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Improvement quality of minced meat using some essential oils

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ABSTRACT

A total of 5250 g fresh minced beef were divided into equal groups (250 g of each). *E.coli* was inoculated into all groups with infective dose 5.36±0.01 log cfu/g except control ones. All samples were treated with thyme oil (0.5%, 1% and 1.5%) and garlic oil (0.5%, 1% and 1.5%) and examined every 48 hrs at 4 ºC of storage temperature. Sensory examination (overall acceptability) and *E.coli* count were conducted. Thyme oil(1% and 1.5%) and garlic oil (1.5%) treated minced beef showed overall acceptability till 8th day of storage. In comparison, thyme oil (0.5%) and garlic oil (0.5% and 1%) showed overall acceptability till 6th day. Furthermore, thyme oil (0.5%, 1% and 1.5%) decreased count of *E.coli*(log cfu/g) from 5.36±0.01 (initial load) to 4.38±0.02, 3.81±0.2 and 3.51±0.14 with reduction percentages 18.28%, 28.92% and 34.51% on 6th,8th and 8th of storage, respectively. Garlic (0.5%, 1% and 1.5%)decreased count of *E.coli* (log cfu/g) to 4.54±0.2, 4.41±0.3 and 3.81±0.1 with reduction percentages 15.30%, 17.72% and 28.92% on 6th, 6th and 8th of storage, respectively. Generally, thyme oil (1.5%) proved to be more efficient than other treatments in reduction of *E.coli* growth in minced beef, therefore, it is recommended to improve safety and extend shelf life of the meat products.

1. INTRODUCTION

Street-vended foods (SVFs) are ready-made instant meals. Meat has high nutritive composition, so it is being the first choice for animal protein by many people all over the world. Meat and meat products are susceptible to microbial and biochemical deterioration, especially during storage, due to their complex composition of saturated and unsaturated lipids, carbohydrates, proteins, vitamins, and pigments (Lombardi-Boccia et al.,2005 ; Lorenzo etal.,2014).After slaughtering of animal, the meat is fabricated into wholesale or retail cuts. Trim and other cuts of meat are undergoing further processing and grounding. This increases surface area of the meat leading to adherence and growth of bacteria (Skandamis & Nychas, 2001 and Donsì et al.,2011). The most important bacterial pathogens in meat and meat products those are responsible for food-borne infections including *E. coli* (Abdaslam-Azza, 2014; Saif-Marwa, 2015).

Pathogenic strains of *E. coli* were divided into (INPEC) causing diarrhea and (EXPEC) including urinary tract infection (UTI), meningitis and septicemia depending on their virulence factors and clinical symptoms (Kaper et al., 2004; Eid and Erfan, 2013).

Essential oils are extracted from several aromatic plants (Saljoughian et al., 2018). They have antioxidant and antibacterial activities that increased their application in the food, cosmetic and pharmaceutical industries (Bakhtiary et al., 2018) and their classification as Generally Recognize as Safe(GRAS).Therefore, they are used as natural food safer additives in food products (Eset al., 2017).

Recently, using of essential oils became the main substitutes for synthetic additives (Vinceković et al., 2017). However, the use of essential oils has an important drawback as most of them have a strong odour, so it is very important to determine the concentration of essential oil that can be used without modifying organoleptic characteristics of the meat (Shen and Kamdem, 2015).

The essential oil of *Thymus vulgaris* contains various levels of thymol and/or carvacrol, phenolic derivatives with strong and widespectrum antimicrobial activity (Nevas, 2004)

The antimicrobial effects of garlic have been attributed to the action of thiosulfinates. Inhibition of certain thiol-containing enzymes in the microorganisms by the rapid reaction of thiosulfinates with thiol groups was assumed to be the main mechanism. Also, Allicin inhibited other bacterial enzymes such as acetate kinase and phosphotransacetyl -CoA synthetase. Further, it inhibited the DNA and protein synthesis, the effect on RNA is suggesting that RNA could be a primary target of allicin (Focke et al.,1990 and Al-Snafi, 2013).

During the last decade, the antimicrobial activity of garlic and garlic derived organosulfur compounds was widely investigated against both food spoilage bacteria and food borne pathogens (Leuschner and Ielsch, 2003).

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The purpose of the current research was to study the antimicrobial effect and acceptability of certain essential oils on reduction of *E. coli* in minced beef.

2. MATERIAL AND METHODS

2.1. Bacterial strain:
Escherichia coli strain (O_{126})10^4cfu/g (Barbosa et al., 2009) was obtainedfrom Media Unite, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt.

2.2.Natural oils:
The ready-made pure herbal oils of thyme (0.5%, 1% & 1.5%) and garlic (0.5%, 1%&1.5%) used in this study were purchased from El Captain Company (CAP PHARM). All the used chemicals were of analytical reagent grade. These oils were stored in amber colored bottles at 4°C until use.

