Antibacterial effect of some plant essential oils against *Salmonella Enteritidis* and *E. coli O157: H7* in minced beef meat during refrigerated storage

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Abstract

Thirty samples (100g each) of minced calf meat were inoculated by *Salmonella Enteritidis* and *E. coli* O157: H7 (15 for each bacterial type) in levels 10^5 cfu/ gm. Both thyme and rosemary oils were used for the treatments of the bacterial contaminated sample in concentrations of 0.1% and 0.5% for each oil. Also, mixture of these oils (0.1% of each) was used for the treatments. Three inoculated minced meat samples were used for each 5 oil treatments for *Salmonella Enteritidis* as well as *E. coli* O157: H7. After bacterial inoculations and mixing with the essential oil solutions, the inoculated meat samples were stored in sterilized polyethylene bags in refrigerator temperature at 4- 6 ºC during the period of the examination. At 2nd, 4th, 6th, 8th, and 10th days after the bacterial inoculations and oil treatments, the bacterial cell count was estimated to evaluate the effects of the essential oil treatments. The more significant lower bacterial counts were detected at 4th day after treatments in inoculated samples with *Salmonella Enteritidis*; while, in samples inoculated with *E. coli* O157: H7, the antimicrobial effects of the used oils expanded from 2nd to 6th day. After that, the bacterial levels gradually increased and exceeded the original inoculated level. Generally, the mixing of thyme and rosemary oils (0.1% for each) exhibited significant antibacterial effects than any used oil alone at concentration 0.1%. On contrarily, this mixture could not declare significant effects comparing with each oil alone at 0.5% concentration. The obtained results declared that the using of thyme oil and/ or rosemary oil at low concentrations (0.1%- 0.5%) for the treatment of minced meat at refrigerator temperature (4- 6 ºC) leads to significant reduction the levels of *Salmonella Enteritidis* and *E. coli* O157: H7 within durations not exceeded 4th and 6th day respectively. Subsequently, the validity time of the tested minced meat was prolonged.

Key words: *E. coli O157: H7, minced meat , Salmonella Enteritidis, antimicrobial effects .*

Introduction

Red meat contains high biological value protein and important micronutrients that are needed for good health throughout life. It also contains a range of fats, including essential omega-3 polyunsaturated fats. Recent analyses have shown that there has been a significant trend to leaner cuts of meat over the past two decades (**Williams *et al*., 2006)**.

Salmonella is one of the most common causes of food poisoning **(Essa *et al*., 2004),** the patient usually complains of headache, vomiting, abdominal pain and fever. In many countries the majority of the recorded food born outbreaks are attributed to Salmonellosis **(Rabsch *et al*., 2001)**. In the recent years, the incidence of salmonellosis caused by *Salmonella Enteritidis* increased world wide **(Humphrey and Jorgensen, 2006).** High incidence of *Salmonella Enteritidis* in minced meat was recorded in many previous studies **(Arsenos *et al*., 2007** and **Wong *et al*., 2007)**. On the other hand, *Escherichia coli* O157: H7 was recognized as a food born pathogen, colonizes in the large intestine and produce shiga like toxin (*Stx*) which is responsible for severe hemorrhagic colitis in human **(Naim *et al*, 2004)**. Cattle are the reservoir of this pathogen, and the consumption of

undercooked meat of bovine origin are considered the main cause of several outbreaks of *E. coli* O157: H7 **(Shekarforoush *et al*., 2007)**.

Essential oils are natural products extracted from vegetal materials. They are used as natural additives in many foods due to their antibacterial, antifungal, antioxidant and anticarcinogenic properties **(Bagambaula *et al*., 2004).** Screening of natural medicinal plants is now being popular because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind **(Naim and Tariq, 2006).** The use of natural antimicrobial compounds is important not only for the preservation of food but also for the control of human and plant diseases of microbial origin **(Bratta *et al*., 1998)**. Recently, there has been considerable emphasis on studies involving essential oils and extracts of spices and their constituents for inhibiting the growth of pathogen microorganisms. It has also been known for some time that certain crude drugs and spices contain substances with antifungal effect in their derivatives **(Deans,. and Svoboda. 1990).**

Thus, the objective of the current investigation is to evaluate the effects of rosemary (*Ros marinus oficinalis*) and thyme *(Thymus vulgaris)* oils to inhibit the growth of *Salmonella Enteritidis* and *E. coli* O157: H7 in the examined minced calf meat samples.

