





Benha University Faculty of Veterinary Medicine Department of Food Control and Hygiene

Assessment of some food poisoning bacteria in ready-to-eat meals

A Thesis Submitted to Faculty of Veterinary Medicine, Benha University

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nhe	nonhemolytic enterotoxin
No	Number
NSW/FA	New South Wales Food Authority
ODC	Ornithine decarboxylase
ONPG	β- galactosidase
PCR	Polymerase Chain Reaction
Ph	Hydrogen Ion Concentration
RTE	Ready-to-eat
S.aureus	Staphylococcus aureus
SEs	S. aureus enterotoxins
SFP	Staphylococcus aureus food poisoning
SVFs	Street-vended foods
TSI	Triple Sugar Iron agar
USDA-FSIS	United States Department of Agriculture
	and Food Safety and Inspection Service
UTI	Urinary tract infection
UV	ultraviolet light
WHO	World Health Organization

7- Summary

Part I:

Various factors contribute to the outbreaks of the food borne illness. The main ones are inadequate food manipulation, improper holding temperatures (failing to properly refrigerate food), inadequate cooking, contaminated equipment (failure to clean and disinfect kitchen or processing plant equipment) and poor personal hygiene.

Therefore, this study was designed to throw spot lights upon presence of food poisoning bacteria in ready to eat foods. The present study was performed on a total of 100 random samples of beef and chicken meat products including Shish tawook , Chicken fajitas, beef fajitas and Hot dog (25 of each) were collected from different restaurants in Menoufiya governorate, Egypt. The samples were transferred in ice box as rapidly as possible to the laboratory under complete aseptic conditions without undue delay and examined microbiologically as quickly as possible for detection of aerobic plate count, coliform count, Staph. aureus incidence and count , isolation and serotyping of E.coli as well as incidence of Bacillus cereus and Clostridium perfringens.

The obtained results revealed that the mean values of APC (CFU/g) were $1.37 \times 106 \pm 1.74 \times 106$ in Shish tawook, $3.24 \times 106 \pm 3.69 \times 106$ in Chicken fajitas, $1.48 \times 105 \pm 1.92 \times 105$ in Beef fajitas and $1.94 \times 105 \pm 2.65 \times 105$ in Hotdog. Also, the Coliform count (CFU/g) were $4.54 \times 104 \pm 8.66 \times 104$, $9.33 \times 104 \pm 1.49 \times 105$, $2.42 \times 104 \pm 1.82 \times 104$ and $1.63 \times 104 \pm 2.64 \times 104$ in Shish tawook, Chicken fajitas, Beef fajitas and Hotdog, respectively. While, the mean values of Staph.aureus count (CFU/g) were $6.33 \times 103 \pm 7.06 \times 103$, $1.60 \times 104 \pm 1.13 \times 104$, $1.91 \times 104 \pm 1.28 \times 104$ and $1.18 \times 104 \pm 8.91 \times 103$ in Shish tawook, Chicken fajitas and Hotdog, respectively.

There is high significant difference at (P < 0.05) appeared between such examined samples.

On the other hand, indicated that the incidence of S. aureus, E.coli, Bacillus cereus and Clostridium perfringens in Shish tawook, Chicken fajitas, Beef fajitas and Hot dog were 8(32%), 10(40%), 6(24%) and 5(20%), 5(20%), 8(32%), 6(24%) and 4(16%), 4(16%), 7(28%), 6(24%) and 5(20%), 6(24%), 6(24%), 4(16%) and 5(20%).

The incidences of E.coli serotypes in the examined samples were 0111:H2 (4%) EHEC, 0113:H4 (4%) EPEC, 026:H11 (4%) EHEC, 0127:H6

(4%) ETEC and 0103 (4%) EHEC in Shish tawook, 0111:H2 (4%) EHEC, 091:H21 (4%) EPEC, 0127:H6 (4%) ETEC, 0119:H6 (8%) EPEC, 0113:H4 (4%) EPEC, 026:H11 (4%) EHEC and 0124 (4%) EIEC in Chicken fajitas, 0111:H2 (4%) EHEC, 0113:H4 (8%) EPEC, 026:H11 (4%) EHEC, 055:H7 (4%) EPEC and 0124 (4%) EIEC in Beef fajitas, 0127:H6 (4%) ETEC, 0119:H6 (4%) EPEC, 055:H7 (4%) EPEC and 0124 (4%) EIEC in Hotdog.

