Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.27, 2014

Effect of Thyme Leaves Extract on Quality of Lamb and Chicken Meat during Storage

Ibrahim A. Baker, Khalil, A.D. Oray and Khabat N. Hussein
Animal Production Department, Faculty of Agriculture and Forestry, University of Duhok, Iraq

Abstract
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 TBA 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: Thyme leaf extract, Lipid Oxidation, Microbial Count, Meat Patties

1. Introduction:

Lipid oxidation is very important to animal industry because it is considered one of the main causes of quality deterioration (Raghavam 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). Herbs and spices have been used since ancients not only for flavoring foods but also for their antiseptic or medicinal properties. The preservative effect of spices and herbs suggests the presence of antioxidative and antimicrobial constituents including phenolics (Maveety, 1938). 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.

2. 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 (4C°), 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 4C°.

Thiobarbituric Acid (TBA) values

Determination of lipid oxidation as thiobarbituric acid (TBA) was carried out by spectrophotometer (6400 – JENWAY, UK) following the method described by Witte *et al,* (1970.

Microbial Count

Microbial count was assessed as recommended by the American Public Health Association for food stuff examination (APHA, 1992).

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 detect significant differences among means of treatment combination (treatments X period).

3. Results and Dissection

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

Thiobarbituric Acid (TBA) values

In the current investigation, TBA values started to increase gradually in all examined samples of both lamb and chicken patties during storage period. TBA 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 TBA 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 TBA 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 would not deceive consumers up to 12 days of storage.

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

TBA 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}
TBA 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}

Means bearing a similar subscripts denote no significant differences otherwise they differ significantly ($P<0.01$)

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 LAB (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}

Means bearing a similar subscripts denote no significant differences otherwise they differ significantly ($P<0.01$)

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}	$61.\pm0.57^{b}$
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}

Means bearing a similar subscripts denote no significant differences otherwise they differ significantly ($P<0.01$)

4. Conclusion

From the result 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.

Acknowledgment:
The authors wish to express their appreciation for Prof. Dr. Jalal E. Alkass, Department of Animal production for his valuable help in reading the manuscript.

Reference:

1- Akthar, M.S., Degaga, B. and Azam, T. (2014) Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review. *Issues in Biological Sciences and Pharmaceutical Research.* Vol.2 (1), pp. 001-007.
2- Amarowicz, R., Egarska, Z., Rafalowski, R., Pegg R.B., Karamac, M. and Ska, A.K. (2009) Antioxidant activity and free radical-scavenging capacity of ethanolic extracts of thyme, oregano, and marjoram. *Eur. J. Lipid Sci. Tech.*, 111: 1111-1117.
3- American Public Health Association, (1992) Compendium methods for microbiological examination of foods, 2nd (ed.), Washington D.C
4- Dorman, H.J.D. and Deans, S.G. (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.*, 88: 308.
5- Duncan, D.B. (1955) Multiple ranges and Multiple test. Biometric, 11; 16.
6- Kassem, G.M., Atta-Alla, O.A. and Ali, F.H.M. (2011) Improving The Quality Of Beef Burger By Adding Thyme Essential Oil And Jojoba Oil.*Archivos de zootecnia,* vol. 60, núm. 231, p. 789.
7- Lacroix, M., Smoragicz, W., Pazdernik, L., Kone, M.I. and Krzystyniak, K. (1997) Prevention of lipid radiolysis by natural antioxidants from rose marry and thyme. *Food Res. Int.*, 30: 457-462.
8- Lawless, J. (1995) The illustrated encyclopedia of essential oils. Element books Ltd. Shaftesbury. UK.
9- Maveety, D.J. (1938) Stabilized Fats and Method for Preventing Rancidity. US patent, *2124706*.
10- Naveena, B.M, Sen, A.R., Kingsly, R.P., Singh, D.B. and Kondaiah, N. (2008) Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. *Int. J. of Food Sci and Tech,* 43:1807-1812.
11- Nguefack, J., Dongmo, J.B., Dakole, C.D., Leth, V., Vismer, H.F., Torp, J. and Nkengfack, A.E. (2009) Food preservative potential of essential oils and fractions from *Cymbopogoncitratus*, *Ocimumgratissimum*and *Thymus vulgaris* against mycotoxigenic fungi. *Int. J. Food Microbiol.,* 131: 151-156
12- Raghavan, S. and Richards, M.P. (2007) Comparison of solvent and microwave extracts of cranberry press cake on the inhibition of lipid oxidation in mechanically separated turkey. *Food Chem*, 102 (3): 818-826.
13- Rhee, K.S. (1989) Chemistry of meat flavour. In: Flavour chemistry of lipid food. MinandSmouse. Champain. pp. 462
14- SAS/STAT, (2002) User Guide for Personal Computers. Release 6.12 SAS. Institute Inc., Cary, NC. U.S.A.
15- Schuler, P. (1990) Natural antioxidants exploited commercially. In: Hudson, B.J.F. (Ed), *Food Antioxidants*. Elsevier Applied Science, London. 99-170.
16- Sebranek, J. and Bacus, J. (2007) Natural and organic cured meat products: regulatory, manufacturing, marketing, quality and safety issues. American Meat Science Association White Paper Series 1. Accessed 2009 March 24.http://www.meatscience.org/pubs/ White%20Papers/wp_001_2007_Natural_Organic_Cured_Meat.pdf
17- Selmi, S. And Sadok, S. (2008)The effect of natural antioxidant (*Thymus vulgaris* Linnaeus) on flesh quality of tuna (*Thunnusthynnus*(Linnaeus)) during chilled storage. *Pan-American Journal of Aquatic Sciences,* 3 (1): 36-45
18- Senatore, F. (1996) Influence of harvesting time on yield and composition of the essential oil of thyme growing wild in Campania (south Italy). *J.Agr. Food Chem.,* 44: 1327-32.
19- Seung-Joo, L., S., Umano, K., Shibamoto, T. and Lee, K. (2005) Identification of volatile components in basil (*Ocimumbasilicum*L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. *Food chemistry*, Vol. 91, Iss.1, pp131-
20- Skerget, M., Kotnik, P., Hadolin, M., Hras, A.R., Simonic, M. and Knez, Z. (2005) Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.*, 89: 191-198.
21- Solomakos, N., Govaris, A., Koidis, P. and Botsoglou, N. (2008) The antimicrobial effect of thyme essential oil, nisin and their combination against Escherichia coli O157:H7 in minced beef during refrigerated storage. *Meat Science*, 80, 159e166.
22- Stahl-Biskup, E. (1991) The chemical composition of thyme oils. A review of the literature 1960-89. *J.*

Essent. Oil Res., 3: 61-82.

23- Verme, S. P. and Sahoo, J. (2000) Improving the quality of ground chevon during refrigerated storage by tocopherol acetate preblending, *Meat Sci.*, 56:403-413.

24- Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J. and Perez- Alvarez, J.A. (2010) Effect of added citrus fibre and spice essential oils on quality characteristics and shelf-life of mortadella

25- Witte, V.C., Krause, G.F. and Baily, M.E. (1970) A new extraction method for determiniy 2-7 hiobarbiturie acid values of pork and beef during storage. *J. Food Sci*., 35:582-585.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Recent conferences: http://www.iiste.org/conference/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar