P	13.64 ±0.50	13.00 ±1.50	13.66 ±0.57	14.00 ±1.50
1	P	13.66 ±0.57	13.33 ±1.52	13.33 ±0.57	14.66 ±1.52
3	P	14.00 ±1.00	13.00 ±1.00	13.66 ±0.57	13.66 ±2.00
0	F	11.10 ±1.00	10.00 ±1.50	11.66 ±1.52	12.33 ±0.57
1	F	11.00 ±1.00	10.66 ±1.52	11.33 ±1.52	12.33 ±0.57
3	F	10.66 ±1.52	12.33 ±2.08	10.33 ±2.88	12.00 ±2.64

M=Moisture; A=Ash; P=Protein; F=Fat

C. Peroxide Value

The peroxide values of chicken nuggets containing different concentrations of thyme essential oil during 6 months storage at -20 ℃ are shown in Table II. Results showed that thyme essential oil was effective in preventing oxidative spoilage. In generally, the oil had a positive effect on shelf life of the product and this effect was directly related to the concentration of essential oil. Considering the essence concentration, no difference was observed between the treated samples and control during the first month of storage but differences among samples can be observed from second month to the end of the storage time. When peroxide values of all samples were compared, control samples showed higher oxidation rate than treated nuggets containing different concentrations of essence throughout the storage, particularly after 2 month to the end of storage period. On the other hand, samples containing 0.1% and 0.2% of essence were significantly different from samples with 0.05% of essence and control samples especially in second and third month of storage. After six months, peroxide value for all three concentrations was significantly different from samples without thyme essential oil. In numerous studies have been found that the ethanolic extracts of sage, basil, thyme and ginger at concentrations of 200mg/ml were much more effective against lipid oxidation than the dried species at concentrations of 10g/kg [24], [25]. It was shown that the inhibitory effect of thyme at 10μg/ml and basil at 50μg/ml were comparable to those of BHT and α-tocopherol at concentrations varying from 10 to 500μg/ml [5].

TABLE II. THE EFFECT OF THYME ESSENTIAL OIL ON THE PEROXIDE VALUE OF CHICKEN NUGGETS STORED AT -20 ℃

month	Concentration (ml/100g)			
	0.05	0.1	0.2	Control
0	3.89 ± 0.20^{a}	4.23 ± 0.67^{a}	4.40 ± 0.33^{a}	4.74 ± 0.61^{a}
1	3.92 ± 0.21^{a}	4.27 ± 0.67^{a}	4.42 ± 0.39^{a}	4.74 ± 0.61^{a}
2	4.37 ± 0.58^{a}	3.22 ± 0.39^{b}	3.02 ± 0.67^{b}	5.45 ± 0.72^{a}
3	3.05 ± 1.15^{ae}	1.95 ± 0.21^{ac}	1.60 ± 0.19^{b}	3.40 ± 0.56^{ade}
4	2.08 ± 0.85^{a}	1.4 ± 0.33^{a}	1.19 ± 0.8^{a}	3.77 ± 1.59^{b}
5	2.5 ± 0.17^{a}	2.78 ± 0.75^{a}	3.07 ± 1.08^{ac}	4.35 ± 0.37^{bc}
6	5.36 ± 0.76^{a}	4.98 ± 0.30^{a}	4.99 ± 0.21^{a}	6.29 ± 0.79^{b}

Different letters in columns indicated significant differences (P<0.05) as determined by analysis of variance.

TABLE III. THE EFFECT OF THYME ESSENTIAL OIL ON THIOBARBITURIC ACID OF CHICKEN NUGGETS STORED AT -20 ℃

Month	Concentration (ml/100g)			
	0.05	0.1	0.2	Control
0	2.10 ± 0.50^{ace}	2.00 ± 0.00^{bce}	1.40 ± 0.40^{bd}	2.50 ± 0.20^{ac}
1	2.17 ± 0.15^{ace}	2.01 ± 0.06^{bce}	1.49 ± 0.42^{bd}	2.58 ± 0.24^{ac}
2	1.81 ± 0.55^{b}	1.79 ± 0.56^{b}	1.64 ± 0.48^{b}	2.36 ± 0.67^{a}
3	1.85 ± 0.48^{bd}	2.75 ± 0.26^{ad}	1.89 ± 0.77^{bd}	3.18 ± 0.55^{ac}
4	3.41 ± 0.15^{a}	2.75 ± 0.00^{b}	2.15 ± 0.21^{b}	3.21 ± 0.02^{a}
5	3.56 ± 0.39^{a}	2.44 ± 0.25^{b}	2.49 ± 0.03^{b}	3.59 ± 0.16^{a}
6	3.58 ± 0.09^{ac}	3.08 ± 0.17^{c}	2.73 ± 0.34^{c}	4.53 ± 0.85^{ab}

Different letters in columns indicated significant differences (P<0.05) as determined by analysis of variance.

