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Antimicrobial Effects of some Essential Oils on the Foodborne Pathogen Campylobacter jejuni

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A B S T R A C T

A total of 1500 g of minced chicken breast meat were divided into 5 equal groups (3x100 g of each). Campylobacter jejuni was inoculated into each group with infective dose 2.5x107cfu/g. The used natural oils were thyme and Coriander oil in concentration of 1% and 2%. All group samples were stored at 4oC in refrigerator until be used and examined every 24 hours for sensory examination (overall acceptability) and Campylobacter jejuni count. The experiment was performed in triplicate. Thyme oil (1 and 2%) decreased count of Campylobacter jejuni (cfu/g) from 3.8x107(initial load) to 7.3x105, and 1.2 x103 with reduction percentages 97.27% and 99.99% on 6th day of storage, respectively. Coriander oil (1 and 2%) decreased count of Campylobacter jejuni (cfu/g) to 3.8x106 and 9.5 x105 with reduction percentages 85.00% and 96.27% on 6th day of storage, respectively. Furthermore, thyme oil (2%) showed overall acceptability till 5th day. While, Coriander oil (2%) showed overall acceptability till 4th day and control group showed overall acceptability till 3rd day. Generally, thyme oils proved to be more efficient than Coriander in reduction of Campylobacter jejuni growth in minced chicken meat; therefore, it is recommended to improve safety of the chicken meat products by essential oils.

Keywords: *Campylobacter jejuni, thyme,coriander, chicken meat, essential oils.*

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1. INTRODUCTION

Chicken meat industry is the biggest supplier of acceptable animal protein with high meat yield, low shrinkage in cooking and great source of amino acids, vitamins and minerals (Oulkeir, *et al.*, 2017). *Campylobacter jejuni* is the major pathogen causing food borne diseases worldwide (Scallan, *et. al.*, 2011). Further, *C. jejuni* is one of the most common causes of bacterial enteritis in human. Nowadays, there is highly interesting of consciousness between almost consumers to consume food with high nutritional value, with natural preservatives and free from chemical preservatives and microbial hazards. In addition to be safer, more healthy and less subject to hazards than food containing artificial food additives especially in meat which is highly susceptible to microbial growth such as different meat types, which consider favorable media for spoilage and food borne diseases in human, resulting in serious health problems (El-Dosoky, *et al.*, 2018).

Increased consumer demand for all natural food products has put pressure on industry and regulatory agencies to closely examine the potential for use of natural antimicrobials that prevent or control the growth of foodborne pathogens and spoilage microorganisms. Although many studies have indicated that EOs have the potential to be used as a natural antimicrobial preservative in the food industry (Djenane *et al*., 2011a,b).

Essential oils, as antimicrobial agents present two main characteristics: first is their natural origin, which means more safety for consumers, and second is that they are considered to be of low risk for resistance development by pathogenic microorganisms.

Coriander EO was tested for antimicrobial activities against *Campylobacter jejuni*, it was found that the oil reduced the bacterial cell loads

(Rattanachaikunsopon and Phum-khachorn, 2010).

Thyme essential oil is stated to posse carminative, antispasmodic, antitussive, secretomotor, bactericidal, expectorant, astringent and anthelmintic properties. It is commonly used in foods mainly for its flavor and aroma (Küçükbay *et al.*, 2014).

Therefore, the goal of the work was to investigate the antibacterial effect of thyme and coriander essential oils in different concentrations (1% and 2%) in control of *C. jejuni* in minced chicken meat at cold storage *(*4 ºC).

2.MATERIAL AND METHODS

2.1 Essential oils:

The ready-made herbal oils of thyme (*Thymus vulgaris*), and Coriander used in this study were purchased from El Captain Company (CAP PHARM) for extracting natural oils, plants and cosmetics, Alobour City-Cairo-Egypt. All the used chemicals were of analytical reagent grade. These oils were stored in amber-colored bottles at 4°C until use.

