Benha University
Faculty of Veterinary Medicine
Food Hygiene and Control Department

Effect of Some natural and chemical preservatives on Shiga toxin producing *E. coli* in minced meat

A Thesis submitted to
Faculty of Veterinary
Medicine Benha University
Presented By

Marwa Magdy Mohamed Abd El Salam
(B.V. Sc., Benha University, 2006)
(M.V. Sc., Benha University, 2015)

For the Degree of Ph. D. V. Sc.

(Meat Hygiene)

Under Supervision of

Prof. Dr. / Hemmat Moustafa Ibrahim

Professor of Meat Hygiene
Course Coordinator of Food Quality and Control Program (FQCP)
Faculty of Veterinary Medicine
Benha University

Prof. Dr. / Mohamed Ahmed Hassan

Professor of Meat Hygiene and Head of Food Hygiene and Control Dept.,
Faculty of Veterinary Medicine
Benha University

Prof. Dr. / Reham Abd El Aziz Amin

Professor.of Meat Hygiene
Faculty of Veterinary Medicine
Benha University

Prof. Dr. / Ahmed Afify Abd El Ghafar

Chief Researcher of Microbiology and Director of Animal Health Research Institute
"Benha Branch"

(2019)

Contents

	Page
1- INTRODUCTION	1
2- REVIEW OF LITERATURE	5
3. MATERIAL AND METHODS	46
3.1. Collection of samples	46
3.2. Bacteriological examination	46
3.3. Experimental part	53
3.4. Statistical analysis	57
4. RESULTS	58
5. DISCUSSION	76
6. CONCLUSION AND RECOMMENDATIONS	93
7.Summary	95
8. REFERENCES	101
9. ARABIC SUMMARY	

List of Tables

Table No	Title	PAGE No
(1)	Statistical analytical results of Aerobic Plate Counts "APC" (cfu/g) in the examined raw minced meat samples at butcher shops .	58
(2)	Analysis of variance (ANOVA) of APC in the examined samples of raw minced meat	59
(3)	Acceptability of the examined raw minced meat samples depending upon their APC/g	59
(4)	Incidence and counts of coliform(cfu/g) in the examined samples of raw minced meat at butcher shops	60
(5)	Acceptability of the examined raw minced meat samples depending upon their coliform/g	61
(6)	Analysis of variance (ANOVA) of coliforms count in the examined samples of raw minced meat.	62
(7)	Incidence of Enteropathogenic *E. coli* in the examined samples of raw minced meat at butcher shops.	63
(8)	Serotyping of *E.coli* isolated from the examined samples of raw minced meat	64
(9)	Acceptability of the examined samples of raw minced meat depending on their contamination with *E. coli*.	65
(10)	Correlation coefficient (r) between incidences of *E. coli* % Vs APC and coliforms count in the examined minced meat samples.	66
(11)	Changes in sensory traits of control and essential oil (1%) treated minced meat stored at 4 ºC .	67
(12)	Changes in sensory traits of control and nisin treated minced meat stored at 4 ºC .	68
(13)	Changes in sensory traits of control and sodium	69

	ascorbate treated minced meat stored at 4 ºC .	
(14)	**Antibacterial activity of some essential oils on viability of *E.coli (O26: H11)* inoculated into minced meat .**	**70**
(15)	**Antibacterial activity of some essential oils on viability of *E.coli (O111: H2)* inoculated into minced meat .**	**71**
(16)	**Antibacterial activity of nisin as chemical preservative on viability of *E.coli (O26: H11)* inoculated into minced meat.**	**72**
(17)	**Antibacterial activity of nisin as chemical preservative on viability of *E.coli (O111: H2)* inoculated into minced meat .**	**73**
(18)	**Antibacterial activity of sodium ascorbate as chemical preservative on viability of *E.coli (O26: H11)* inoculated into minced meat.**	**74**
(19)	**Antibacterial activity of sodium ascorbate as chemical preservative on viability of *E. coli (O111: H2)* inoculated into minced meat .**	**75**

