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## Control of *Bacillus Cereus* in Minced Meat Using some Essential Oils

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### ABSTRACT

The effect of different essential oils (Cinnamon, Clove and Marjoram) on viability of *B. Cereus* inoculated into minced meat was studied. The minced meat was inoculated by *B. cereus* at intensity of  $3 \times 10^6/g$  and classified into 4 groups. The first groups act as control(untreated) and the other 3 groups were treated with cinnamon, clove and marjoram by concentration 1% examined at zero, 2, 4 & 6 days at chilling temperature ( $4^\circ C$ ). *B. Cereus* count were  $3.0 \times 10^6 \pm 0.5 \times 10^6$  for control & treated samples at zero day. While at 2<sup>nd</sup> days *B. Cereus* count were  $2.9 \times 10^6 \pm 0.3 \times 10^6$  with reduction % 3.3 for control sample,  $1.8 \times 10^5 \pm 0.2 \times 10^5$  with reduction % 94.0 for treated samples by cinnamon,  $8.5 \times 10^4 \pm 2.1 \times 10^4$  with reduction % 97.0 for treated samples by clove &  $6.7 \times 10^3 \pm 1.5 \times 10^3$  with reduction % 99.0 for treated samples by marjoram. Moreover, the result obtained in 4<sup>th</sup> day of storage *B. Cereus* count were  $2.7 \times 10^6 \pm 0.3 \times 10^6$  with reduction % 10.0 for control sample,  $7.9 \times 10^4 \pm 1.4 \times 10^4$  with reduction % 97.4 for treated samples by cinnamon,  $5.2 \times 10^3 \pm 1.0 \times 10^3$  with reduction % 99.8 for treated samples by clove in contrast marjoram had complete inhibition to *B. Cereus* at 4<sup>th</sup> day of storage. Concerning to the result obtained in 6<sup>th</sup> day of storage *B. Cereus* count were  $2.6 \times 10^6 \pm 0.2 \times 10^6$  with reduction % 13.3 for control sample,  $3.3 \times 10^4 \pm 0.6 \times 10^4$  with reduction % 98.9 for treated samples by cinnamon & clove had complete inhibition to *B. Cereus* at 6<sup>th</sup> day of storage. So in the current study marjoram is the best essential oil which make complete inhibition to *bacillus cereus* at 4<sup>th</sup> day of storage. followed by clove which make complete inhibition to *bacillus cereus* at 6<sup>th</sup> day of storage. followed by cinnamon.

**Key words:** Cinnamon, Clove, Marjoram, *Bacillus Cereus*.

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### 1.INTRODUCTION:

Essential oils and their components commonly used as flavoring in the food industry also exhibit some antibacterial,

antifungal, and antioxidant properties. The primary constituents of essential oils are terpenoids and terpenes. Essential oils can also contain Aliphatic Hydrocarbons, Acids,

Alcohols, Aldehydes, Acyclic esters or lactones. (Tajkarimi *et al.*, 2010).

Cinnamon and thyme oils can be used as natural meat preservatives with anti-microbial activities against food borne pathogens, and therefore may be useful in maintaining the meat quality, extending shelf life of meat products, preventing economic loss and providing the consumer with food containing natural additives, which might be seen more healthful than synthetic origin. (Salem-Amany *et al.*, 2017).

A piece of each of fresh raw meat (100 g) was cut with a sterile scalpel and put under the UV light in the cabinet for 20 minutes in order to reduce the number of the microorganisms attached to its surface.

#### 2.2. Preparations of inocula (Tassue *et al.*, 1995):

*Bacillus Cereus* strains were obtained from Food Analysis Center, Faculty of Veterinary Medicine, Benha University. Bacteria were sub-cultured on Brain-Heart Infusion (BHI) broth and incubated for 24 hours at 37°C. The cells were harvested by centrifugation (3000×g, 15 min), washed twice, and resuspended with saline (NaCl, 0.85%, w.v).

#### 2.3. Inoculation of minced meat with tested bacteria:

For inoculation of the minced meat, 1 ml of the dense suspension  $3 \times 10^6$  /g for *Bacillus cereus* was employed.

#### 2.4. Essential oil extraction:

The extraction of active ingredients from dry Cinnamon, Garlic and Thyme plants were done according to the technique developed by Tandon and Rane (2008). In brief, the dried plant material was size reduced with milling using hammer mill. Extraction of the plant

material was carried out by immersion in absolute methanol for three days with agitation using automatic shaker.