2.2 Bacterial strains:

Campylobacter jejuni reference strain, used in this study, was obtained from Bacteriology Unit, Reference Laboratory For Veterinary Quality Control of Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt. The cell count was adjusted to 10^6 cfu/ml because the infective dose is $>10^5$cfu/g (Tribble et al., 2009)

2.3 Experimental application:

A total of 1500g of the fresh minced chicken breast meat was purchased from shops in El Menofia Governorate. Samples were divided into 2 main treated groups and control ones (300g of each). *Campylobacter jejuni* was inoculated into each group with infective dose $>10^5$cfu/g as described by Tribble *et al.* (2009). The used Essential oils were added according to the following order:

Control +ve: inoculated with the tested culture *(Campylobacter jejuni)* without treatment with EOs.

First group: Treated with thyme oil (1%) and (2%) of thyme oil (3x100g).

Second group: Treated with Coriander oil (1%) and (2%) of Coriander oil (3x100g).

The inoculated samples were packed in polyethylene bag, labeled and stored in refrigerator chamber at 4 ºC till be used. The inoculated groups were examined every 24 hours for:

- Initial load of *Campylobacter* was detected before and after addition of essential oils according to (ISO, 2006)

Campylobacter count was estimated at zero days (after 3 hours of inoculating the oils) and every 24 hrs to evaluate the effect of the essential oils treatments.

- Organoleptic examination (color, odor, texture and overall acceptability) according to Penny *et al.* (1993).

The criteria used as the basis of the organoleptic assessment and the rating system were provided in table (A). A nine-point hedonic scale (1: poor; 9: excellent) was used in the evaluation as illustrated in table (A) (Patsias *et al.,* 2006).

overall acceptability of artificially inoculated minced chicken breast meat samples with *Campylobacter jejuni.*

Thyme (2%) showed overall acceptability extended to 5th day of storage. While, lower concentration (1%) showed overall acceptability extended till 4th day. In comparison, coriander (2% and 1%) showed overall acceptability extended to 4th day. In contrast, the control group showed overall acceptability extended to 2nd day.

2.4. Statistical analysis:

The obtained results were statically evaluated by application of analysis of variance (ANOVA) test according to *Feldmanet al. (2003).*

Table (A): Score System for Sensory Evaluation (Patsias *et al.*, 2006).

Scoring			
Excellent :	9	Fair :	4
Very very good :	8	Poor:	3
Very good :	7	Very poor :	2
Good :	6	Very very poor :	1
Medium :	5		

Tests were performed in triplicate

3. RESULTS

Table (1) illustrated the effects of various concentrations of thyme and Coriander on overall acceptability of artificially inoculated minced chicken breast meat samples with *Campylobacter jejuni.*

Thyme (2%) showed overall acceptability extended to 5th day of storage. While, lower concentration (1%) showed overall acceptability extended till 4th day. In comparison, coriander (2% and 1%) showed overall acceptability extended to 4th day. In contrast, the control group showed overall acceptability extended to 2nd day.

Table (1): Overall acceptability of cold stored (4ºC) minced chicken breast meat inoculated with *Campylobacter jejuni* and treated with different concentrations of thyme and Coriander at cold storage (4ºC)

Groups	oil conc.	Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day
Control	–	7	6	5	4	4	1	1
Thyme	1%	7	8	8	6	5	4	4
	2%	7	8	8	7	6	5	4
Coriander	1%	7	7	6	5	5	4	4
	2%	7	7	6	5	5	4	4

- Zero days: Definitely after 30 minutes of oils injection.
- Score System for Sensory Evaluation:

9: Excellent
8: Very very good
7: Very good
6: Good
5: Medium
4: Fair
3: Poor
2: Very poor
1: Very very poor

Tables (2 and 3) illustrated the antimicrobial effect and reduction percentages of Thyme oil (1% and 2%) and coriander (1% and 2%) on *Campylobacter jejuni* artificially inoculated into minced chicken breast meat samples

Thyme oil (1% and 2%) decreased count of *Campylobacter jejuni* (log CFU/g) from 25 x 10^6± 1.02 x 10^7 (initial load) to 7.3 x 10^{5a}±3.6x 10^5 and 2.5x 10^{3a}± 1.2 $X10^3$ with reduction percentages 97.27 % and 99.99 % on 6th day of storage, respectively.