2.2. Collection of samples
A grand total of 5250 g of the fresh minced beef used in this study was purchased from butcher shops in El Menofiya Governorate. To eliminate natural microbial populations, the purchased meat was sterilized with ultraviolet light at wavelengths 385 nm for 30 minutes (Morsy et al., 2014).

2.3. Experimental application:
Accurately, 5250g fresh minced beef samples were divided into untreated and treated equal groups (250g each) and inoculated with E.coli(5.36±0.1 log cfu/g)(initial load) and then mixed with certain concentrations of tested natural oils according to following order:
Control +ve inoculated with E.coli only. Phosphate buffer saline was used for treatment of control (untreated) samples.
A: Treated with thyme oil 0.5%. B: Treated with thyme oil 1%. C: Treated with thyme oil 1.5%. D: Treated with garlic oil 0.5%. E: Treated with garlic oil 1%. F: Treated with garlic oil 1.5%.
The inoculated samples were stored at 4ºC in refrigerator and examined every 48 hours during storage until spoilage of samples. The experiment was conducted in triplicate according to Kantachote and Charernjiratrakul (2008).

2.4. Sensory examination (overall acceptability) was applied according to Hemin (2013).

2.5. Preparation of sample was done according to ISO (1999).
2.6. E. coli count was performed according to FAD (2001).

2.7. Statistical analysis:
The obtained results were statistically evaluated by application of analysis of variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

Results in table (1) illustrated the effects of various concentrations of thyme and garlic oils on overall acceptability of artificially inoculated minced beef samples with *E.coli*.

Thyme oil(1% &1.5%) and garlic oil (1.5%) showed overall acceptability extended to 8th day of storage. In contrast, thyme oil (0.5%) and garlic oil (0.5% and 1%) showed overall acceptability till 6th day of storage. while, control group showed overall acceptability till 4th day.
Table (2) and Table (3) illustrated the antimicrobial effects and reduction % of various concentrations of thyme and garlic oils on counts of *E.coli* artificially inoculated into minced beef.
Thyme oil (0.5%, 1%& 1.5%) decreased count of *E.coli* (log cfu/g) from5.36±.01 (initial load) to 4.38±.02,3.81±0.2 and 3.51±0.14 with reduction of 18.28%, 28.92% and34.51% on 6th,8th and 8th of storage, respectively. Garlic oil (0.5%, 1%& 1.5%)decreased count of *E.coli* (log cfu/g) to 4.54±0.2,4.41±0.3 and 3.81±0.1with reduction of 15.30%,17.72% and 28.92% on 6th,6th and 8th of storage, respectively. In control group *E. coli* count increased from5.36±.01 log cfu/g (initial load) to 6.16±0.1on4th day.
On regard table (2), the differences between the effects of various concentrations of thyme and garlic oils on counts of *E.coli* (log cfu/g) artificially inoculated into minced meat samples were significant ($P \leq 0.05$).

Table 1 Sensory evaluation of the control(untreated) and treated samples of minced beef during cold storage at 4ºC.

Group	zero day	2nd day	4th day	6th day	8th day	10th day
Control	5	4	3	2	1	1
Thyme 0.5%	5	4	4	3	2	1
Thyme 1%	5	4	4	3	3	2
Thyme 1.5%	5	5	4	4	3	2
Garlic 0.5	5	4	3	3	2	1
Garlic 1%	5	4	4	3	2	1
Garlic 1.5%	5	4	4	3	3	2

(5) Very acceptable, (4) Acceptable, (3) Middle, (2) Unacceptable, (1) Rejected (Hemin, 2013).

Table 2 Mean values of *E. coli* count (log cfu/g) of the examined control(untreated) and treated samples of minced beef during cold storage at 4ºC.

Groups	zero day	2nd day	4th day	6th day	8thday
Control	5.36±0.1^{a}	5.65±0.3^{b}	6.16±0.1^{d}	Spoiled	spoiled
Thyme 0.5%	5.36±0.1^{a}	5.24±0.2^{ab}	4.62±0.1^{dc}	4.38±.02^{fc}	spoiled
Thyme 1%	5.36±0.1^{a}	5.16±0.1^{c}	4.52±0.1^{e}	4.24±0.2^{fd}	3.81±0.2^{fe}
Thyme1.5%	5.36±0.1^{a}	5.12±0.1^{c}	4.42±0.1^{e}	4.12±0.15^{fd}	3.51±0.14^{fe}
Garlic 0.5%	5.36±0.1^{a}	5.25±0.2^{ab}	4.76±0.2^{dc}	4.54±0.2^{fc}	spoiled
Garlic 1%	5.36±0.1^{a}	5.24±0.1^{c}	4.61±0.1^{dc}	4.41±0.3^{fc}	spoiled
Garlic 1.5%	5.36±.01^{a}	5.19±0.2^{c}	4.48±0.1^{e}	4.32±0.2^{fd}	3.81±0.1^{fe}

Initial load of *E .coli* at zero hour =5.36±.01^{a} log CFU/g. The values represent Mean ± SD of three experiments. Means within a column followed by different letter are significantly different ($P \leq 0.05$).

