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Natural preservatives in raw chicken meat

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ABSTRACT

Cinnamon and clove essential oils were evaluated for their effects on the growth and survival of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) artificially inoculated into raw chicken meat. Fresh minced chicken meat samples were inoculated with (6 log cfu/g) of *S. aureus* and *E. coli*. Cinnamon and clove oils were added to the minced chicken meat samples to achieve final concentrations of 0.5% and 1 %. Sensory (color, odor and texture) and bacteriological analyses were conducted after 24 hrs for *E.coli* and every 48 hrs for *S. aureus* during cold storage at 4 °C. The results concluded that the addition of clove oil by 1% concentration had a powerful effect in reducing *S. aureus* that can cause food poisoning, while cinnamon oil 1% had good effect on *E. coli* but had strong flavor so from this study the authors found that clove oil at 1 % and cinnamon at 0.5 % had improved the flavor and extended the shelf life of fresh chicken meat.

1. INTRODUCTION

Poultry meat is a very popular food and is preferred by consumers due to low production cost, low fat content and high nutritional value. Considering the fact that poultry meat belongs to perishable foods, the main concern of industries is the shelf life extension of the poultry meat. Modern trends to achieve this target include the application of the hurdle technology concept and the use of natural food preservatives so as to sustain minimal processing and also to provide protection from both spoilage and pathogenic microorganisms (Khiari *et al.*, 2014).

Staphylococcal food-borne disease (SFD) is one of the most common food-borne diseases worldwide, resulting from the consumption of food that already contaminated by preformed *S. aureus* enterotoxins. This toxin resulting in sudden onset of nausea, vomiting and abdominal cramps (Unal and Cinar, 2012), while *E. coli* is often found in our surrounding environment because of fecal contamination (Ferens and Hovde *et al*., 2011)and It is characterized by causing abdominal cramping, pain, fever, nausea , vomiting ,hemorrhagic colitis and bloody diarrhea (Abongo and Momba, 2009).

Cinnamon is considered a common spice with strong antimicrobial and antioxidant activity. It belongs to the family *Lauraceae* and possesses significant biological activities (antitumor, antifungal, cytotoxic and antimutagenic) attributed to the cinnamaldehyde. The ability of this extract to retard lipid oxidation is attributed to the ability of its phenolic constituents to quench reactive oxygen species (Bonilla and Sobral, 2016).

Clove essential oil has a wide spectrum of actions not only antibacterial, antiviral, antifungal and antiprotozoal, but also have beneficial effects on the cardiovascular and immune system. It has the ability to inhibit the growth of *S. aureus* in meat products. The antibacterial activity of clove is attributed to eugenol with a small addition of cariophyllene and humulene (Daniel *et al*., 2009).

Therefore, the aim of the present work was to evaluate the efficacy of clove and cinnamon essential oils as antimicrobial agents in chicken minced meat by screening their bactericidal activities against *S. aureus* and *E.coli.*

2. MATERIAL AND METHODS

2.1. Samples:

Sixty samples of raw chicken meat (breast and thigh) were collected from different chicken retails in Gharbia governorate, Egypt. All collected samples were separately kept in a sterile plastic bag and transferred in an ice box to the laboratory under complete aseptic conditions without undue delay and examined as quickly as possible. The samples were subjected to the bacteriological examination for *S. aureus* and *E. coli* with serological examination for isolated *E. coli*.

2.2. Natural oils:

The essential oils (cinnamon and clove) were purchased from "The Agricultural Research Centre, Dokki, Egypt "which were produced by hydro-distillation method.

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2.3. Experimental application:

In three trials, a total 3600 gm of fresh chicken breast were purchased directly from different local markets in Gharbia governorate, Egypt . In each trial, the samples (1200 gm) were divided into six equal groups (100 gm each) for S. aureus trial and other six equal groups (100 gm each) for E. coli trial. All samples were washed in sterile distilled water and were properly labeled as: control (-ve), control (+ve), cinnamon oil (0.5% and 1%) and clove oil(0.5% and 1%). Each group of artificially inoculated chicken breast samples with previously isolated S. aureus and E.coli O119 (10^6 cfu/ml) were inoculated by essential oils (cinnamon and clove ,which dissolved in tween 80) at room temperature (25 ± 1 ℃) except control samples. All the samples treated with essential oils and the control groups were packed in polyethylene bags; labeled and stored at 4 ºC.