Coriander oil (1% and 2%) decreased count of *Campylobacter jejuni* (log CFU/g) from 25 x 10^6± 1.02 x 10^7 (initial load) to 3.8x 10^{6b}±2.1x 10^5 and 9.5x 10^{5a}±1.2x 10^5 with reduction percentages 85 % and 96.27 % on 6th day of storage, respectively.

Table (2): Antimicrobial effect of different concentrations of thyme and Coriander essential oils on *Campylobacter jejuni* count artificially inoculated in cold stored (4ºC) minced chicken breast meat.

Treated groups / Durations	Control	Thyme oil 1%	thyme oil 2%	Coriander oil 1%	Coriander 2%
Zero day^{NS}	3.8x 10^{7a} ±6.2 $X10^6$	2.8 x 10^{7a} ± 3.1 x 10^6	1.6 x 10^{7a} ±1.6x 10^6	3.4x 10^{7a} ±7.1x 10^6	2.9 x 10^{7a} ±1.4x 10^6
2nd day^{++}	1.7x 10^{8a} ±2.5$X10^7$	8.3x 10^{6b} ±2.4x 10^6	5.5x 10^{6b} ± 1.2 x 10^6	2.4x 10^{7b} ±5.3x 10^6	8.5 x 10^{6b} ±0.5x 10^6
4th day^{+}	8.5x 10^{8a} ±3.3x 10^8	9.1 x 10^{5b} ±3.6 x 10^5	8.4x 10^{4b} ±1.7x 10^4	6.4x 10^{6b} ±2.3 x 10^6	2.5x 10^{6b} ±6.1x 10^5
6th day^{++}	-	7.3 x 10^{5a} ±3.6x 10^5	2.5x 10^{3a} ± 1.2 $X10^3$	3.8x 10^{6b} ±2.1x 10^5	9.5x 10^{5a} ±1.2x 10^5

- Initial load of *campylobacter jejuni*= 25 x 10^6± 1.02 x 10^7 CFU/g
- Means within the same row not bearing the same superscripts are significantly different.

NS = Non significant differences.
+ = Significant differences (P<0.05)
++ = High significant differences (P<0.01)

Table (3): Reduction % of *Campylobacter jejuni* count artificially inoculated into cold stored (at 4ºC) minced chicken breast meat samples treated with different concentrations of *thyme* and Coriander essential oils.

Treated groups / Durations	*thyme* oil 1%	*thyme* oil 2%	Coriander oil 1%	Coriander oil 2%
Zero day	30.77	53.85	7.69	26.92
2nd day	68.86	80.00	19.23	68.07
4th day	96.46	99.67	76.15	89.62
6th day	97.27	99.99	85.00	96.27

4. DISCUSSION

Campylobacter jejuni has emerged as one of the most important bacterial causes of foodborne diseases in the world. It is responsible for severe gastrointestinal symptoms characterized by diarrhea, fever, abdominal cramps, nausea, and in certain cases by post infectious sequelae, like Guillain-Barré syndrome and Miller- Fisher syndrome (Dingle et al., 2001). The most important sources of infection are under cooked contaminated poultry meat (Skarp et al., 2016). There are undoubtedly permanently presenting food safety problems caused by *C. jejuni*, which are further aggravated by an increasing spread of antibiotic resistance among the isolates. For this reason, the search for alternative antimicrobials, both for prevention and therapy, has a pivotal role. With their remarkable antibacterial features, essential oils may represent a possible solution for these problems.