Material and Methods

A- Bacterial strain preparation

1- *Salmonella Enteritidis*

Salmonella Enteritidis strain, isolated from chicken meat in aprevious study was prepared for the current study. The bacterial cells were pelleted by centrifugation at 5000 rpm for 15 minutes at 5°C, washed twice in 10 ml of 0.01 M phosphste buffered saline (PBS), PH 7.0 and diluted to 1.0 X 10^8 cfu/ ml in PBS for the inoculation of minced beef meat samples. Cell counts were determined by serial dilution and subsequence enumeration on tryptone soy agar **(Govaris *et al*., 2010)**.

2- *E. coli* O157: H7

An isolate of *E. coli* O157: H7 strain from meat samples in a previous study was prepared. Stock cultures were maintained at –70 ºC on tryptic soy agar slants (Oxoid) and were activated in tryptic soy broth (pH 7.0) at 35ºC. Culture was transferred by loop inocula twice at 24 hr intervals to 100 ml of tryptic soy broth in 250 ml Erlenmeyer flasks. Appropriate dilutions were performed in peptone water 0.1% in order to obtain high inoculum level (10^7 -10^8 CFU/ml) populations **(Arias *et al*., 2001)**.

B- Essential oils

The pure rosemary (*Ros marinus oficinalis*) and thyme *(Thymus vulgaris)* oils were purchased from local essential oil companies in Egypt. The essential oils diluted to 0.5% and 0.1% by mixing with diluted propylene glycol in the sterilized distilled water as an inert substance act as dissolving agent for oil without any harmful effects on the food **(Ozcan *et al*., 2003)**.

C- Minced meat preparation and bacterial inoculation

Thigh muscles meat samples were taken from carcasses of approximately 2 years old calf. After 1 hour post slaughter in a local abattoir, and transferred to the laboratory under refrigerated conditions within 30 minutes. Minced calf meat was prepared under sterile condition. After the aseptic removal of the outer surface, the inner core of the sterile meat was aseptically minced by means of a sterile steel meat grinder autoclaved at 121ºC for 15 minutes, prior to meat inoculation with pathogen and the addition of rosemary and/ or thyme oils. The minced meat was also examined for the presence of *Salmonella Enteritidis* and *E. coli* O157: H7 as described in microbiological analysis section. Thirty treated samples (100g) for each were contaminated and well mixed with the bacterial inoculums from each studied microorganism in levels 10^5 cfu/ gm.

D- Essential oils treatment

Both thyme and rosemary oils were used for the treatment of the bacterial contaminated samples in concentrations of 0.1% and 0.5% for each oil. Furthermore, mixture of these oils (0.1% of each) were used also for the treatment. Three inoculated minced meat samples

were used for each 5 oil treatments for *Salmonella Enteritidis* and *E. coli* O157: H7. Thus 30 samples were used for the 2 examined bacterial types. After bacterial inoculations and mixing with the essential oil solutions, the inoculated meat samples were stored in sterilized polyethylene bags in refrigerator temperature at 4- 6 ºC during the period of the examination. At 2nd, 4th, 6th, 8th, and 10th days after the bacterial inoculations and oil treatments, the bacterial cell count was estimated to evaluate the effects of the essential oil treatments.

