Received: 22 April 2023, Accepted: 10 May 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 6, 1079-1083

Effect of Thyme Oil and Acetic Acid on The Quality and Shelf Life of Fresh Meat

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Abstract

Meat industry is beginning to view meat shelf life as a serious issue. Organic acids and essential oils with antibacterial activities improve preservation of meat safety. Thus, the current study aimed to assess the preservation advantages of thyme oil, acetic acid, and a mixture of thyme oil and acetic acid (2% of each), as well as their effects on sensory characteristics, pH, total volatile basic nitrogen (TVBN), and thiobarbituric acid reactive substances (TBARS) of raw beef meat. The current study's findings demonstrated that treating raw beef meat with thyme oil, acetic acid at a concentration of 2% separately successfully lowered levels of APC, pH, TVBN, and TBARS and prolonged shelf life to 15 days when stored at 4°C. While the treatment with a combination of thyme oil and acetic acid at a concentration of 2% outperformed other treated and control groups leading to improving shelf life, and quality of raw beef meat. In conclusion, natural and organic preservatives may be utilized as an alternative to maintain meat and their products quality and extend their shelf life.

KEYWORDS
Meat quality, Shelf life, Thyme oil, Acetic acid, Aerobic bacterial count.

INTRODUCTION

By the year 2050, it is expected that there will be 9 billion individuals in the world. All agricultural food commodities will need to expand due to the population's dramatic increase (United Nations, 2015; Wicks *et al*., 2019). In the world's human nutrition, beef is very important. Meat contaminated with microbes and chemicals at every stage of production (Tshabalala *et al*., 2021). As mentioned above, when consumers make purchases, the shelf life is just as significant as the nutrient quality. To assure food safety and shelf life extension, numerous food preservation techniques are used in the meat industry. Among these are organic acids for preserving food quality and increasing the shelf life of perishable food ingredients as well as bioactive natural chemicals found in meat plants (Vieira *et al*., 2021).

Numerous essential oils including thyme oil have antibacterial activity because they degrade the bacterial lipid bilayer and encourage the breakdown of the cell membrane (Radünz *et al*., 2020). As natural antimicrobials in reformulated meat products, organic acids might be a good choice. They have demonstrated a blatant level of effectiveness, although flavor and color alterations may need to be considered. In terms of fighting bacteria, organic acids and their salts work quite well and have been regarded as the most widely used meat preservatives (Sohaib *et al*., 2016; Ben Braek and Smaou, 2021). They also have many benefits, such as they are generally recognized as safe, no upper limit on the amount that can be consumed each day, low cost, and easiness of manipulation (Santiesteban-López *et al*., 2022).

Both conventional and modern techniques are still used to delay the microbiological and biochemical deterioration of beef products (Luong *et al*., 2020). One of the greatest indicators of microbiological meat spoilage is the aerobic plate count (APC), while the best indicators of chemical meat spoilage are pH, total volatile basic nitrogen (TVBN), and thiobarbituric acid reactive substances (TBARS) (EOS, 2013; Tornuk *et al*., 2015). Consequently, to extend the shelf life of beef meat by preventing bacterial development and chemical deterioration, the current study aimed to evaluate whether thyme oil and acetic acid may be utilized as preservatives.

MATERIALS AND METHODS

Samples collection and processing

One kilogram of fresh beef steaks was bought from a butcher shop in EL Beheira Governorate, Egypt. As soon as it was practical, the gathered samples were stored in sterile plastic bags and an icebox before being transported to the lab. Within an hour, samples were delivered right away to Damanhur lab of Animal Health Research Institute for analysis. The beef meat steaks were divided into four equal portions (250 g). The first portion served as control sample and was submerged in sterile, deionized water without any additives, while the other three groups were submerged in 5ml at concentrations of 2% thyme essential oil, 2% acetic acid, and 2% mixture of thyme oil and acetic acid for 30 minutes at 25°C, respectively. A total bacterial count was conducted on all samples kept at 4°C for 15 days starting on the first day, and then regularly every 3 days until decomposition was

ISSN: 2090-6277/2090-6269/

seen in each group from day 0 to day 15.

Determination of aerobic plate count

According to the same procedures used for ground beef products, the beef sample's aerobic plate count (APC) was evaluated (Sabike *et al*., 2015). Each sample was homogenized at concentrations of 10% then diluted ten times in a row before being divided into two separate sterile Petri dishes, each of which received 1 ml of each dilution. Incubation at 37°C for 24 hours followed by the solidification of the inoculation plates (ISO, 2013).