Table III shows the changes in thiobarbituric acid within the six months of storage. TBA is the second breakdown products of lipid oxidation, especially malonaldehyde, and widely used as an indicator of degree of lipid oxidation. It can be observed that while the TBA values of all samples gradually increased during 6 months of storage, there was a significant increase in TBA value of control samples compared to treated ones probably because of faster conversion of primary oxidation products such as peroxides to secondary oxidation products. Among all tested concentrations of *Zataria multiflora* essential oil, supplementation of 0.2% of essence showed the highest activity in prevention of secondary oxidation process, whereas supplementation of 0.05% of essence tended to exhibit the lowest preventive effect. In the first month, no significant difference was shown between the control and treated samples with 0.05% essential oil whereas samples with 0.1 and 0.2% of essence were statistically different from control. During the second and third months, no significant differences were shown between treated samples and to the end of storage time, concentration of 0.1 and 0.2% of essence showed the lowest TBA values. These results were in agreement with Medina *et al.* findings who reported that oxidative stability of restructured nuggets was influenced by the type of antioxidant and its concentration [26]. Viuda-Martos *et al.* showed that the addition of *thyme* essential oil to cooked meat products (bologna sausage) was a viable alternative for increasing their oxidative stability [27]. Babaie *et al.* demonstrated significant antioxidant activity of the methanolic extract of *Zataria multiflora* by evaluating the inhibitory activity against DPPH radical, Total Antioxidant Power (TAP) and Thiobarbituric Acid Reactive Substances (TBARS) in the serum of treated rats [28]. Karimian *et al.* [28] have also shown that *Zataria multiflora* oil possesses nitricoxide and malondialdehyde scavenging properties and could prevent nitro oxidative stress and lipid peroxidation [29]. Shabanpoor *et al.* showed that, TBA of rainbow trout fillet increased gradually during storage but this index in treated samples with *Zararia multiflora boiss* extract was significantly ($P \leq 0.05$) lower than the control [21]. Studies on the oxidative stability of chicken nuggets using other spices are also noticeable. For example, chicken nuggets containing ground mustard or clove powder showed lower ($p \leq 0.05$) TBA values throughout the storage period due to the antioxidant properties of these spices [30].

Given that the concentration of 0.2% of essential oil showed better results in terms of the oxidation stability of the samples, we were examined the only samples containing this concentration of essence in sensory evaluation. Table IV shows the percentage of acceptability obtained for the sensory attributes of the nuggets treated with 0.2% essential oil after 3 and 5 months storage. To find a compromise between effective doses of flavoring agents like essential oils and sensory acceptability is a difficult task. Some researchers showed better sensory characterization of minced beef or sheep meat treated with 0.8-1% of oregano essential oil in comparison to control [31]. Similarly, Ouattara *et al.* reported that the addition of *thyme* essential oil at 0.9% had no negative effect on the flavor and the appearance of cooked shrimps [32]. Other authors regarded the concentrations of essential oils in meat close to 1% as unacceptable [33], [34]. According to our results (Table IV), treated samples with 0.2% essence showed higher acceptability in all sensory characteristics tested in this study than control samples. Therefore, addition of this concentration of *thyme* essential oil can simultaneously improve the oxidative stability and sensorial perception of product in case of studied parameters.

TABLE IV. ACCEPTABILITY (%) OF THYME TREATED NUGGETS IN TERMS OF SENSORY CHARACTERISTICS DURING 3 AND 5 MONTHS STORAGE AT -20 ℃

Organoleptic Properties	3 months storage		5 months storage	
	Treated	Control	Treated	Control
Odor	53.33%	46.66%	80%	20%
Hardness	80%	20%	80%	20%
Fatness	80%	20%	66.66%	33.33%
homogeneity perception	66.66%	33.33%	73.33%	26.66%
Saltiness	80%	20%	60%	40%

IV. CONCLUSIONS

In conclusion, this research demonstrated that two concentrations (0.1 and 0.2%) of *Zataria multiflora boiss* essential oil had strong retarding effect on inhibition of lipid oxidation in chicken nuggets (although addition of 0.1% was less effective than addition of 0.2% of essence) while lower concentration (0.05%) of was less effective. Considering the consumer preference for natural additives, such as natural antioxidants, *thyme* essential oil could be used as natural antioxidant for inhibiting of

industrially chicken nuggets lipid oxidation while providing more sensorial acceptability. The results of this research suggest that essential oil obtained from *Zararia multiflora Boiss* leaf can be successfully used as a natural antioxidant substituted for synthetic antioxidant such as BHT in meat industry.

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