



Antimicrobial effect of some natural oils on *Bacillus cereus* in minced beef

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ABSTRACT

A total of 1800 g fresh minced beef were divided into 6 equal groups (3x100 g of each). *Bacillus cereus* was inoculated into each group with infective dose 29×10^7 cfu/g. The used natural oils were thyme oil (0.6% and 1%), cinnamon oil (0.6% and 1%) and mixture of them (0.4 % from each). The inoculated samples were stored at 4°C in refrigerator until be used. The inoculated groups were examined every 24 hours for sensory examination (overall acceptability) and *B. cereus* count. The experiment was performed in triplicate. Thyme oil (0.6 and 1%) decreased count of *B. cereus* (cfu/g) from 2.2×10^8 (initial load) to 5.8×10^7 and 8.8×10^6 with reduction percentages 73.63% and 96% on 5th day and 6th day of storage, respectively. Cinnamon oil (0.6 and 1 %) decreased count of *B. cereus* (cfu/g) to 9.8×10^7 and 6.1×10^7 with reduction percentages 55.45% and 72.27% on 3rd day and 5th day of storage, respectively. Mixture of (thyme and cinnamon oils) decreased count of *B. cereus* (cfu/g) to 8.6×10^6 with reduction percentage 96.09% on 6th day of storage. In control group *B. cereus* count increased from 2.2×10^8 (initial load) to 5.1×10^8 on 3rd day. Furthermore, thyme oil (1%) and mixture treated minced beef showed overall acceptability till 6th day of storage. In comparison, thyme oil (0.6%) and cinnamon oil (1%) showed overall acceptability till 5th day. While, cinnamon oil (0.6%) and control group showed overall acceptability till 3rd day. Generally, mixture of thyme and cinnamon oils (0.4% of each) proved to be more efficient than other concentrations in suppression of *B. cereus* growth in minced beef, Therefore, It is recommended to improve safety of the meat products.

Keywords: *Bacillus cereus*, antimicrobial effect, meat products, natural oils.

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1. INTRODUCTION

Meat consumption is important for human growth, development and maintenance of health, which is why safety of meat and meat products is of growing concern in modern society (Pereira and Vicente, 2013).

A major issue related to meat consumption is the presence of pathogens among them, the causative agents of food borne diseases

(Sofos, 2008), for which raw meat provides an ideal growth medium.

Bacillus cereus is one of the organisms that cause problems to the food industry both by deteriorating the products (Eneroth et al., 2001), and by endangering people's health upon consuming contaminated foods (Ghelardi et al., 2002).

Bacillus cereus is widely spread in the environment and raw foods especially rice, meat and meat products, vegetables and desserts made with corn starch (Wyatt, 1992). Practices such as inadequate cooling after heat treatment are indicated as the main factors contributing to the spread of the microorganism in these foods (Harmon and Kautter, 1991; Nortjé, 1999).

In recent years, the use of natural antibacterial agents has been proposed as an alternative for the inactivation of vegetative cells and bacterial spores in food systems (Burt 2004; Cetin-Karaca and Newman, 2015; Martins et al., 2014).

Essential oils are currently in demand; both in industry and academic research, and their antimicrobial properties against food spoilage microorganisms have been investigated in many studies (Burt, 2004; Hernandez-Ochoa et al., 2011).

Thyme essential oil has antibacterial and antifungal activities of and its potential for use as a natural food additive, due to its inhibitory effects on the growth of susceptible and multidrug-resistant pathogenic and non-pathogenic food spoilage bacteria and fungi (Sabina Anzlovar et al., 2014).

Cinnamon (*Cinnamomum zeylanicum* or *Cinnamomum verum*), rich in essential oils (EO), belongs to Lauraceae family and usually grows in South and South-East Asia. *C. zeylanicum* oil has strong antimicrobial activity amongst essential oils of plants from Lauraceae family (Raharivelomanana et al., 1989; Mishra et al., 2008).

It has been shown that combining different antimicrobial substances can lead to a broad spectrum of activity which can increase their effectiveness in foods (Ghraiiri and Hani, 2013; Goni et al., 2009; Gutierrez et al., 2008).

The goal of this research was to study the antimicrobial effect and acceptability of

certain essential oils on *B. cereus* inoculated in minced beef.

2. Materials and methods

2.1. Bacterial strain:

Bacillus cereus strain was obtained from Animal Health Research Institute (AHRI), Dokki, with recommended dose (29 x 10⁷ CFU/ml (8.64 log CFU/ml) as recorded by McFarland's nephelometer standards according to Slabyj et al. (2003).

2.2. Natural oils:-

Thyme oil (0.6 and 1%), Cinnamon oil (0.6 and 1 %) and mixture of Thyme and Cinnamon oils (0.4 % of each). The oils were purchased from Harraz Planta Medical group company.

