EFFECT OF THYME LEAVES EXTRACT ON QUALITY OF LAMB AND CHICKEN MEAT DURING STORAGE

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ABSTRACT:

It has been known that thyme have antioxidant capacity and antimicrobial activity against different bacteria, therefore

this investigation was designed to study the effect of adding thyme extract (0, 250, 500 and 1000 ppm) on the oxidative rancidity and microbial growth on lamb and chicken patties stored at 4C° for 12 days. The result revealed that malondialdehyde (MDA) values started to increase during storage periods, of both lamb and chicken patties. Extract of thyme apparently retarded significantly ($p<0.01$) oxidative rancidity and microbial growth as well in both lamb and chicken patties. Thus it can be concluded that addition of thyme especially at a rate of 500 ppm is effective as compared to untreated patties against oxidation and microbial growth.

KEYWORDS: *Lipid Oxidation, Microbial Count, Thyme leaf extract, Meat Patties*

INTRODUCTION:

Lipid oxidation is very important to animal industry because it is considered one of the main causes of quality deterioration (Raghavan and Richards, 2007), particularly when the oxidized products develop undesirable flavors, unpleasant taste, rancid odors, discolorations, and other forms of spoilage (Schuler, 1990).Beside lipid oxidation, microbial activity is another primary cause of deterioration of many foods, often is responsible for the loss of quality and safety and shortening of shelf life(Rhee, 1989).

Synthetic chemicals have been popularly applied as antioxidants and antimicrobial. However, in response to recent demand from consumers for natural products, and their willingness to pay significant premiums for natural foods (Sebranek and Bacus, 2007), therefore the meat and poultry industry is actively seeking natural solutions to minimize oxidative rancidity and increase the shelf life of their products (Naveena *et al.,* 2008). Antioxidants are of great importance in terms of preventing oxidative stress that may cause several degenerative diseases. The primary sources of antioxidants are plants; the preservative effect of plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues (Javanmardi *et al*,2003).

Thyme have been used as flavoring agents in meat and meat products (Lawless, 1995). Additionally, it has been known that thyme have antioxidant capacity due to its contents of flovnoids (Lacroix *et al*., 1997),as well as the volatile oil components of thyme have been known to have antimicrobial activity against different bacteria and fungi species (Dorman and Deans 2000; Nguefack *et al.*, 2009).

In the view of the above considerations, the reported study was aimed to evaluate the antibacterial and antioxidant activities of ethanol extraction of *Thymus vulgaris* leaves in minced lamb and chicken meat.

MATERIALS AND METHODS

Extraction of thyme leaf

Thyme vulgaricus leaves used in this work were obtained from Doski area at Duhok province Kurdistan region of Iraq. Leaves were washed, dried at room temperature, followed by grinding. One hundred gm of the ground leaves were extracted with 1000 ml of 70% (v/v) aqueous ethanol in a closed conical flask for 24 hr at room temperature in the dark. The extract was filtered through cheese cloth and the residue was re-extracted three times using the same solvent. The filtrate obtained was evaporated in a vacuum oven at 40c°,and frozen until use. High performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan) was used to detect the active compound of the extract.

Preparation of meat Patties

Samples of meat were obtained from each of leg karadi lambs and the thigh of chicken. After chilling for 24 hr at (4°C), the connective tissues and fat were trimmed off from the leg samples, and the skin was removed from the de-bond thigh. The samples were cut into pieces, and then minced using meat grinder. Minced meat (1kg each) were divided into four treatment, the first untreated (control) and the remaining samples were blended was 250, 500, and 1000ppm thyme leaves extract ((TLE) respectively. Patties (100g) were formed using a meat former, and placed on plastic foam meat trays, wrapped with polyethylene film and stored for 12 days at 4°C.

Determination MDA values

The MDA values were determined according to the method described by Witte *et al*. (1970). Twenty grams of the minced meat were blended with 50 ml of cold solution containing 20% trichloroacetic acids in 2 M phosphoric acids. The resulting slurry was then transferred into a 100 ml with distilled water, homogenized by shaking and filtered through Whatman no.1 filter paper. Five ml of the filtrate was then pipette into a test tube while another 5 ml of fresh chilled 2-Thiobarbituric acid (0.005 M in distilled water) was added. The test tube was shaken well and placed in the dark at room temperature (25°C) for 15- 17 h to develop the color reaction. The absorbance was measured using spectrophotometer (6400-JENWAY) at 530 nm to calculate the MDA values. The MDA value was expressed as mg malondialdehyde /kg meat, which was calculated by multiplying the absorbance by 5.2 factors as follows:

MDA (mg malondialdehyde /kg meat) = A530 × 5.2

Microbial Count

Microbial count was determined as recommended by the American Public Health Association for food stuff examination (APHA, 1992). Total plate count (TPC) was determined on nutrient agar medium, and the plates of different dilutions were incubated at 37°C for 48 h. The average number of colonies per countable plate as well as the total number of colonies per gram (CFU/g) was determined. MacConky agar medium was used for determination of coliform bacteria, and the inoculated plates were incubated at 37°C for 48 h. Psychrophilic bacteria(PSY) were determined on nutrient agar medium, and the plates, and the inoculated plates were inoculated at 7°C for 10 days.

