AJMBR

AlQalam Journal of Medical and Biological Research

AlQalam Journal of Medical and Biological Research,2017;1(1):48-52
LN: 207/2015

Available Online: http://www.utripoli.edu.ly

Microbiological Quality and Detection of the Genes Stx1 and Stx2 in Fresh Sausage Marketed in Tripoli, Libya

Ben Hamza I[1]*, Elshrek Y[2], Abojnah Y[2], Al gazali M[3], Ibrahim K[4].

[1] Microbiological Department, Enviroment General Authority, Tripoli, Libya.

[2] Food Science Department Faculty of Agriculture University of Tripoli Libya.

[3] Microbiological Laboratory, Biotechnology Research Center - Tripoli Libya.

[4] Genetic Laboratory, Biotechnology Research Center - Tripoli Libya.

Email: *imanhmz@gmail.com*

Received:30,07,2017 ***Accepted: 02, 08, 2017***

ABSTRACT

Background: *In this study, samples of fresh sausage (n=100) were collected randomly from meat shops in four different areas of Tripoli (Alhoot market, Aldhra market, Grgarsh and Alsyahia). All samples were analyzed for the presence of the following: the total aerobic plate count, Coliforms, Escherichia coli and E .coli O157:H7. The mean of total plate count for fresh sausage (Almargaz) was 2.5 x 10^8 CFU/g, with a range from 7 x 10^4 to7.5 x 10^9 CFU/g, with no significant differences at level (P< 0.05) between the regions of study. The mean of the most probable number of Coliforms was 1.4x10^3 Cell/gram (g), ranging from 1.6 x 10^2 to 2.4 x 10^3 Cell/g, indicating a significant difference at level (P< 0.01). The percentage of Escherichia coli contaminated sausage samples was 81%, with no significant differences in level (P< 0.01) between the regions of the study. The percentage of E .coli O157:H7 was contaminated sausage samples 48% with no significant differences at level (P< 0.05). The percentages of Stx1 gene in E. coli O157:H7 were 37.5% in the Aldhra Market, 25% in Hoot market and Grgarsh, while was 12.5% in Alsyahia. On the other hand the high percentages of Stx2 % were detected 27.5% in both Alsyahia and Grgarsh, 24.2% in Aldhra Market, while was 20.6% in the Alhoot market.*

Keywords: ***Total aerobic plate count, Coliforms, Escherichia coli/, E .coli O157:H7, Stx1, Stx2.***

INTRODUCTION

Fresh sausages are highly perishable and serve as suitable substrate for several spoilage and pathogenic microorganisms, due to their high moisture content and abundance of essential nutrients [1]. Previous studies from different countries indicated the prevalence of Enterohemorrhagic *Escherichia coli* (EHEC) in meat products being as follows, 0 -14% in ground meats, 0 - 8% in fresh meats [2], and 5%-7% of red meat products [3], [4] reported that total microbial counts in fresh sausages ranged from 1.0 x 106 to 9.3 x 10^8 CFU/g, with an average of 8.5 x 10^7 CFU/g. In addition, Coliforms counts ranged from 3.0 x 10^3 to 1.1 x 10^8 /g with an average of 2.4 x 10^5 /g, while thermotolerant (fecal) Coliforms ranged from 1.0 x 106 to 9.3 x 10^7 /g, with an average of 1.3 x 10^5 /g.

Cite this article: Ben Hamza I, Elshrek Y, Abojnah Y, Al gazali M, Ibrahim K. Microbiological Quality and Detection of the Genes Stx1 and Stx2 in Fresh Sausage Marketed in Tripoli, Libya. Alq J Med Bio Res, 2017;1(1):48-52.

Some pathogenic bacteria were isolated from sausage samples, especially, *E. coli* O157 and *Salmonella* sp. with a percentage of 39.3 % and 2.10 %, respectively .In little more than a decade PCR has evolved from being a technique used by a few molecular biologists to a universally accepted tool for rapid diagnosis of pathogenic bacteria like *Escherichia coli* (*E. coli*) where the pathogenic and nonpathogenic strains of *E. coli* are difficult to distinguish using selective cultivation methods, whereas pathogenic properties are reliably identified through detection of toxin genes [5].
One of these pathogenic strains of *E. coli* is *Escherichia coli* O157:H7, which is an emerging food pathogen that was first identified as a cause of illness in 1982. Same applied methods focused on three types of primary food products; namely poultry-carcass rinse, swab, and milk [6]. The aim of this study is to detect the extend

contamination and the existence of Stx genes (Stx1 & Stx2) of the pathogenic bacteria *E. coli* O157:H7 on raw fresh sausage samples (Almergaz). Using specific primers and PCR is suggested, which will be helpful, for the detection of *E. coli* O157:H7 in a faster way.

METHODOLOGY

Collection of samples

One hundred (100) fresh sausage samples were collected from butcher's shops in four regions in Tripoli city (Alhoot market, Aldhra market, Gargarsh and Alsyahia). Samples were placed in a sterile ice container and transferred to the Microbiology Laboratory (Biotechnology Research Center) within 30 – 45 min. These samples were collected through a six months period (January – June) in the year 2010.