All groups were sensory examined for color, odor and texture every day (24 hrs) during storage period. *S. aureus* count was conducted on Baird Parker Plate media every 48 hrs during that period, while *E. coli* count was recorded on E.M.B media every 24 hrs , during the same period, using the serial dilutions and spread plate technique (Jay, 1992).

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) revealed that mean value of S. aureus in examined chicken meat samples were 5.00 ± 4.37 log cfu/g represented by 5.20 ± 4.65 log cfu/g in breast and 4.80 ± 4.22 log cfu/g in thigh samples. While the prevalence % of *E. coli* in same examined samples was (42.8%) in breast and (62.5%)in thigh samples.

The results recorded in table (2) revealed that E. coli serologically detected as O119, O125 and O111 in a percent 40, 10 and 20%, respectively.

Table 1 Statistical analytical results of *S. aureus* and *E. coli* count (log cfu/g) of the examined samples of chicken meat.

Samples	No. of examined samples	Mean value of *S. aureus* ± S.E	Prevalence % of *E.coli*
Breast	28	5.20 ± 4.65	42.8
Thigh	32	4.80 ± 4.22	62.5
Total	60	5.00 ± 4.37	53.3

The value represent mean ± SD Of three experiments.

Table 2 Serotyping of isolated *E. coli* (n=10).

Serotypes	No. of E.coli isolates	percent %
O119	4	40
O125	1	10
O111	2	20

Result in table (3) and table (4) showed the antimicrobial effects and reduction percentage of various concentrations of clove and cinnamon oils on S. aureus count artificially inoculated into chicken breast minced meat. Clove oil (0.5% and 1%) decreased the count of *S. aureus* (log cfu/g) from 8.70 ± 7.76 log cfu/g (initial load) to 5.60 ± 5.06 log cfu/g and 3.50 ± 3.10 log cfu/g with reduction percentages 35.6% and 60% on 8^{th} day and 12^{th} day of storage, respectively. Cinnamon oil (0.5% and 1%) decreased the count of *S. aureus* (log cfu/g) from 8.70 ± 7.76 log cfu/g (initial load) to 6.60 ± 6.07 log cfu/g and 5.50 ± 5.18 log cfu/g with reduction percentages 24% and 37% on 6^{th} day of storage, respectively.

The results recorded in table (5) revealed that the shelf life of examined samples artificially inoculated with *S. aureus* and treated with cinnamon (1%), clove (0.5%) and clove (1%) reached 6, 9 and 12 days of storage, respectively .

Moreover, the results in table (6) and table (7) illustrated that the antimicrobial effects and reduction percentage of various concentrations of clove and cinnamon oils on *E. coli* count artificially inoculated into chicken breast minced meat using clove oil (1%) revealed that it decreased the count of *E. coli* (log cfu/g) from 6.70 ± 6.06 log cfu/g (initial load) to 5.70 ± 5.06 log cfu/g with reduction percentages 15% on 1shday of storage, while clove oil (0.5%) showed no reduction in count of *E. coli*. Cinnamon oil (0.5% and 1%) decreased the count of *E. coli* (log cfu/g) from 6.70 ± 6.06 log cfu/g (initial load) to 3.70 ± 3.06 log cfu/g and 0 log cfu/g with reduction percentages 45% and 100% on 5thday of storage, respectively.

Furthermore, the results recorded in table (8) revealed that the shelf life of examined samples artificially inoculated with *E. coli* and treated with cinnamon (1%) and cinnamon (0.5%) reached 5 and 4 days of storage, respectively .

4. DISCUSSION

Staphylococci exist in air, dust, equipment, food, environmental surfaces, humans and /or animals. Staphylococci usually present in the nasal passage, throat, on the hair and skin (FDA, 2012). *S. aureus* which is the third most common cause of food poisoning in the world (Acco *et al*., 2003).Different stages of slaughter such as scalding, de-feathering and chilling may affect the prevalence and bacterial load of *S. aureus* on the carcasses (Spescha *et al.*, 2006).