The multiplex PCR technique for Bacillus cereus was used to recognize presence of virulence genes encoding two enterotoxins (nonhemolytic enterotoxin [NHE] and cytotoxin K [CytK]) in isolated strain from readyto- eat food. The results revealed that the all isolated Bacillus cereus strains were positive for the nhe and cytk yielded a consistent fragment at 766 and 421bp, respectively.

Finally, the obtained results in the present study concluded that the examined Chicken fajitas samples showed higher contamination than other products. While S.aureus is the most microorganisms isolated from all examined samples. So, it has further evidence that the undesirable level of contamination which might have acquired from the environment and to obtain wholesome, safe and sound meat, the Hazard Analysis and Critical Control Point system (HACCP) must be adopted.

Part II:

A three trial based experiment was designed to evaluate the antibacterial effect of different concentrations (0.5%, 1% and 1.5%) of thyme and garlic essential oils against reference strain of E. coli inoculated into minced beef during cold storage at 4 °C and their role in enhancement of sensory characters.

From the obtained results, it was found that the sensory properties of the samples were enhanced by using essential oils as thyme and garlic. Moreover, by increasing the concentrations of thyme and garlic oils, there were more enhancements in sensory properties of the samples as samples containing 1.5% oils, demonstrated the highest enhancement of sensory attributes, while the samples treated with 0.5% of the same oils demonstrated the lowest enhancement.

Moreover, the obtained results revealed that each of thyme and garlic EOs has antimicrobial effect against E. coli as:-

The initial counts of E. coli in minced beef samples after inoculation was 5.36±.01a log10cfu/g. E. coli counts in the control samples increased

and were 5.36 ± 0.1 , 5.65 ± 0.3 and 6.16 ± 0.1 log10 cfu/g at zero, 2nd and 4th days respectively, and spoiled at 6th of inoculation.

By using the concentration of 0.5% thyme, E. coli counts were 5.36 ± 0.1 , 5.24 ± 0.2 , 4.62 ± 0.1 and $4.38\pm0.2 \log 10$ cfu/g at zero, 2nd, 4th and 6th days of inoculation respectively, with reduction percentage 2.24%, 13.80% and 18.28% at 2nd, 4th and 6th days of inoculation respectively, but spoiled at 8th day of inoculation. Where, at the concentration of 1% thyme, E. coli counts were 5.36 ± 0.1 , 5.16 ± 0.1 , 4.52 ± 0.1 and 4.24 ± 0.2 log10 cfu/g at zero, 2nd, 4th and 6th days of inoculation respectively, with reduction percentage 3.73%, 15.67% and 20.89% at 2nd, 4th and 6th days of inoculation. At the concentration of 1.5% thyme, E. coli counts were 5.36 ± 0.1 , 5.12 ± 0.1 , 4.42 ± 0.1 , 4.12 ± 0.15 and 3.51 ± 0.14 log10cfu/g at zero, 2nd, 4th, 6th and 8th days of inoculation respectively, with reduction percentage 4.43%, 17.54%, 23.13% and 34.51% at 2nd, 4th, 6th and 8th days of inoculation respectively, but spoiled at 10th day of inoculation.

By using the concentration of 0.5% garlic, E. coli counts were 5.36 ± 0.1 , 5.25 ± 0.2 , 4.76 ± 0.2 and $4.54\pm0.2 \log 10 cfu/g$ at zero, 2nd, 4th and 6thdays of inoculation respectively, with reduction percentage 2.05%, 11.20% and 15.30% at 2nd, 4th and 6th days of inoculation respectively, but spoiled at 8th day of inoculation. At the concentration of 1% garlic, E. coli counts were 5.36 ± 0.1 , 5.24 ± 0.1 , 4.61 ± 0.1 and $4.41\pm0.3 \log 10$ cfu/g at zero, 2nd, 4th and 6thdays of inoculation respectively, with reduction percentage 2.24\%, 13.99% and 17.72% at 2nd, 4th and 6th days of inoculation respectively, but spoiled at 8th day of inoculation. At the concentration of 1.5% garlic, E. coli counts were 5.36 ± 0.1 , 5.19 ± 0.2 , 4.48 ± 0.1 , 4.32 ± 0.2 and $3.81\pm0.1 \log 10cfu/g$ at zero, 2nd, 4th, 6th and 8th days of inoculation respectively, with reduction percentage 3.17%, 16.42%, 19.40% and 28.92% at 2nd, 4th, 6th and 8th days of inoculation respectively, but spoiled at 10th day of inoculation.

Finally, the present study allowed concluding that thyme oil (1.5%) proved to be more efficient in suppression of E.coli growth in minced meat. So, the use of thyme oil (1.5%), as it is safe antimicrobial agent against E.coli, is therefore recommended to improve safety of meat products.