2.3. Experimental application:

Accurately, 1800 g fresh minced beef samples divided into 6 equal groups (3x100 g each) were inoculated with *B. Cereus* and then treated with certain concentration of tested natural oils according to the following order: control+ve inoculated with *B. cereus* only.

A:Treated with thyme oil 0.6 %.

B:Treated with thyme oil 1%.

C:Treated with cinnamon oil 0.6 %.

D:Treated with cinnamon oil 1 %.

E:treated with 0.4 % Thyme oil + 0.4% Cinnamon oil.

The inoculated samples were stored at 4°C in refrigerator to be examined after 3 hours and every day (24 hrs) intervals during storage until spoilage of samples for:

Sensory examination (overall acceptability) according to Hemin (2013).

Bacillus cereus count according to FDA (2001).

The experiment was performed in triplicate.

2.4. Statistical analysis:

The obtained results were statistically evaluated by application of analysis of

variance (ANOVA) test according to Feldman et al., (2003).

3. RESULTS

Table (1) illustrated the effects of various concentrations of thyme and cinnamon oils on overall acceptability of artificially inoculated minced beef samples with *B. cereus*.

Thyme oil (1 %) and mixture group showed overall acceptability extended to 6th day of storage. While, thyme oil (0.6 %) and cinnamon oil (1%) showed overall acceptability till 5th day. In contrast, cinnamon oil (0.6%) and control group showed overall acceptability till 3rd day.

Table (2) and Table (3) illustrated the antimicrobial effects and reduction percentage of various concentrations of thyme and cinnamon oils on counts of *B. cereus* artificially inoculated into minced beef.

Thyme oil (0.6 and 1%) decreased count of *B. cereus* (cfu/g) from 2.2×10^8 (initial load) to 5.8×10^7 and 8.8×10^6 with reduction percentages 73.63% and 96% on 5th day and 6th day of storage, respectively. Cinnamon oil (0.6 and 1 %) decreased count of *B. cereus* (cfu/g) to 9.8×10^7 and 6.1×10^7 with reduction percentages 55.45% and 72.27% on 3rd day and 5th day of storage, respectively. Mixture of (thyme and cinnamon oils) decreased count of *B. cereus* (cfu/g) to 8.6×10^6 with reduction percentage 96.09% on 6th day of storage. In control group *B. cereus* count increased from 2.2×10^8 (initial load) to 5.1×10^8 on 3rd day.

On regard table (2), the differences between the effects of various concentrations of thyme and cinnamon oils on counts of *B. Cereus* (cfu/g) artificially inoculated into minced meat sample were significant different at ($P < 0.05$).

Table 1: The effects of various concentrations of essential oils on overall acceptability of the examined minced beef meat samples during cold storage at 4oC.

Days	Control samples	Thyme oil (0.6%)	Thyme oil (1%)	Cinnamon oil (0.6 %)	Cinnamon oil (1%)	Mixture (0.4+0.4%)
Zero	5	5	5	5	5	5
2 nd	4	4	4	4	4	5
3 rd	3	4	4	3	4	4
4 th	2	3	4	2	3	4
5 th	1	3	3	2	3	3
6 th	1	2	3	1	2	3
7 th	1	1	1	1	1	2
8 th	1	1	1	1	1	1

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

Table 2: The effects of various concentrations of thyme and cinnamon oils on counts of *B. cereus* (cfu/g) artificially inoculated into minced beef samples.

Days	Control* Samples	Thyme oil* (0.6%)	Thyme oil* (1%)	Cinnamon* oil (0.6 %)	Cinnamon* oil (1%)	Mixture* (0.4+0.4%)
Zero	$2.2 \times 10^8 \pm 0.5 \times 10^8$					
2 nd	$3.5 \times 10^8 \pm 1.1 \times 10^8$	$1.8 \times 10^8 \pm 0.7 \times 10^8$	$1.7 \times 10^8 \pm 0.8 \times 10^8$	$2.1 \times 10^8 \pm 0.3 \times 10^8$	$2 \times 10^8 \pm 0.3 \times 10^8$	$1.2 \times 10^8 \pm 0.9 \times 10^8$
3 rd	$5.1 \times 10^8 \pm 1.8 \times 10^8$	$8.3 \times 10^7 \pm 2.9 \times 10^7$	$8.1 \times 10^7 \pm 2.3 \times 10^7$	$9.8 \times 10^7 \pm 3.8 \times 10^7$	$8.9 \times 10^7 \pm 2.2 \times 10^7$	$7.6 \times 10^7 \pm 3.1 \times 10^7$
4 th	spoiled	$7.7 \times 10^7 \pm 2.4 \times 10^7$	$7.5 \times 10^7 \pm 1.9 \times 10^7$	spoiled	$7.9 \times 10^7 \pm 1.9 \times 10^7$	$5.1 \times 10^7 \pm 1.8 \times 10^7$
5 th	spoiled	$5.8 \times 10^7 \pm 1.2 \times 10^7$	$5.5 \times 10^7 \pm 0.7 \times 10^7$	spoiled	$6.1 \times 10^7 \pm 1.7 \times 10^7$	$2.5 \times 10^7 \pm 0.4 \times 10^7$
6 th	spoiled	spoiled	$8.8 \times 10^6 \pm 3.2 \times 10^6$	spoiled	spoiled	$8.6 \times 10^6 \pm 1.8 \times 10^6$
7 th	spoiled	spoiled	spoiled	spoiled	spoiled	spoiled
8 th	spoiled	spoiled	spoiled	spoiled	spoiled	spoiled