Physicochemical examination

Sensory analysis

According to Lawless and Heymann (2010), fifteen trained adult panelists were asked to assess the sensory qualities of samples of raw beef meat. The samples were blind coded using unique codes. While the samples were still fresh, individuals were asked to assign each of the total acceptances as a score. A nine-point descriptive scale was utilized. For the evaluation of flavour, texture, and general acceptability, scores of 7-9 denoted "very good" quality, 4.0-6.9 "good" quality, and 1.0-3.9 "spoiled" quality.

pH measurement

Utilizing an electrical pH meter (Bye model 6020, USA), the pH value was calculated (ISO, 1979). To calibrate the pH meter, two buffer solutions with known pH values were used. Then, the pH electrode was cleaned with neutralized water and added to the homogenate when the temperature control system was adjusted.

Total volatile basic nitrogen measurement (TVBN)

The procedure for calculating total volatile nitrogen (TVBN) in beef meat was validated by the Food and Agriculture Organization (FAO, 1980).

Thiobarbituric acid reactive substances measurement (TBARS)

According to Vyncke (1970), the thiobarbituric acid (TBA) assay was performed. Using a blender, the sample (20g) was homogenized for 2 minutes after adding 100 ml of a 7.5% trichloroacetic acid solution. Then filtration of the homogenate was applied. In a test tube with a screw top, 5ml of the filtrate was mixed with 5ml of TBA reagent (0.02M TBA) following filtration. The test tubes were immersed in water for 40 minutes, after which a spectrophotometer was used to detect the absorbance at 538 nm. The value of TBARs was given as milligrams of malonaldehyde (MA) per kilogram of beef.

Microbial analysis

Following ICMSF, (1996) a total bacterial count (TBC) was conducted. Duplicated sterile plates with plate count agar were inoculated with 1 ml of a predefined prepared dilution. The plates were incubated at 37°C for 24 hours after the inoculums were dispersed using sterile bent glass. The aerobic plate count (APC) per gram of the sample was determined and recorded after counting plates with 25–250 colonies.

Statistical analysis

An analysis of one-way variance was performed on the gathered data (ANOVA) using SPSS Version 25 (SPSS Inc. Chicago, IL, USA). To compare the means of treatments, a statistical model utilizing Duncan's multiple-range tests was performed. At $P<0.05$, there were significant differences.

RESULTS AND DISCUSSION

The shelf life of a meat product refers to how long it will maintain its high quality (Li *et al*., 2022). Growing public awareness of the harmful effect of chemical preservatives necessitates research about plant-based natural preservatives (Ali *et al*. 2018; Nieto 2020). The purpose of this study was to establish the impacts of acetic acid and/or thyme essential oil on the physicochemical properties of beef and to assess the antibacterial advantages of these treatments.

The most popular techniques for judging the quality and spoilage of meat products were found to be sensory evaluations carried out by a panel of many experts (Luong *et al*., 2020; Ruiz-Capillas *et al*., 2021). Table 1 summarizes the sensory properties of treated and untreated beef meat samples that were stored at 4°C and exposed to 2% thyme oil, 2% acetic acid, and 2% combination of thyme oil + acetic acid. According to sensory evaluation, beef meat samples treated with thyme oil 2%, acetic acid 2%, or a combination of thyme oil and acetic acid (2% of each) maintained their acceptable quality until days 12 and 15; however, the means of the columns with the same superscript do not significantly differ ($P>0.05$). On days 12 and 15 the treated groups showed good sensory characteristics (texture, flavor, and overall acceptability), however, control beef meat samples showed poor sensory characteristics. These results show how the antibacterial capabilities of thyme oil and acetic acid applied to beef meat samples affect the meat's sensory quality (Sirocchi *et al*., 2017).

The oxidation of lipids and pigments, as well as fatty liberation, are thought to be the main factors affecting product appearance and flavour scores when they are being stored (Sharma *et al*., 2017).

Natural preservatives may change the product's original flavour, some customers consider them unacceptable (Zhang *et al*., 2017). But some EO components, like 0.3% thyme essential oil, can also offer food with distinctive flavor (Boskovic *et al*., 2017).