E- Microbiological analysis

1- *Salmonella Enteritidis*

Each sample was diluted by the addition of 90 ml of 0.1% peptone- water and homogenized for 2 minutes. Then the homogenate was serially (10 folds) diluted in 0.1% peptone- water. Sample dilution (0.1 ml) of minced meat was plated on selective XLD agar in duplicate. Populations of *Salmonella Enteritidis* were determined after incubation of plates at 37 ºC for 24 h. The presence of *Salmonella Enteritidis* was determined after incubation of plates at 37 ºC for 24h **(Govaris *et al.*, 2010).**

2- *E. coli* O157: H7:

Each sample was diluted by the addition of 90 ml of 0.1% peptone- water and homogenized for 2 minutes. Then the homogenate was serially (10 folds) diluted in 0.1% peptone- water. Volumes of 0.1 ml of diluted samples were surface plate in duplicate onto eiosin methyline blue enumerated after incubation for 24 h at 37 ºC **(Shekar-foroush *et al.*, 2007)**.

F- Statistical analysis

Statistical analysis of data was conducted using "Statistic for animal and veterinary science" **(Petric and Watson, 1999)**.

Results and Discussion

Table 1. The mean counts ±S.E. (cfu/ gm.) of the inoculated *Salmonella Enteritidis* in the minced beef meat samples after treatments by the essential oils (n = 3 for each treatment).

Counts & duration / Essential oils & concentrations		The mean counts of *Salmonella Enteritidis*				
		2nd day	4th day	6th day	8th day	10th day
Thyme oil	0.1%	$3.5 X10^4 \pm 2.3X 10^{4\,l}$	$3.5 X10^3 \pm 8.6X 10^{2\,p}$	$9.1 X10^4 \pm 7.9X 10^{4\,j}$	$2.4 X10^5 \pm 1.8X 10^{5\,f}$	$4.0 X10^5 \pm 3.4X 10^{5\,c}$
	0.5%	$1.0 X10^4 \pm 4.9X10^{3\,mno}$	$3.0 X10^2 \pm 1.1X 10^{2\,p}$	$2.8 X10^3 \pm 4.3X 10^{2\,p}$	$2.1 X10^5 \pm 6.2X 10^{4\,g}$	$2.7 X10^5 \pm 2.6X 10^{5\,e}$
Rosemary oil	0.1%	$1.5 X10^4 \pm 4 X10^{3\,m}$	$5.0 X10^3 \pm 1.1X 10^{3\,op}$	$5.7 X10^4 \pm 4.2X 10^{4\,k}$	$3.9 X10^5 \pm 3.5X 10^{5\,c}$	$7.1 X10^5 \pm 6.4X 10^{5\,b}$
	0.5%	$1.2 X10^4 \pm 8.7 X10^{3\,mn}$	$2.7 X10^3 \pm 2.1X 10^{3\,p}$	$1.1 X10^4 \pm 5.8X 10^{3\,mno}$	$1.0 X10^5 \pm 5.6X 10^{4\,i}$	$3.6 X10^5 \pm 1.2X 10^{5\,d}$
Thyme +Rosemary	0.1%	$1.48 X10^4 \pm 9.4 X10^{3\,m}$	$9.6 X10^2 \pm 5.1X 10^{2\,p}$	$5.9 X10^3 \pm 4.5X 10^{3hop}$	$1.4 X10^5 \pm 6.7X 10^{4\,h}$	$7.2 X10^5 \pm 6.3X 10^{5\,a}$
Initial count	$10^{5\,i}$					

N.B.: Different letters within the Table means significant variations between the values of the bacterial counts ($P \leq 0.01$).

The obtained results in Table (1) revealed significant reduction of *Salmonella Enteritidis* count in minced meat after treatments by the used essential oils as previously reported in another studies by **Del Campo *et al.* (2000)** and **Abderrahmane *et al.* (2012).** At the 2nd day after treatments, the bacterial number recorded significant slight low levels comparing with the initial count. Meanwhile, the more significant lower bacterial counts were detected at 4th day. The bacterial number was raised gradually from 6th day, till exceeded the initial count at 8th and 10th days. Moreover, the concentration 0.5% of both thyme and rosemary oils recorded more significant antibacterial effects than that of the concentration 0.1% at 6th, 8th and 10th days. Concerning the comparison between the effects of the 2 used essential oils on the *Salmonella Enteritidis* levels, rosemary 0.1% at 2nd, 6th and 0.5% at 8th day exhibit more significant antibacterial effect than those of thyme oil. On contrast, thyme oil, 0.5% at 6th day, 0.1% at 8th day and in the 0.1% and 0.5% at 10th day declared significant higher antibacterial effect than the other oil. Therefore, we could conclude the absence of the clear difference between the two examined essential oils as antibacterial agent on *Salmonella Enteritidis* in minced meat. Furthermore, the mixing of the two examined oils in concentration 0.1% exhibited slight more antibacterial effect on *Salmonella Enteritidis* than each oil alone.