#### 2.5. Mixing of minced with essential oils: essential oil from Faculty of Veterinary Medicine, Banisuif University.

The inoculated samples were divided into 4 groups; the 1<sup>st</sup> was untreated control, while the 2<sup>nd</sup> group was mixed with cinnamon extract (1%) for 15 minutes, the 3<sup>rd</sup> was mixed with clove extract (1%) for 15 min and the last 4<sup>th</sup> group was mixed with Marjoram extract (1%) for 15 minutes. The control and treated minced meat samples were labeled and packaged as triplicates, then stored at  $2 \pm 1^\circ\text{C}$  inside the refrigerator. All groups (either control or treated) were subjected to microbiological assessment at day zero (within 2 hours after treatment) then periodically every 2 days (0, 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> days).

#### 2.6. Enumeration of the tested bacteria:

In order to enumerate *B. Cereus*, 100 µl of a suitable dilution of the bacteria grown in BHI broth were surface plated on PEMBA. Enumerations were carried out after incubating of the plates at 37°C for 24 hours.

### 3. RESULTS

Table (1) showed the viability of *B. Cereus* inoculated into minced meat by intensity of  $3 \times 10^6$  /g on control samples. *B. Cereus* count were  $3.0 \times 10^6 \pm 0.5 \times 10^6$  at zero day,  $2.9 \times 10^6 \pm 0.3 \times 10^6$  at 2<sup>nd</sup> day with reduction % (3.3%),  $2.7 \times 10^6 \pm 0.3 \times 10^6$  at 4<sup>th</sup> day with reduction % (10.0%) &  $2.6 \times 10^6 \pm 0.2 \times 10^6$  at 6<sup>th</sup> day reduction % (13.3%).

Concerning to the result obtained in table 2, showed that the effect of cinnamon (1%) on viability of *B. cereus* inoculated into minced

meat. *B. Cereus* count were  $3.0 \times 10^6 \pm 0.5 \times 10^6$  at zero day,  $1.8 \times 10^5 \pm 0.2 \times 10^5$  at 2<sup>nd</sup> day with reduction % (94%),  $7.9 \times 10^4 \pm 1.4 \times 10^4$  at 4<sup>th</sup> day reduction % (97.4%) &  $3.3 \times 10^4 \pm 0.6 \times 10^4$  at 6<sup>th</sup> day reduction % (98.7%).

While the result obtained in table 3, showed that the effect of clove (1%) on viability of *B. cereus* inoculated into minced meat. *B. cereus* count were  $3.0 \times 10^6 \pm 0.5 \times 10^6$  at zero day,  $8.5 \times 10^4 \pm 2.1 \times 10^4$ , at 2<sup>nd</sup> day with reduction % (97.2%),  $5.2 \times 10^3 \pm 1.0 \times 10^3$  at 4<sup>th</sup>

day reduction % (99.8%) & complete destruction of *B. cereus* at 6<sup>th</sup> day.

More ever the result obtained in table 4, showed that the effect of marjoram (1%) on viability of *B. cereus* inoculated into minced meat. *B. cereus* count were  $3.0 \times 10^6 \pm 0.5 \times 10^6$  at zero day,  $6.7 \times 10^3 \pm 1.5 \times 10^3$  at 2<sup>nd</sup> day with reduction % (99.7%) & complete destruction of *B. Cereus* at 4<sup>th</sup> day.

Table (1): Effect of different essential oils (1%) on viability of *B. cereus* inoculated into minced meat by intensity of  $3 \times 10^6$  /g (n=5).

Treatment Storage time	Control	
	Count	R %*
Zero time	$3.0 \times 10^6 \pm 0.5 \times 10^6$	-
2 <sup>nd</sup> day	$2.9 \times 10^6 \pm 0.3 \times 10^6$	3.3
4 <sup>th</sup> day	$2.7 \times 10^6 \pm 0.3 \times 10^6$	10.0
6 <sup>th</sup> day	$2.6 \times 10^6 \pm 0.2 \times 10^6$	13.3

R % \*= Reduction %

Table (2): Effect of Cinnamon oil (1%) on viability of *B. cereus* inoculated into minced meat by intensity of  $3 \times 10^6$  /g (n=5).

Treatment Storage time	Cinnamon	
	Count	R %
Zero time	$3.0 \times 10^6 \pm 0.5 \times 10^6$	-
2 <sup>nd</sup> day	$1.8 \times 10^5 \pm 0.2 \times 10^5$	94.0
4 <sup>th</sup> day	$7.9 \times 10^4 \pm 1.4 \times 10^4$	97.4
6 <sup>th</sup> day	$3.3 \times 10^4 \pm 0.6 \times 10^4$	98.9

R % \*= Reduction %



*Cereus* at 6<sup>th</sup> day of storage followed by cinnamon.

## 5. REFERENCES

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