One of the fundamental factors that affect how long meat will stay fresh is its pH; at the moment of slaughter, the pH of beef is roughly 7.0; after that, it droped to 5.8-5.3 for 18 to 40 hours. Microorganisms that cause spoiling grow more quickly when the pH is higher (Hazards and Panel, 2016). Comparing the basic pH values for raw beef meat to those described in the literature, they were deemed normal (Fleck *et al*., 2015; Triki *et al*., 2018). Table 2 shows the changes in the pH value of the meat among study groups as follows: the initial pH on zero-day was 5.85±0.12 in group I (control) and 5.63±0.02 in group II (thyme oil 2%). The pH in both gradually increases with the increase of the storage days. Also, the pH value of groups III, and IV (acetic acid 2%, Mixture of Thyme oil 2% and acetic acid 2% respectively) were increased gradually with increasing the storage days. The pH of meat increase after refrigeration storage due to lipid/protein degradation that caused by chemicals, microorganisms, and physical damage, Triki *et al*. (2018). The mean, calculated in triplicate ($n = 3$), is displayed in the results as a mean value with standard error. Significant variations between the samples at $P<0.05$ are shown by different letters in the same storage period. The pH value showed reduction and the viability of raw beef meat in groups II, III, and IV was kept for the 15th days (5.90±0.01, 5.32±0.10, and 5.28±0.14, respectively), which was substantially (P<0.05) different from the control sample (6.93±0.14) for the same storage time. This is most likely because EOs limit lipid/protein degradation caused by chemicals, microorganisms, and physical damage, leading to reduces pH rise and prevents the production of nitrogen and highly alkaline volatile base like ammonia (Badee *et al.*, 2014). Contrary to Triki *et al.* (2018) who reported that the pH of various types of meats increases after refrigeration storage, the pH in the control group decreased during storage.

A type of meat degradation called lipid oxidation causes meat to eventually deteriorate their sensory and nutritional value, which affects the responding of consumers to the product (Amaral *et al.*, 2018). To check whether beef meat is rancid or has started to spoil, it must be tested for TBARS, which must not be more than 0.9 mg/kg (EOS, 2013). Table 3 show the effects on TBARS values of raw beef samples over 15 d of storage at 4°C. The TBARS values over 0 to 15 days of storage ranged from 0.43 to 1.06 mg of MA/100 g of meat in the control group. TBARS values increased during storage in all studied groups, the highest TBARS value was observed in the control group on day 15. In general, adding thyme essential oil and/or acetic acid to raw meats decreases the TBARS values. Results are presented as a mean value ± SE and reflect the mean completed in triplicate (n = 3). Mean values in the same storage period bearing the same superscript do not differ significantly (P>0.05). When compared to Egyptian TBARS standards (EOS, 2013), on day zero, the control samples' average TBARs value was 0.43±0.02 and 1.06±0.04 on day 15, both of which were above the maximum recommended

Table 1. Sensory assessment of examined raw beef meat samples during refrigerated storage (4°C) for 15 days (Mean ± SE).

	Control	Thyme oil 2%	Acetic acid 2%	A mixture of Thyme oil 2% +Acetic A 2%
Flavour				
Day 0	7.02±0.02^a	7.85±0.05^a	7.25±0.07^a	7.89±0.01^a
Day 3	6.86±0.02^a	7.51±0.02^a	7.23±0.04^a	7.75±0.08^a
Day 6	6.25±0.05^b	7.19±0.09^a	7.07±0.01^a	7.54±0.05^a
Day 9	5.63±0.03^c	6.89±0.05^a	6.75±0.04^a	7.35±0.09^a
Day 12	Spoiled	6.75±0.07^a	6.54±0.02^a	7.04±0.06^a
Day 15	Spoiled	6.65±0.08^b	6.23±0.04^b	6.96±0.09^a
Texture				
Day 0	7.35±0.09^a	7.65±0.03^a	7.44±0.05	7.74±0.03
Day 3	7.14±0.07^a	7.43±0.05^a	7.17±0.05	7.55±0.07
Day 6	7.07±0.01^a	7.17±0.08^a	7.10±0.05	7.42±0.05
Day 9	5.51±0.02^b	7.05±0.01^a	6.45±0.04	7.15±0.06
Day 12	Spoiled	6.77±0.04^b	6.10±0.05	6.99±0.03
Day 15	Spoiled	6.58±0.05^b	6.01±0.01	6.75±0.06
Overall acceptability				
Day 0	7.03±0.06^a	7.24±0.04^a	7.10±0.02^a	7.35±0.06^a
Day 3	6.74±0.07^a	6.89±0.06^a	6.76±0.05^a	6.95±0.08^a
Day 6	6.35±0.05^a	6.76±0.05^a	6.46±0.07^a	6.85±0.09^a
Day 9	5.27±0.04^b	6.65±0.04^a	6.41±0.01^a	6.77±0.07^a
Day 12	Spoiled	6.60±0.03^a	6.38±0.05^a	6.69±0.05^a
Day 15	Spoiled	6.05±0.05^a	5.91±0.04^a	6.59±0.08^a

Data represents the mean ± SE values. In each column, different letters mean statistically significant differences at P < 0.05.