Table 2. The mean counts ±S.E. (cfu/ gm.) of the inoculated *E. coli* O157: H7 in the minced beef meat samples after treatments by the essential oils (n = 3 for each treatment).

Counts & duration / Essential oils & concentrations		**The mean counts of *E. coli* O157: H7**				
		2nd day	4th day	6th day	8th day	10th day
Thyme oil	0.1%	$5.7 X10^4 \pm 1.7X 10^{4\,k}$	$2.8 X10^4 \pm 6.9X 10^{3\,mn}$	$3.7 X10^4 \pm 1.6X 10^{4\,lm}$	$1.6 X10^5 \pm 7.0X 10^{4\,g}$	$1.5 X10^6 \pm 7.0X 10^{5\,b}$
	0.5%	$4.5 X10^3 \pm 2.8X10^{3\,o}$	$2.1 X10^4 \pm 9.8X 10^{3\,n}$	$2.7 X10^4 \pm 1.3X 10^{4\,mn}$	$4.5 X10^4 \pm 2.9X 10^{4\,kl}$	$4.4 X10^5 \pm 2.8X 10^{5\,d}$
Rosemary oil	0.1%	$7.0 X10^4 \pm 2.2 X10^{4\,j}$	$4.4 X10^4 \pm 3.3X 10^{4\,kl}$	$7.2 X10^4 \pm 2.7X 10^{4\,j}$	$2.4 X10^5 \pm 2.2X 10^{5\,e}$	$2.7 X10^6 \pm 2.6X 10^{6\,a}$
	0.5%	$5.5 X10^3 \pm 1.8 X10^{3\,o}$	$5.5 X10^3 \pm 2.7X 10^{3\,o}$	$3.0 X10^3 \pm 1.0X 10^{3\,o}$	$9.1 X10^4 \pm 5.4X 10^{4\,i}$	$1.9 X10^5 \pm 1.0X 10^{5\,f}$
Thyme +Rosemary	0.1%	$3.4 X10^3 \pm 2.3 X10^{3\,o}$	$6.0 X10^3 \pm 2.9X 10^{3o}$	$2.0 X10^4 \pm 1.1X 10^{4\,n}$	$1.3 X10^5 \pm 5.4X 10^{4\,h}$	$1.3 X10^6 \pm 8.4X 10^{5\,c}$
Initial count	$10^{5\,i}$					

N.B.: Different letters within the Table means significant variations between the values of the bacterial counts ($P \leq 0.01$).

From the results achieved in Table (2) the significant antimicrobial effects of the used essential oils on *E. coli* O157: H7 in minced meat were evident. These results coincided with those recorded by **Krisch *et al.* (2010)** and **Duckova *et al.* (2012)**. Generally, the more significant antimicrobial effects of the essential oils were recorded at 2nd, 4th and 6th days with slight variations within the mentioned durations (Table 2). The values of *E. coli* O157: H7 were gradually increased after 8th and 10th days till exceeded the initial count (10^5 cfu/ gm). On the other aspect, the concentration 0.5% of the both used essential oils recorded more significant antimicrobial effect than the concentration 0.1% at all examined durations. Regarding, the comparison between the effects of the two used essential oils on the *E. coli* O157: H7 count, the obtained results exhibited clear fluctuations between the effects of both essential oils on the bacterial levels. Thus, we could conclude the absence of the considerable variations between the two used oils as antimicrobial effects on *E. coli* O157: H7. On the other hand, the mixing of thyme and rosemary oils (0.1% for each) exhibited significant antibacterial effects than any of the used oils alone at concentration 0.1%. On contrarily, this mix could not declared significant effects comparing with each oil alone at 0.5% concentrations.
From aforementioned results, it is evident that the using of thyme oil and/ or rosemary oil at low concentrations (0.1%- 0.5%) for treatment minced meat at refrigerator temperature (4-6ºC) leads to reducing the levels of *Salmonella Enteritidis* and *E. coli* O157: H7 within durations not exceeding 6 days. Subsequently, the validity time of the tested minced meat was prolonged.