Table 3: Reduction % in counts of *B. Cereus* count artificially inoculated into minced meat samples treated with different concentrations of thyme and cinnamon oils.

Days	Thyme(0.6 %)	Thyme(1 %)	Cinnamon (0.6%)	Cinnamon (1%)	Mixture(0.4% +0.4%)
2 nd	18.18%	22.73	4.55%	9.09%	45.45%
3 rd	62.27%	63.18%	55.45%	59.55%	65.45%
4 th	65%	65.91%	Spoiled	64.09%	76.81%
5 th	73.63%	75%	Spoiled	72.27%	88.64%
6 th	Spoiled	96%	Spoiled	Spoiled	96.09%
7 th	Spoiled	Spoiled	Spoiled	Spoiled	Spoiled
8 th	Spoiled	Spoiled	Spoiled	spoiled	Spoiled

4. DISCUSSION

Bacillus cereus which frequently associated with food borne diseases (Borge et al., 2001), also it can be found in the natural environment (soil, water and air) and isolated from various foods, including meat and meat products (Konuma et al., 1988; Pirhonen et al., 2005).

There are two forms of the intoxication: one cause diarrhea, starting from 6 to 15

hours after consumption, and the other cause vomiting and nausea, starting from 30 minutes to 6 hours after consumption. Symptoms in both forms last about 24 hours. Everyone is susceptible to *B. cereus* food poisoning. Some isolates of *B. cereus* can grow at refrigerated temperature (Valeroa et al., 2007) and spore can survive at high temperature.

Natural products and naturally derived compounds from plants may have

applications in controlling pathogens in foods (Davidson, 1997; Bowles and Juneja, 1998).

Thyme and cinnamon EOs have gained greater acceptance among food technologists due to their better sensory evaluation and antimicrobial properties (Fischer and Phillips, 2006).

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).

The major component of cinnamon, cinnamaldehyde, possesses antimicrobial effects on microorganisms, as it inhibited cell wall biosynthesis, membrane function, and specific enzyme activities. More specific cellular targets of cinnamaldehyde are still required to be studied in detail (Shreaz et al., 2016).

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 except mixed 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 mixed group (thyme oil 0.4% +cinnamon oil 0.4%) then in thyme oil-treated minced beef samples, while slight improvement in acceptability of cinnamon oil minced beef samples as compared with 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, Sallem-Amany

et al., (2010) indicated that sensory properties of minced beef samples during cold storage (4°C) were enhanced by treatment minced beef by different concentrations of thyme oil (0.5%, 1%, 1.5%) compared to the untreated (control) samples and sample contain 1.5% thyme oil revealed best enhancement of sensory properties than sample 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 and 1.5% cinnamon oil, demonstrated the highest enhancement of sensory attributes, while the samples treated with 1% of thyme and 0.5% of cinnamon oils demonstrated lower enhancement.

These results are not agreed with those obtained by Solomakos et al., (2008) and Giatrakou et al., (2010) who found 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) and also not agreed with Agnieszka et al., 2012 whose results in sensory testing, were 9 out of the 10 persons rejected the meat stored in modified atmosphere with thyme oil due to unacceptable odor, and 6 due to bad taste.

From the obtained results in Table(2), the mixed sample (0.4% thyme+0.4% cinnamon oils) has the best antimicrobial activity against *B. cereus* then thyme oil (1%), thyme oil (0.6%), cinnamon oil (1%) , cinnamon oil (0.6%), respectively.

These findings were nearly similar with those obtained by Lu Fei et al., (2011) whose findings were that the combined application of cinnamon oil with thyme oil displayed an additive effect against *B. cereus*, but not agreed with Valero and Salmeron, (2003) whose results were that among all the examined oils against *B. cereus*, the essential oil of cinnamon was the most effective,

followed by the essential oil of oregano and thyme.

Finally, the present study allowed to conclude that thyme oil (0.4%) in combination with cinnamon oils(0.4%)proved to be more efficient than usage of them alone in suppression of *B. Cereus* growth in minced meat. So, the use of mixture of thyme and cinnamon oils (0.4% of each), as it is safe antimicrobial agent against *B. Cereus*, is therefore recommended to improve safety of meat products.

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