Table 2. Mean values show the pH levels of raw beef meat samples changed while being stored at 4°C for 15 days.

Storage days/ groups	pH values ± SE					
	0	3	6	9	12	15
Control	5.85±0.12a	5.93±0.07a	5.98±0.14a	6. 23±0.24a	6. 54±0.13a	6.93±0.14a
Thyme oil 2%	5.63±0.02b	5.68±0.04b	5.75±0.01b	5.79±0.01 b	5.84±0.01b	5.90±0.01b
acetic acid 2%	4.42±0.14c	4.63±0.16c	5.09±0.12c	5.18±0.09c	5.26±0.15c	5.32±0.10c
A mixture of Thyme oil 2% & acetic acid 2%	4.32±0.13d	4.54±0.07d	5.06±0.13d	5.14±0.15d	5.21±0.14b	5.28±0.14d

Data represents the mean ± SE values. In each column, different letters mean statistically significant differences at P < 0.05.

Table 3. Mean TBARS readings of raw beef meat samples stored at 4°C for 15 days.

Storage days/ groups	TBA mg Malonaldehyde/Kg ±SE TBARS (mg MA/100g)					
	0	3	6	9	12	15
Control	0.43±0.02a	0.53±0.03a	0.64±0.03a	0.78±0.03a	0.92±0.04a	1.06±0.04a
Thyme oil 2%	0.39±0.121b	0.43 ± 0.14b	0.52 ± 0.12b	0.64 ± 0.13b	0.76 ± 0.05b	0.92 ± 0.12b
acetic acid 2%	0.37±0.03c	0.39±0.04c	0.43±0.03c	0.44±0.04c	0.45±0.02c	0.47±0.02c
A mixture of Thyme oil 2% & acetic acid 2%	0.33 ± 0.13d	0.36 ± 0.11d	0.39 ± 0.10d	0.41 ± 0.13d	0.43 ± 0.12d	0.45 0.11d

Data represents the mean ± SE values. In each column, different letters mean statistically significant differences at P < 0.05.

Table 4. Mean TVBN (mg N/100 g meat) readings of raw beef meat samples stored at 4°C for 15 days.

Storage days/groups	Total volatile basic nitrogen value TVBN (mg N/100g meat)					
	0	3	6	9	12	15
Control	13.5±2.0a	15.1±0.75a	17.0±1.48a	19.3±0.85a	21.2±1.14a	23.8±1.71a
Thyme oil 2%	12.2±1.25 b	14.4±1.33 b	16.2±1.22 b	17.7±1.15 b	19.3±1.30 b	21.1±1.30 b
acetic acid 2%	10.0±2.07b	11.3±0.45c	12.2±0.51c	14.2±1.14c	15.3±1.3c	18.2±1.68c
A mixture of Thyme oil 2% & acetic acid 2%	09.0±1.06d	10.0±1.06d	11.4 ± 0.10d	12. 2 ± 0.13d	14.3 ± 0.12d	16.0±1.06d

Data represents the mean ± SE values. In each column, different letters mean statistically significant differences at P < 0.05.

Table 5. Total aerobic plate count APC (log_{10}cfu/g) of raw beef meat samples stored at 4°C for 15 days.

Storage days/ groups	Total aerobic plate count APC (log_{10}cfu/g)					
	0	3	6	9	12	15
Control	3.92±0.43a	4.74±0.16a	5.84±0.24a	6.65±0.18a	7.86±0.17a	8.27±0.35a
Thyme oil 2%	3.70±0.45b	3.83±0.16 b	4.89±0.16 b	5.12±1.15 b	5.45±1.30 b	6.12±1.30 b
acetic acid 2%	3.51±0.42c	3.62±0.12c	4.73±0.17c	4.75±0.25c	4.78±0.32c	5.42±0.13c
A mixture of Thyme oil 2% & acetic acid 2%	3.42±0.35d	3.49±0.14d	3.52±0.15d	4.11±0.20d	4.21±0.20d	4.75±0.20d

Data represents the mean ± SE values. In each column, different letters mean statistically significant differences at P < 0.05.