Different mechanisms are known and have been hypothesized to play a role in their antimicrobial effects (Judit *et al*.,2016). The hydrophobic feature of certain components can cause partition of the lipids in the bacterial cell membrane, resulting in increased membrane permeability, lipid depolymerization, and therefore, disturbance in coordinated ion flow leading to decreased membrane potential and a halt in ATP synthesis. Other EO components are able to interfere with cell wall proteins usually involved in the transport of essential molecules into the cell. The exact mode of antibacterial action of most EOs, however, has not been entirely elucidated until now, although some studies have emphasized the possible importance of pore formation and subsequent oxidative stress in this process (Burt , 2004). Coriander (Coriandrum sativum L.), is a soft growing plant about50 cm tall and has potent volatile leaf oil and a stronger aroma which have a different taste from the seeds. Its flavor diminished by gain or loss temperature and there were it usually consumed raw. Raw coriander leaves are92% water, 4% carbohydrates, 2% protein, and less than1% fat in addition to its high content in vitamin;(A, C, K) with dietary fiber, and many minerals; (calcium, selenium, iron, magnesium and manganese).Thyme is an aromatic green herb characterized by its aromatic flavor. Thyme retains its flavor on drying better than many other herbs. Oil of thyme, the essential oil of common thyme (Thymus vulgaris), contains 20–54% thymol (Ibrahim and El-Shehawy, 2013). Thyme essential oil also contains a range of additional compounds, such as p-cymene, myrcene, borneol, and linalool (Friedman et al., 2002).

Antimicrobial activities of the essential oils of various herbs were investigated by Abdollah *et al*. (2010) against *C. jejuni* and *C. coli* isolated from chicken meat. The results indicated that the EOs of these plants exerted remarkable activity against *C. jejuni* and *C. coli* and, therefore, they could be used as natural anti-*Campylobacter* additives in meat. Several recent studies described in detail the antimicrobial properties of some essential oils against *C. jejuni*, which may be envisaged as natural alternatives to chemical based antibacterial for food safety and preservation (Nannapaneni *et al*., 2009; Djenane *et al*., 2011d).

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 of storage 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 than in coriander oil-treated samples. These results agreed with those obtained by Dorman and Deans (2000) and Lambert et al. (2001) studied a lot of herbs EOs antimicrobial effect and concluded that thyme consider one of the widest spectrum antibacterial herb. These inhibition effect refers to the presence of the hydroxyl group at a different location on the phenolic ring which able to disintegrate the outer cell membrane of the Gram negative bacteria which allow releasing lip polysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP.

From the obtained results in Table (2), Thyme oil (1 and 2%) decreased count of *Campylobacter jejuni* (cfu/g) from $3.8x10^7$(initial load) to $7.3x10^5$, and $1.2 x10^3$ with reduction percentages 97.27% and 99.99% on 6th day of storage, respectively. Coriander oil (1 and 2%) decreased count of *Campylobacter jejuni* (cfu/g) to $3.8x10^6$ and $9.5 x10^5$with reduction percentages 85.00% and 96.27% on 6th day of storage, respectively. These findings were nearly similar with those obtained by (Karami-Obsoo, 2010) who studied the antimicrobial effect of thyme EO on gram – ve bacteria. Delaquis et al. (2002), Duman et al. (2010) and Silva et.al. (2011) reported weak antibacterial effect of coriander (Coriandrum sativum) essential oil against Gram-positive and Gram-negative bacteria due to its content of

linaloolEO which affect the bacterial cell membrane permeabilization and damage bacterial cell wall.

Finally, the present study allowed to conclude that thyme oil is proved to be more efficient than usage of Coriander are in suppression of *Campylobacter jejuni* growth in minced chicken meat and it is safe antimicrobial agent against *Campylobacter jejuni*, is therefore recommended to improve safety of chicken meat.

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