List of Figures

Fig No.	Title	PAGE No.
(1)	Mean value of APC (cfu/g) in the examined samples of raw minced meat	58
(2)	Incidence of accepted and unaccepted minced meat according to APC contamination..	60
(3)	Mean value of Coliforms count/g in the examined samples of raw minced meat	61
(4)	Incidence of accepted and unaccepted minced meat according to coliform contamination	62
(5)	Incidence of *E. coli* isolated from the examined samples of raw minced meat	63
(6)	Incidence and serotyping of *E.coli* isolated from the positive examined samples of raw minced meat	65
(7)	Incidence of accepted and unaccepted minced meat according to *E. coli* contamination	66
(8)	Reduction of *E.coli O26:H11* after addition of different essential oils	70
(9)	Reduction of *E.coli O111:H2* after addition of different essential oils	71
(10)	Reduction of *E.coli O26:H11* after addition of different concentration of nisin	72
(11)	Reduction of *E.coli O111:H2* after addition of different concentration of nisin	73
(12)	Reduction of *E.coli O26:H11* after addition of different concentration of Sodium ascorbate	74
(13)	Reduction of *E.coli O111:H2* after addition of different concentration of Sodium ascorbate	75

List of Abbreviations

µg/ml	**Microgram/milliliter**
AHRI	**Animal Health Research Institute**
ANOVA	**Analysis Of Variance**
APC	**Aerobic Plate Count**
CDC	**Centers for Disease Control and Prevention**
CFU/g.	**Colony Forming Unit/gram**
E.coli	***Escherichia coli***
EOS	**Egyptian Standard Specifications**
Eos	**Essential oils**
FAO	**Food Agriculture Organization**
FDA	**Food and Drug Administration**
GIT	**GastroIntestinal tract**
HACCP	**Hazard Analysis and Critical Control Point**
ICMSF	**International Commission on Microbiological Specification for Food microorganisms**
IU	**International Unit**
MIC	**Minimum Inhibitory Concentration**
MLD	**Minimum lethal Dose**
MPN	**Most Probable Number**
PL	**Permissible Limit**
ppm	**part per million**
SE	**Standard Error**
TSC	**Tryptose Sulphate Cycloserine**
UK	**United Kingdom**
USA	**United States of America**
WHO	**World Health Organization**

7. Summary

Shiga toxin (stx)–producing *E. coli* (STEC) considered as one of the most important pathogens and the presence of them in meat products constitutes serious problems for consumers. So, the present study was conducted to evaluate the safety of minced meat at Kaliobia Governorate by studying the prevalence of *E. coli*, mainly STEC strains in minced meat and to evaluate the efficacy of both natural and chemical preservatives on them.

- The bacteriological examination of minced meat samples collected from different localities (Benha city, Toukh center and Moshtohor village) revealed that, the mean value of APC and Coliforms count in fresh minced meat samples collected from Benha city were $2.85x10^5 \pm 0.44x10^5$ and $6.91x10^2 \pm 1.12x10^2$, respectively; for fresh minced meat samples collected from Toukh center were $7.31\times10^5 \pm 1.06\times10^5$ and $9.53\times10^2 \pm 2.01\times10^2$, respectively; for fresh minced meat samples collected from Moshtohor village were $1.67\times10^6 \pm 0.29\times10^6$ and $2.86\times10^3 \pm 0.49\times10^3$, respectively.
- Regarding to *E. coli* species isolation, the results appeared that, 31 isolates of *E. coli* were isolated from examined minced meat samples represented as 6 (17.1%) in Benha city with serotypes 1 O26 : H11,2 O111 : H2,1 O113 : H2and 2 O128 : H2; 9 (25.7%) in Toukh center with serotypes 3 O26 : H11, 1 O91 : H21, 1 O104 : H4, 2 O111 : H2 , 1 O125 : H21 , 1 O146 : H21; and 16 (45.7) in Moshtohor village with serotypes 3 O26 : H11, 1 O55 : H7, 1O86,2 O91 : H21, 4 O111 : H2 ,1 O124,2 O127 : H6, 1 O128 : H2and 1 O153 : H2 , also, 63 samples out of 105 ones were accepted as they were free from *E. coli* isolates.

- Regarding to studying the antibacterial activity of different essential oils (Clove, Garlic and Thyme 1%); on viability of *E. coli* strains, the results cleared that, the *E. coli* O26:H11 and O111:H2 counts in examined minced meat samples were as follow:

- At zero time, the mean value of both *E. coli* serotypes counts for control and treated samples was $5.0\times10^{6}\pm 0.91\times10^{6}$.
- In control samples, *E. coli* O26:H11and *E. coli* O111:H2 counts were 2.61% and 3.80% as reduction % with mean values of $4.87\times10^{6}\pm 0.75\times10^{6}$; and $4.81\times10^{6}\pm 0.69\times10^{6}$, respectively, after 3rd owing to the effect of chilling at 4˚C during storage then spoilage of minced meat samples occurred 6th and 9th days, respectively .The shelf life of control samples was very short (3 days) in comparison with essential oils (9 days).Concerning to addition of clove oil 1%, *E. coli* O26:H11 counts were decreased by 45.40%; 82.16% and 99.85% as reduction % with mean values of $2.73\times10^{6}\pm 0.49\times10^{6}$; $8.92\times10^{5}\pm 1.57\times10^{5}$ and $7.14\times10^{3}\pm 1.22\times10^{3}$ and *E. coli* O111:H2 counts were decreased by 27.78%; 63.40% and 99.14% as reduction% with mean values of $3.61\times10^{6}\pm 0.52\times10^{6}$, $1.83\times10^{6}\pm 0.35\times10^{6}$ and $9.28\times10^{4}\pm 1.84\times10^{4}$ after 3rd; 6th and 9th days, respectively. Meanwhile, the addition of Garlic oil 1% revealed the decrease of *E. coli* O26:H11 counts by 67.86 % and 98.22 %as reduction % with mean values of $1.65\times10^{6}\pm 0.36\times10^{6}$ and $5.43\times10^{4}\pm 0.89\times10^{4}$ after 3rd and 6th. But, at the 9th day of storage, it had complete inhibited effect (100%) on *E. coli* O26:H11 strains that cannot be detected in treated samples. But for *E. coli* O111:H2 the counts declined by 58.39%, 85.68% and 98.87% as reduction % with mean values of $2.08\times10^{6}\pm 0.26\times10^{6}$, $7.16\times10^{5}\pm 1.35\times10^{5}$ and $5.63\times10^{4}\pm 0.80\times10^{4}$ after 3rd, 6th and9th day respectively. Moreover, the addition of Thyme oil 1% revealed

the decrease of *E. coli* O26:H11 counts by 98.17% as reduction %with mean value of $9.16\times10^4\pm 2.01\times10^4$ after 3rd day of storage and after that, it had complete inhibited effect on *E. coli* O26:H11 strains which cannot be detected in treated samples. But for *E. coli* O111:H2 the counts declined by 93.16% and 99.82% as reduction % with mean values of $3.42\times10^5\pm 0.59\times10^5$and $9.06\times10^3\pm 2.10\times10^3$after 3rd and 6th But, at the 9th day of storage, it had complete inhibited effect(100%) on *E. coli* O111:H2 strains that cannot be detected in treated samples.

- Regarding to studying the antibacterial activity of Nisin with different concentrations (10 ppm, 30 ppm and 50 ppm) on viability of *E. coli* strains, the results cleared that, the *E. coli* O26:H11 and O111:H2 counts in examined minced meat samples were as follow:
- In control samples, *E. coli* O26:H11and *E. coli* O111:H2 counts were 1.38%; and 1.80% as reduction % with mean values of $4.93\times10^6\pm 0.80\times10^6$; and $4.91\times10^6\pm 0.76\times10^6$ respectively, after 3rd owing to the effect of chilling at 4˚C during storage then spoilage of minced meat samples occurred 6th and 9th days, respectively .The shelf life of control samples was very short (3 days) in comparison with Nisin (9 days).
- The addition of 10 ppm Nisin, *E. coli* O26:H11 counts were decreased by 29.60 %; 64.80% and 90.72% as reduction % with mean values of $3.52\times10^6\pm 0.60\times10^6$, $1.76\times10^6\pm 0.28\times10^6$ and $4.64\times10^5\pm 0.71\times10^5$ and *E. coli* O111:H2 counts were decreased by 20.81%; 59.79 % and 80.10 % as reduction % with mean values of $3.96\times10^6\pm 0.54\times10^6$; $2.01\times10^6\pm 0.33\times10^6$ and $9.95\times10^5\pm 2.14\times10^5$after 3rd; 6th and 9th days, respectively. Meanwhile, the addition of 30 ppm Nisin, *E. coli* O26:H11 counts were decreased by 58.00 %; 81.62 % and 92.04 % as reduction % with mean

values of $2.10\times10^6\pm 0.32\times10^6$; $9.19\times10^5\pm 2.05\times10^5$ and $3.98\times10^5\pm 0.24\times10^5$ and *E. coli* O111:H2 counts were decreased by 45.60 %; 72.43 % and 82.21 % as reduction % with mean values of $2.72\times10^6\pm 0.43\times10^6$, $1.38\times10^6\pm 0.21\times10^6$ and $8.89\times10^5\pm 1.37\times10^5$ after 3rd; 6th and 9th days, respectively. Moreover, the addition of 50 ppm Nisin, *E. coli* O26:H11 counts were decreased by 70.39 %; 87.41 % and 95.86% with mean values of $1.48\times10^6\pm 0.26\times10^6$; $6.30\times10^5\pm 1.18\times10^5$and $2.07\times10^5\pm 0.15\times10^5$ and *E. coli* O111:H2 counts were decreased by 61.80%, 81.20% and 89.67%as reduction % with mean values of $1.91\times10^6\pm 0.32\times10^6$, $9.40\times10^5\pm 1.24\times10^5$and $5.16\times10^5\pm 0.73\times10^5$after 3rd; 6th and 9th days, respectively.