Statistical Analysis

General linear model (SAS 2002) was used to estimate best linear unbiased (BLUE) for main effects and their interaction, on all studied traits. Duncan multiple range tests (Duncan, 1955) was performed to detect significant differences among means of ($p < 0.05$) treatment combination (treatments X period).

RESULTS AND DISCUSSION:

The active compounds present in thyme leaf extract are Carvacrol ,Thymol , 1-8-Cinote,P-Cymene,Borneol(Table 1). Similar, active anti oxidative constituents of thyme leaf extract were found by other investigates (Seung-Joo *et al.,* (2005); Solomakos *et al.,* 2008.

Table (1): The active compounds of thyme leaf extract (TLE)

The active compounds Of (TLE)	Retention time of standard (min)	Retention time of sample (min)	Concentration mg/100gm thyme leaf extract
P-Cymene	1.05	1.08	0.131
Thymol	2.34	2.32	0.133
Carvacrol	2.98	2.99	0.200
1-8-Cinote	4.08	4.08	0.322
Borneol	4.91	4.92	0.134

Malondialdehyde Levels

In the current investigation, MDA values started to increase gradually in all examined samples of both lamb and chicken patties during storage period. MDA of lamb patties in the untreated samples started to increase significantly ($p<0.01$) from their initial values (0.157) at day 1 to reach 5.71 mg MDA/kg meat at day 12 of storage, while the maximum values for patties treated with 250, 500 and ppm thyme were 1.290, 1.018 and 1.216 mg MDA/kg meat, respectively at the end of storage period. A similar trend was also observed in the chicken patties but to a lesser extent. Hence the MDA value was increased from 0.649 at day 1 to 1.000 mg MDA/kg meat at the end of storage period. While the corresponding value of treated chicken samples with 250, 500 and 1000 ppm thyme were 0.899, 0.805 and 0.850 mg MDA/kg meat, respectively. Thus treated both lamb and chicken samples with thyme extract apparently retarded significantly ($p < 0.01$) oxidative rancidity compared to untreated control samples. Moreover, it seems from the results presented in Table (2) that the maximum retardation of oxidative rancidity in lamb patties (80.3%) and chicken patties (19.5%) was occurred when the concentration of the thyme extract used was 500 ppm. Such results may be due to the antioxidant effect of thyme extract, which is related to scavenger nature of its flavonoids and phenolic content (Stahl-Biskup, 1991; Senatore, 1996; Skerget *et al.*, 2005, Amiarowicz *et al.*,2009 and Kassem *et al*., 2011).This result was consistent with studies in beef burger (Kassem *et al.*, 2011), and tuna fish (Selmi and Sadok, 2005). Furthermore, since MDA values are considered as indicators of rancidity in fat products.Verme and Sahoo (2000) stated that MDA concentration between 1.0 and 2.0 mg/kg as threshold values for rancidity, therefore the lamb and chicken patties treated with thyme extract in present study would not deceive consumers up to 12 days of storage due to the antioxidant activity of thyume.

Table (2): Effect of thyme leaf extracts (TLE) on changes in MDA (mg malondialdehyde / kg meat) values of Chicken and lamb meat during storage at 4°C for 12 days

MDA Chicken				
Treatment	Day 1	Day 4	Day 8	Day 12
Control	0.649 ± 0.003^{g}	0.719 ± 0.0019^{f}	0.899 ± 0.036^{b}	1.000 ± 0.019^{a}
250 ppm (TLE)	0.735 ± 0.03^{f}	0.790 ± 0.002^{fd}	0.850 ± 0.019^{bc}	0.899 ± 0.012^{b}
500 ppm (TLE)	0.649 ± 0.02^{g}	0.761 ± 0.007^{fde}	0.798 ± 0.007^{cd}	0.805 ± 0.008^{c}
1000 ppm (TLE)	0.707 ± 0.019^{f}	0.752 ± 0.003^{fde}	0.782 ± 0.004^{fd}	0.850 ± 0.005^{bc}
MDA lamb				
Treatment	Day 1	Day 4	Day 8	Day 12
Control	0.197 ± 0.012^{g}	3.430 ± 0.083^{c}	4.250 ± 0.167^{b}	5.17 ± 0.105^{a}
250 ppm (TLE)	0.220 ± 0.010^{g}	0.543 ± 0.0001^{f}	1.359 ± 0.103^{d}	1.29 ± 0.106^{d}
500 ppm (TLE)	0.314 ± 0.016^{g}	0.548 ± 0.011^{fi}	1.058 ± 0.049^{e}	1.018 ± 0.066^{e}
1000 ppm (TLE)	0.300 ± 0.011^{g}	0.595 ± 0.010^{fi}	1.123 ± 0.022^{k}	1.216 ± 0.008^{ed}

*The same letters mean no significant difference between the mean of treatment.
*The different letters mean significant difference between the mean of the treatment ($P<0.05$)

Microbial changes

Microbial quality of lamb and chicken patties was assessed through estimation of TPC, PSY and coliform bacteria. Results, presented in Table (3) revealed that in control untreated lamb patties there was a significant ($p < 0.01$) steady rise during storage period up to 12 days from their initial values in TPC (0.61 vs. 81 x 10^5), PSY (0.43 vs. 75 x 10^5) and coliform (1.3 vs. 106 x 10^3). A similar trend was also observed in untreated chicken patties. TPC, PSY and coliform was raised from 8.4, 6.2 x 10^5 and 22.0 x 10^3, respectively at day 1 to reach 107, 93 x $10^5$and 83 x 10^3 at day 12 of storage (Table 4). Also, the results reveal that addition of thyme extract resulted in a significant ($P < 0.01$) reduction on all counts of studied bacteria. The highest decline was recorded in treated samples of both lamb and chicken patties with 500 ppm thyme, which amounted to 75% in TPC, 72% in PSY and 57.8% in coliform in lamb patties and 61.7% in TPC, 64% in PSY and 70.7% in coliform in chicken patties. Similarly, Kassem *et al.,* (2011) indicated that addition of thyme essential oil resulted in a significant reduction of microbial load in beef burger. Also, the addition of rosemary or thyme EO to fine paste meat products, has been effective against aerobic bacteria and PSY (Viuda-Martos *et al.,* 2010).

Such results may emphasize the antimicrobial activity of phenolic compound in thyme extract mainly Carvacrol ,Thymol , 1-8-Cinote,P-Cymene, Borneol through its effect directly on the cell membrane of the microorganism by causing an increase in the permeability and leakage of vital intracellular constituents, and finally disrupt the cell respiration and microbial enzyme system (Akthar *et al.,* 2014).

Table (3): Total plate count (T.P.C), psychrophilic bacteria count(PSY) and coliform count as affected by different level of Thyme leaf extract on lamb patties stored for 12 days at 4°C

T.P.C. x 10^5 lamb				
Treatment	Day 1	Day 4	Day 8	Day 12
Control	0.61±0.005^{j}	4.3±0.057^{h}	57±0.577^{b}	81±0.577^{a}
250 ppm (TLE)	0.33±0.005^{j}	2.7±0.057^{i}	17±0.577^{f}	51±0.577^{c}
500 ppm (TLE)	0.31±0.005^{j}	2.5±0.057^{i}	15±0.577^{g}	31±0.577^{e}
1000 ppm (TLE)	0.33±0.005^{j}	2.2±0.0^{i}	16±0.00^{fg}	39±0.577^{d}
PSY x 10^5 lamb				
Treatment	Day 1	Day 4	Day 8	Day 12
Control	0.43±0.017^{j}	3.9±0.057^{h}	49±0.577^{b}	75±0.577^{a}
250 ppm (TLE)	0.3±0.005^{j}	2.3±0.057^{i}	15±0.577^{f}	45±0.577^{c}
500 ppm (TLE)	0.28±0.0^{j}	2.1±0.057^{i}	13±0.0^{g}	27±0.577^{e}
1000 ppm (TLE)	0.31±0.005^{j}	2.6±0.00^{i}	13±0.0^{g}	35±0.577^{d}
Coliform x 10^3 lamb				
Treatment	Day 1	Day 4	Day 8	Day 12

Control	1.3±0.057^{j}	10.5±0.288^{g}	61±0.577^{b}	106±0.577^{a}
250 ppm (TLE)	0.38±0.11^{j}	3.5±0.057^{i}	11.5±0.288^{g}	32±0.577^{d}
500 ppm (TLE)	0.31±0.005^{j}	5.5±0.152^{h}	15±0.577^{f}	31±0.577^{d}
1000 ppm (TLE)	0.6±0.0^{j}	5.2±0.0^{h}	18.5±0.288^{e}	39±0.577^{c}

*The same letters mean no significant difference between the mean of treatment.
*The different letters mean significant difference between the mean of the treatment (P<0.05)

Table (4): Total plate count (T.P.C), psychrophilic bacteria count (PSY) and coliform count as affected by different level of Thyme leaf extract on chicken patties stored for 12 days at 4°C