Mean values of *S. aureus* in examined chicken meat samples were nearly similar to the results conducted by El Allaoui *et al*. (2013) and Saad *et al.* (2018) and were higher than Abo El- Enean-Hanan *et al.* (2008) and Abd El-Rahman *et al*. (2010), but these results were lower than Ahmad *et al.* (2013).

The reason for the relatively high prevalence of *S. aureus* in this research may be due to the bad personal hygiene of the workers and the traditional hand evisceration of chicken carcasses predominantly practiced in Egypt.

Prevalence of *E. coli* in examined chicken meat samples were nearly similar to that obtained by Rahman *et al.* (2017) (49.02%) in chicken meat and Xuan Binh *et al.* (2017) 43.3%. Meanwhile, the current study was higher than that conducted by Abd El-Rahman *et al.* (2010) (10.6%) and Shaltout *et al*. (2018) (37,7%). Moreover, these results were lower than that conducted by Eyi-Ayla and Arslan-Seza (2012) (87.5%) and Hmd-Randa *et al.* (2017) (82%). High prevalence of *E.coli* is good indicator to fecal contamination, water pollution and lack of sanitary condition during hand evisceration and washing of chicken carcasses. Clove oil is obtained from distillation of the flowers, stems, and leaves of the clove tree (Eugenia aromatica).

Table 3 The effects of various concentrations of essential oils on S. aureus count (log cfu/g) of the examined chicken minced meat samples during cold storage at 40 °C.

Treated samples	========== Storage days ==========						
	0 day	2nd day	4th day	6th day	8th day	10 day	12 day
Clove 0.5%	8.7± 7.76	7.7 ±7.06	6.8 ±6.06	5.7±5.06	5.6 ±5.06	7.6 ±7.19	8.7 ±8.06
Clove 1%	7.6 ±6.94	6.7 ±6.06	5.6±4.94	4.7±4.16	4.6±4.06	3.7±3.06	3.5±3.1
Cinnamon 0.5%	8.7± 8.06	8.5±7.76	7.6 ±7.06	6.6 ±6.07	7.6 ±7.07	8.6±8.33	8.8± 8.07
Cinnamon 1%	8.6± 7.94	8.6± 8.06	6.7 ±6.06	5.5±5.18	6.6 ±6.16	8.6± 8.06	8.8± 8.06

The value represent mean ± SD Of three experiments.

Table 4 Reduction % in S. aureus count of the examined chicken minced meat samples during cold storage at 4ºC.

Groups	0 day	2nd day	4th day	6th day	8th day	10 day	12 day
Clove 0.5%	--	11.5	22	34.5	35.6	12.64	--
Clove 1%	12.64	23	35.63	46	47	57.5	60
Cinnamon 0.5%	--	2	12.64	24	12.64	1	--
Cinnamon 1%	1	1	23	37	24	1	--

No reduction.(--)

Table 5 Sensory evaluation of treated chicken minced meat samples with various concentrations of essential oils for S. aureus artificially inoculated in minced chicken meat samples during cold storage at 40 °C.

Groups	0 day	2nd day	4th day	6th day	8th day	10 day	12 day
Clove 0.5%	8	7	6	5	4	3	2
Clove 1%	9	9	8.5	8	6	5.5	5
Cinnamon 0.5%	8	7	6	4.5	3	2	1
Cinnamon 1%	8	7	5	4	3	2	1

Score System for Sensory Evaluation (Pearson and Tauber, 1984):

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

Table 6 The effects of various concentrations of essential oils on *E. coli* count (log cfu/g) of the examined chicken minced meat samples during cold storage at 4^0C.