- Regarding to studying the antibacterial activity of Sodium ascorbate with different concentrations (550 ppm, 650 ppm and 750 ppm) on viability of *E. coli* strains, the results cleared that, the *E. coli* O26:H11 and O111:H2 counts in examined minced meat samples were as follow:
- In control samples, *E. coli* O26:H11and *E. coli* O111:H2 counts were. 1.02%; and 1.59% as reduction % with mean values of $4.95\times10^6\pm 0.74\times10^6$; and $4.92\times10^6\pm 0.83\times10^6$respectively, after 3rd owing to the effect of chilling at 4˚C during storage then spoilage of minced meat samples occurred 6th and 9th days, respectively .The shelf life of control samples was very short (3 days) in comparison with Sodium ascorbate (9 days).
- The addition of 550 ppm sodium ascorbate, *E. coli* O26:H11 counts were decreased by 17.20 %, 52.19 % and 63.41 % as reduction % with mean values of $4.14\times10^6\pm 0.71\times10^6$; $2.39\times10^6\pm 0.45\times10^6$and $1.83\times10^6\pm 0.27\times10^6$and *E. coli* O111:H2 counts were decreased by 10.39%; 41.62% and 56.80% as reduction % with

mean values of $4.48\times10^6\pm 0.69\times10^6$; $2.92\times10^6\pm 0.51\times10^6$and $2.16\times10^6\pm 0.34\times10^6$ after 3rd, 6th and 9th days, respectively. Meanwhile, the addition of 650 ppm Sodium ascorbate, *E. coli* O26:H11 counts were decreased by 19.40%, 61.03% and 61.03% as reduction % with mean values of $4.03\times10^6\pm 0.78\times10^6$; $1.95\times10^6\pm 0.24\times10^6$and $1.32\times10^6\pm 0.18\times10^6$ and *E. coli* O111:H2 counts were decreased by 16.20%, 48.81% and 62.23% as reduction % with mean values of $4.19\times10^6\pm 0.67\times10^6$; $2.56\times10^6\pm 0.42\times10^6$and $1.89\times10^6\pm 0.25\times10^6$after 3rd, 6th and 9th days, respectively. Moreover, the addition of 750 ppm Sodium ascorbate, *E. coli* O26:H11 counts were decreased by 23.18%; 67.79% and 81.28% as reduction % with mean values of $3.84\times10^6\pm 0.57\times10^6$; $1.61\times10^6\pm 0.30\times10^6$ and $9.36\times10^5\pm 2.11\times10^5$and *E. coli* O111:H2 counts were decreased by 18.80%, 60.17% and 71.78% as reduction % with mean values of $4.06\times10^6\pm 0.79\times10^6$; $1.99\times10^6\pm 0.28\times10^6$ and $1.41\times10^6\pm 0.19\times10^5$after 3rd, 6th and 9th days, respectively.

- In addition, the mean value of both *E. coli* serotypes counts at zero time showed no changes since reduction effect of all essential oils; Nisin and Sodium ascorbate were not initiated yet and all of them were more effective on *E. coli* O26:H11than *E. coli* O111:H2.
- From the obtained results, it was found that the sensory properties of the samples were enhanced by using essential oils as (Clove, Garlic and Thyme), Nisin and Sodium ascorbate . Moreover, addition of Thyme 1% leads to enhancement in sensory properties of the minced meat samples more than Garlic and Clove 1% , on other hand addition of Nisin and Sodium ascorbate leads to enhancement in sensory properties of minced meat samples more than essential oils.

Finally, addition of essential oils leads to extension of shelf life of minced meat in comparison with storage at 4˚C. Moreover, Thyme oil 1% was considered the best added preservative as it was the most effective on *E. coli* strains which cannot be detected in treated samples at 9^{th} day of storage.

Therefore, it was concluded that, *E. coli* is meat borne pathogen of public health important and the hygienic measures are suggested for obtaining meat products with controlled bacterial pathogens to be fit for human consumption.