T.P.C. x 10^5 chicken				
Treatment	Day 1	Day 4	Day 8	Day 12
Control	8.4±0.05^{f}	9.2±0.11^{f}	13±1.15^{e}	107±0.57^{a}
250 ppm (TLE)	4.3±0.0^{g}	4.7±0.057^{g}	10±1.15^{f}	61.±0.57^{b}
500 ppm (TLE)	2.5±0.11^{g}	2.7±0.057^{g}	3.5±0.057^{g}	26±1.15^{d}
1000 ppm (TLE)	3.6±0.0^{g}	3.5±0.11^{g}	3.7±0.057^{g}	45±1.73^{c}
PSY x 10^5chicken				
Treatment	Day 1	Day 4	Day 8	Day 12
Control	6.2±0.05^{ef}	8.5±0.12^{e}	7±0.57^{ef}	93±0.57^{a}
250 ppm (TLE)	3.1±0.0^{g}	3.4±0.11^{g}	4.8±0.03^{fg}	55±2.88^{b}
500 ppm (TLE)	2.2±0.11^{g}	2.2±0.057^{g}	2.5±0.11^{g}	26±0.0^{d}
1000 ppm (TLE)	3.2±0.11^{g}	2.2±0.11^{g}	2.5±0.11^{g}	37±1.73^{c}
Coliform x 10^3 chicken				
Treatment	Day 1	Day 4	Day 8	Day 12
Control	22±0.57^{g}	31±0.57^{e}	35±0.57^{d}	83±1.73^{a}
250 ppm (TLE)	11±0.57^{i}	21±0.57^{g}	28±0.0^{f}	57±0.57^{b}
500 ppm (TLE)	10±0.57^{i}	17±0.57^{h}	21±0.57^{g}	35±0.57^{d}
1000 ppm (TLE)	12±0.0^{i}	22±1.15^{g}	26±0.0^{f}	44±1.15^{c}

*The same letters mean no significant difference between the mean of treatment.
*The different letters mean significant difference between the mean of the treatment (P<0.05)

CONCLUSION

From the results obtained in the current work, it can be concluded that adding thyme extract especially at a rate of 500 ppm to lamb and chicken patties is effective in retarding oxidation rancidity and microbial growth for storage period of 12 days at 4ºc.

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کارتێکرنا زێدەکرنا پوختەیێ (مستخلص) جاترێ د جورێ گوشتێ بەرخا و مریشکاندا د دەمێ کوگەهکرنێدا

پوختە:

ئەڤ تاقیکرنە هاتیە ئەنجامدان بو خواندنا کارتێکرنا زێدەکرنا پوختەیێ جاترێ (0 . 250 . 500 . 1000 پارچە ژ ملیونێ) د زنخاتیا ئەکسەدێ و وەراربوونا میکروبی و خەلەکێت گوشتێ بەرخا و مریشکا بێت کوگەهکری لژێر نمرا گەرماتیا (4م) د ماوێ (12) روژان دا ، دەرئەنجامان ئاماژە ب زێدەبوونا TBA دا دماوێ کوگەهکرنێ دا یا هەردوو خەلەکێت گوشتێ بەرخی و مریشکێ دکەت.هەروەسا دیاربوو کو زێدەکرنا پوختەیێ جاترێ بوویە دەرئەنجامێ راوستیانا ئاستێ (أ < 01، 0) د پروسەیا زنخاتیا ئەکسەدێ دا و وەرارا میکروبیدا د هەردوو جورێن گوشتیدا و ل سەر ڤی بنەمای ئەم دشێن بێژین زێدەکرنا پوختەیێ جاترێ و تایبەت ل ڕێژەیا (500 پارچە ژ ملیونێ) چالاکترینانە بەراورد دگەل معاملا کونترولێ.

تأثير اضافة مستخلص الزعتر في نوعية لحوم الحملان والدجاج خلال الخزن

المستخلص :

تم تصميم هذه التجربة لدراسة تأثير اضافة مستخلص الزعتر (0 , 250 , 500 , 1000 جزء من المليون) في التزنخ التأكسدى والنمو الميكروبي في أقراص لحوم الحملان والدواجن المخزونة بدرجة حرارة 4 ْم ولمدة 12 يوم .اشارت النتائج بان قيم TBA بدات بالازدياد خلال مدة الخزن لكلا اقراص لحم الحملان والدواجن .كما يتضح بأن اضافة مستخلص الزعتر قد أدى الى تثبيط معنوي (أ < 0.01) لعملية التزنخ التأكسدى والنمو الميكروبي في كلا نوعين من اللحوم . وعليه يمكن الأستنتاج بان اضافة مستخلص الزعتر و خاصة عند معدل 500 جزء من المليون يعد الاكثر فعالية مقارنة بمعاملة السيطرة.