Table 3 Reduction % of *E. coli* count(log cfu/g) artificially inoculated into minced beef samples treated with different concentrations of thyme and garlic oils.

Groups	Concentration	2nd day	4nd day	6th day	8th day
Thyme oil	0.5%	2.24	13.80	18.28	spoiled
	1%	3.73	15.67	20.89	28.92
	1.5%	4.43	17.54	23.13	34.51
Garlic oil	0.5%	2.05	11.20	15.30	spoiled
	1%	2.24	13.99	17.72	spoiled
	1.5%	3.17	16.42	19.40	28.92

4. DISCUSSION

The meat preservatives prevent microbial activity that responsible for deterioration and spoilage of meat and meat products (Yadav and Singh, 2004), but their carcinogenic nature is the major problem for their application. So, natural compounds derived from herbs or plants are suggested to be used either completely or partially substituting chemical preservatives (Gammariello et al., 2008; Hyldgaard et al., 2012).

Natural products and naturally derived compounds from plants may have applications in controlling pathogens in foods (Davidson, 1997; Bowles and Juneja, 1998).

Garlic oil is rich in organosulfur compounds and their precursors "allicin, dially sulfide & diallyltrisulfides" (Ankri and Mirelman, 1999) inhibiting the growth of a lot of pathogens as *E. coli* and *S. aureus* by reacting with their cystine, inactivating the thio-containing enzymes or affecting the metabolism of lipids (Song et al., 2004).

Sensory evaluation is an easy, quick and efficient method and used to evaluate the degree of meat freshness depend on organoleptic characteristics such as color, odor, texture and overall acceptability of the product (Haq et al., 2013).

There was a decline of acceptability began after the first day of storage with marked reduction of odor, color, texture and overall acceptability values in the control samples at the 4th day except thyme oil (1.5%) samples the decrease of acceptability began after the 2nd day (Table 1).

Furthermore, the obtained results indicated that the best acceptability quality was attained at thyme oil (1.5%) treated minced beef samples then in garlic oil(1.5%) treated samples, while slight improvement in acceptability of garlic oil (0.5%) minced beef samples as compared control samples.

These results agreed with those obtained by Sasse et al. (2009) who reported that many herbs and spices as thyme contain antioxidant components that improve both color and flavor stability in meat. Also, Salem–Amany et al. (2010) indicated that sensory properties of minced beef samples during cold storage (4ºC) were enhanced by treatment minced beef by different concentrations of thyme and garlic oils (0.5%, 1%, 1.5%) compared to the untreated (control) samples and samples contain 1.5% thyme and garlic oils revealed best enhancement of sensory properties than samples contain 0.5% of the same oil, and those obtained by Shaltout et al., (2017) whose results were that meat samples containing 2% thyme oil demonstrated the highest enhancement of sensory attributes, while the samples treated with 1% of thyme oil demonstrated lower enhancement.

These results are disagreed with those obtained by Solomakos et al. (2008) and Giatrakou et al. (2010) who found the effect of *Thymus vulgaris* EO on meat was acceptable concerning odor and taste in the range of 0.2 to 0.6% but unacceptable at 0.9% on minced beef (Solomakos et al., 2008). Also, Agnieszka et al. (2012) recorded 9 out of 10 persons rejected the meat stored in modified atmosphere with thyme oil due to unacceptable odor, and 6 due to bad taste.

The results in Table (2)showed that the control samples had the highest counts of *E. coli* at any time of cold storage compared to other treatments. Thyme essential oil showed maximum antibacterial activity followed by garlic oil. The inhibition of *E. coli* is related to the concentration of the studied essential oils, since they declined and even inhibited completely, when increasing the concentration of the studied essential oils. As the thyme oil (1.5%) has the best antimicrobial activity against *E. coli* than that obtained by (0.5% and 1%). Furthermore, the high concentration of garlic oil (1.5%) has a higher inhibitory effect than that obtained by lower concentration (0.5% and 1%).

The major active compound of thyme is thymol, which exerted its antimicrobial action through binding to membrane proteins by hydrophobic bonding and hydrogen bonding, and then changing the permeability of the membranes (Burt, 2004).

These finding were nearly similar with those obtained by Abd EL Fatah- Hend (2016) who found that thyme oil (1.5%) had the best antimicrobial activity against *E.coli* than that obtained by (0.5% and 1%) and the high concentration of garlic oil (1.5%) has a higher inhibitory effect than that obtained by lower concentration (0.5% and 1%).

The low effectiveness of garlic oil in comparison with thyme oil could be attributed to the losses of volatile sulfur compounds, which have high biological activity, during distillation, and also due to the nature of garlic oil itself, which is volatile and hydrophobic (Pranoto et al., 2005).

Finally, the present study allowed to conclude that thyme oil (1.5%) proved to be more efficient in suppression of *E.coli* growth in minced meat. So, the use of thyme oil (1.5%), as it is safe antimicrobial agent against *E.coli*, is therefore recommended to improve safety of meat products.

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