limit. The mixture of thyme oil and acetic acid (2% of each) and the acetic acid 2% treated samples had the lowest TBARs values on zero days, which were 0.33±0.13 and 0.37±0.03, respectively. The values of the TBARs in the treated groups were lower than the control group at the same storage period at 4°C. TBARs values of the three groups of beef samples (thyme oil 2%, acetic acid 2%, and mixture of thyme oil 2% and acetic acid 2%) showed 0.92±0.12, 0.47±0.02, and 0.45±0.11 respectively on day 15 of storage. The rate of MDA production was decreased in the group treated with mixture of thyme oil and acetic acid (2% of each) in comparing with treated groups with thyme oil or acetic acid separately, however the difference wasn't significant (P>0.05) between the untreated samples and the treated samples. This result is consistent with the findings of Alsaiqali *et al*. (2016) who found that thyme oil considerably lowers the TBA levels compared to control samples. According to Kassem *et al*. (2011), after six days of storage at 4°C, meat treated with 0.05% thyme oil had minimal TBA levels. These findings were in contrast to another study by Sharafati-Chaleshtori *et al*. (2015) who found no appreciable decrease in TBA levels after adding various quantities of essential oil to raw beef burgers while storing them at 4°C for 12 days. Furthermore, TBA was shown to gradually decrease in groups that received acetic acid treatments at 1% and 2%, according to Saleh *et al*. (2022).

One chemical indicator for determining the level of meat microbiological quality is total volatile basic nitrogen (Luong *et al*., 2020). According to Egyptian Standards (EOS, 2013), TVBN accumulations of more than 20 mg N/100 g in raw samples show that beef products are unsuitable due to related breakdown. As displayed in Table 4, shows the impact of thyme oil, acetic acid, and a mixture of thyme oil and acetic acid (2% of each) on the TVBN level of beef meat samples stored at 4°C. The TVBN values of the beef samples in each treatment group increased as the storage time increased. The beef samples of control group reached 23.8 mg N/100 g on the 15th day of storage. When compared to the preservation effect of a single thyme oil 2%, acetic acid 2%, and the mixture of acetic acid 2% and thyme oil 2%. TVBN values of the three groups of beef samples (thyme oil 2%, acetic acid 2%, and mixture of thyme oil 2% and acetic acid 2%) showed 21.1 mg N/100 g, 18.2 mg N/100 g, and 16 mg N/100 g respectively on the 15th day of storage. Mean values in the same storage period bearing the same superscript do not differ significantly (P<0.05). The degradation of lipids and proteins by chemical, microbial, and physical processes is related to the synthesis of nitrogen and highly alkaline volatile bases, such as ammonia (Luong *et al*., 2020). The findings of this study corroborate earlier findings that show EOs, especially thyme essential oil, are effective at preventing unwanted microbiological and chemical alterations, including TVBN in beef products (Sharma *et al*., 2017; Huang *et al*., 2021).

The microbiological condition of raw beef meat is assessed using the aerobic plate count, a broad measure of the overall level of microbial contamination of meat (Kim *et al*., 2018). Statistically significant (P<0.05) differences between control and treated meat samples with thyme oil and/ or acetic acid to the average level of different microbial counts over storage time are shown in Table 5. The highest inhibition of total aerobic bacterial count growth (3.42 log_{10}cfu/g and 4.75 log_{10}cfu/g) was found in meat containing a mixture of thyme oil, 2% and acetic acid 2% on zero and the 15th days of storage, respectively . On the other hand, the control group showed the highest total aerobic bacterial count growth on the 15th day of storage (8.27 log_{10}cfu/g), which exceeded the suggested maximal limit for total APCs in raw beef samples which was 5 log CFU/g. The increase in storage time produced an increase in APCs and the increase was more rapid in the control sample. The APC values were lower than 5 log CFU/g on day 15 in samples treated with a mixture of thyme oil and acetic acid (2% of each). Between treated and untreated beef meat samples, there were significant differences in APC (P <0.05). The mixture, as demonstrated in the current study, had the best inhibitory effects. These findings are in line with other studies and point to the potential use of essential oils, particularly thyme oil, as natural preservatives in a range of meat products (Nieto, 2020; Huang *et al*., 2021; Hammoudi Halat *et al*., 2022). Also, treatment of beef meat samples with acetic acid reduced microbial count (EL-Tabiy and Soliman, 2011).

CONCLUSION

The current study's results showed that treating raw beef meat with thyme oil, acetic acid, or a mixture of thyme oil and acetic acid (2% of each) significantly reduce the levels of APC, pH, TVBN, and TBARS and extended shelf life up to 15 days at 4°C. Regarding the sensory quality, shelf life, and antibacterial activity of raw beef meat, the combination of thyme oil and acetic acid (2% of each). Outperformed the single treatment of thyme oil or acetic acid. The current study presents effective natural and organic preservative substitutes that, in the future, might take the place of undesirable synthetic substances while also lowering antibiotic resistance. Consequently, they might preserve meat and meat products quality, prolong their shelf life, and avoid economic loss.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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