Treated samples	========== Storage days ==========					
	0 day	1st day	2nd day	3rd day	4th day	5th day
Clove 0.5%	6.7 ±6.06	6.7 ±6.06	6.8 ±6.16	8.7±7.94	8.8±7.94	spoiled
Clove 1%	6.7 ±6.06	5.7 ±5.06	6.7 ±6.16	8.7 ±7.94	8.8 ±7.94	spoiled
Cinnamon 0.5%	5.8 ±5.06	5.6 ±5.06	5.6 ±4.94	4.7 ±4.16	4.7 ±3.94	3.7 ±3.06
Cinnamon 1%	5.7 ±5.07	4.7 ±4.06	3.6 ±3.07	2.7 ±2.06	2.6 ±2.06	Non detectable

The value represent mean ± SD of three experiments (initial count =6.7 ±6.06)

Table 7 Reduction % in E.coli count of the examined chicken minced meat samples during cold storage at 40 °C.

Groups	0 day	1st day	2nd day	3rd day	4th day	5th day
Clove 0.5%	--	--	--	--	--	--
Clove 1%	--	15	--	--	--	--
Cinnamon 0.5%	13.5	16.4	16.4	30	30	45
Cinnamon 1%	15	30	46	60	61	100

No reduction (--)

Table 8 Sensory evaluation of treated chicken minced meat samples with various concentrations of essential oils for E. coli artificially inoculated in minced chicken meat samples during cold storage at 40 °C.

Groups	0day	1st day	2nd day	3rd day	4th day	5th day
Clove 0.5%	6	5	4.5	3	2	1
Clove 1%	6	5	4	3	2	1
Cinnamon 0.5%	6	5.5	5	4	4	3.5
Cinnamon 1%	6	6	5.5	5	4	4

Score System for Sensory Evaluation (Pearson and Tauber, 1984):

6: Excellent	5: Very good	4: Good
3: poor	2: Very poor	1: Very very poor

Amongst other components of the clove oil is the phenylpropene eugenol which is responsible for the distinctive aroma of the clove oil, with eugenol making up about 70-90% of the clove oil (Safrudin *et al*., 2015). Clove oil has been reported to have various degrees of antimicrobial activity against pathogens (Mishra and Kalyani, 2014).

Clove 1% cause obviously inhibition of *S. aureus* growth beginning from 0 day till end of storage period so high concentration of clove oil (1%) more effective in

decreasing *S. aureus* count than low concentration (0.5%) . The antimicrobial action of clove oil is related to its ability to inactivate microbial adhesion, enzymes and cell envelope proteins (Marí *et al.*, 2004).

While cinnamon oil 1 % cause slight inhibition of *S. aureus* growth at 4th day and 6th day then count increase again.

These results were in agreement with results recorded by Hassanien-Fatin *et al.* (2016) and Abd El – Hamed-Naglaa *et al*.(2017). The results of my study revealed that clove oil potentially effective against *S. aureus* than cinnamon oil and were agree with that of Al-dhaher -Zainab (2008).

On the other side cinnamon oil is more effective against *E.coli* than clove oil and these results were agree with that obtained by Adesiji *et al*. (2015) and Raeisi *et al*. (2015) who mentioned that cinnamon essential oil showed higher antimicrobial activity against *E. coli*, but were in disagreement with Asha *et al*. (2014)**,** who found that clove oil had high antibacterial activity against *E. coli*.

The results of my study revealed that clove oil more effective against *S. aureus* than *E.coli* and were agreet with that recorded by Hoqu *et al*. (2008) and Nzeako *et al*. (2006)

Gram positive bacteria are more sensitive than Gram negative to antimicrobial compounds of spice, such as phenols, aldehydes, ketones and terpenes (Nychas, 1995). In general, the difference lies in that the Gram negative bacteria cell wall is thinner than the Gram positive's cell wall, also the Gram negative cell wall has an outer membrane with a high percent of lipids that protects the cell wall taking into account that the cell wall is essential for keeping the cell integrity (Dourou *et al*., 2009).

A wide variety of microorganisms including *S. aureus* produce lipolytic enzymes that hydrolyze lipids, producing readily oxidizable substrates that have a rancid odor. *E. coli* produce proteolytic enzymes that hydrolyze proteins in foods leading to offensive odor. Most groups of microorganisms can spoil food by growing on the surface. Similarly, color changes in food can occur because of the surface growth of microorganisms (Sperber, 2009).

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