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التأثير المضاد البكتيري لبعض الزيوت النباتية الأساسية على ميكروب السالمونيلا إنترتيديز و ميكروب الإيكولاي في اللحم البقري المفروم خلال فترات التخزين في الثلاجة

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الملخص العربى

تم إجراء هذه الدراسة لبيان مدى تأثير بعض الزيوت الأساسية مثل زيت الزعتر و زيت الروزماري على مستويات بكتيريا سالمونيلا إنترتيديز و إي كولاي O157:H7 في درجة حرارة المبردات. تم إعداد 30 عينة لحم بقري مفروم (كل عينة 100 جم) و تم حقن العينات بنوعي البكتيريا المذكورين بتركيز 10^5 لكل نوع (بمعدل 15 عينة لكل نوع بكتيريا)، و تمت معالجة اللحم المفروم و المحقون بالميكروبات المذكورة بنوعي الزيوت المستخدمين بتركيزي 0.1%، 0.5% لكل نوع بالإضافة لخليط من نوعي الزيوت بتركيز 0.1% لكل منهما، و تم استخدام 3 عينات لكل معالجة من المعالجات الخمسة ليكون مجموع العينات لكل نوع بكتيريا هو 15 بإجمالي 30 عينة لنوعي البكتيريا، تم حفظ العينات في الثلاجة عند درجة حرارة 4- 6 م°. تم فحص العينات و إجراء عمليات العد البكتيري لها بعد 2، 4، 6، 8، 10 أيام من المعالجات و تم إجراء الدراسة الإحصائية و قد أسفرت النتائج عن الآتي. أثبتت الدراسة الأثر الإيجابي للمعالجة بزيتي الزعتر و الروزماري في تقليل أعداد نوعي البكتيريا محل الدراسة بشكل معنوي مقارنة بالعدد المحقون، و قد كانت أفضل النتائج في هذا التقليل عند اليوم الرابع بالنسبة لميكروب سالمونيلا إنترتيديز و خلال المدة من اليوم الثاني لليوم السادس فيم يخص بكتيريا إي كولاي O157:H7. من ناحية أخرى، بمقارنة كفاءة نوعي الزيتين المستخدمين كمضاد للبكتيريا، لم يظهر أي نوع من نوعي الزيتين كفاءة واضحة كمادة مضادة للبكتيريا مقارنة بالنوع الآخر، كما كان تركيز 0.5% أكثر تأثيرا كمضاد للبكتيريا من تركيز 0.1% خاصة مع ميكروب إي كولاي O157:H7. و الجدير بالذكر أن خلط نوعي الزيوت محل الدراسة بتركيز 0.1% قد أظهر تحسن معنوي كمضاد بكتيري مقارنة بكل نوع منفرد من نوعي الزيوت عند تركيز 0.1%، و لم يكن هناك تأثير معنوي لخلط الزيتين (0.1%) مقارنة بكل نوع منفرد عند تركيز 0.5%، و كان هذا التأثير أكثر وضوحا مع بكتيريا إي كولاي O157:H7. من الدراسة نستخلص أن زيتي الزعتر و الروزماري بتركيزات منخفضة لها تأثير معنوي مضاد لنوعي البكتيريا محل الدراسة في لحم البقر المفروم مما يجعلنا نوصى باستخدام تلك الزيوت لزيادة فترات صلاحية اللحوم المفرومة في درجات حرارة التبريد.