Microbiological analysis

Total plate count (TPC) and Total Coliforms as well detection of fecal Coliforms of *E. coli*, and *E. coli* O157:H7 were conducted according to microbiological standard methods described in [7], [8].

Preparation of bacteria for PCR

E. coli O157:H7 culture that was identified by Latex agglutination test was grown in Nutrient Agar for 24 h at 37°C, a well isolated colony was picked and the bacterial cells were suspended in 10ml sterile normal saline and used as a DNA template for PCR. The bacterial suspension was transported to the Genetic Engineering Department (Biotechnology Research Center) for DNA extraction and PCR analysis.

DNA extraction

The method used for DNA extraction was, according to protocol [9], with some modifications.

Determination of dna quantity and quality

The method used was according to [1].

Preparation of primer

The primers shown in table (1) designed according to previous study [12], and diluted with T.E buffer before use.

Table 1: The sequences of Primers designed

Prime Set		Size (bp)
stx1F	CAGTTAATGTGGTGGCGAAG	513 b p
stx1R	CTGTCACAGTAACAAACCGT	-
stx2F	TTCTTCGGTATCCTATTCCC	-
stx2R	ATGCATCTCTGGTCATTGTA	482 b p

Polymerase chain reaction (PCR)

The method for the Preparation of the reaction mixture was that used according to [11].The final volume of each reaction mixture was 25µL. Table (2) illustrates the program of thermocycler for each PCR reaction.

Table 2: The program of thermocycler for each PCR reaction

First Denaturation step at 95° C for 5 minutes	
30 cycles were repeated for each reaction	Denaturation step 1 at 95° C for 1 min
	Annealing at 58°C for 1.5 min
	Extension at 72°C for 1.5 min
Final Extension at 72°C for 5 min.	

Detection of PCR products by agarose gel electrophoresis

The last step in the procedure was the detection of PCR products by agarose gel to indicate the presences or absent of stx1, stx2. The electrical power supply was adjusted to 80V of voltage, 200mA of current for a period of 15min before it was turned on. Gel electrophoresis result was photographed under UV light and documented [11].

Statistical analysis

The data obtained in this study for total plate count, fecal coliform count, *Escherichia coli*, *E. coli* O157:H7 and STX gene were statistically analyzed using the Completely Randomized Design [CRD] and Analysis of Variance (ANOVA). Duncan table test was used to compare the average at ($P \leq 0.05$).

RESULTS AND DISCUSIONS

Total plate count

The results of the TPC of sausage samples for all the regions of the present study, are shown in Table (3).

Table 3: Total plate count for sausage in four different of the regions

Region	Mean of total plate count (CFU/g)	Minimum (CFU/g)	Maximum (CFU/g)
Al dhra Market	3.19 x10^8	7x10^4	6 x10^9
Al hoot market	5 x10^7	8x10^4	3 x10^8
Al syahia	6 x10^8	39 x10^5	7.5 x10^9
Grgarsh	4 x 10^7	14x10^4	4 x10^8
Total of all regions	2.5 x10^8	7 x 10^4	7.5x10^9

According to Libyan National Center for Standardization and Metrology [13], regarding microbiological quality of fresh beef sausage; the maximum number of TPC must not exceed 10^7, the present study results showed very high number of TPC. The highest level for TPC in the present study is due to the unsanitary conditions during processing, handling, preparations poor personal hygiene and unsanitary habits for people who handle sausages. Beside that It may be due to slaughterhouses hygienic quality.

Estimate the most probable number (mpn) of coliforms.

Coliform bacteria can be served as indicators of human or animal fecal contamination during meat and meat products processing. MPN is commonly used to estimate the numbers of Coliform bacteria in food and in water. Table (4) shows the average means of (MPN) of Coliforms for all regions of this study was 1.4x10^3 Cell/g, and their range between 1.6 x 10^2 to 2.4 x 10^3 Cell/g. These mean was generally lower than the results of [1] who found that the Coliform bacteria counts ranged from 3 x 10^3 to 1.1 x 10^8 Cell /g. with an average of 2.4 x 10^5 Cell /g, The results of this study were higher than [14] that indicate for MPN Coliform bacteria in sausages should be less than 1.0x10^2 Cell/g.

Table 4: The average, maximum and minimum mean of MPN of Coliforms bacteria for all samples in four different regions of the study.

Region	Mean of *Coliform* counts (Cell/g)	Minimum (Cell/g)	Maximum (Cell/g)
Aldhra Market	^{a}1.5 x10^3	2.4x10^2	2.1 x10^3
Al hoot market	^{b}16 x10^3	4.6x10^2	2.4 x10^3
Al syahia	^{a}1.5 x10^3	1.6x10^2	2.4 x10^3
Grgarsh	^{b}1.1 x10^3	4.6 x10^2	1.1x10^3
Total of samples all regions	1.4 x10^3	1.6x10^2	2.4x10^3

[a, b] Values in the same Colum with different superscripts are significantly different at level (P<0.01).

Detection of Escherichia coli

E. coli is often used as an indicator of fecal contamination because it is abundant in human and animal feces and not usually found in other niches. It is used to indicate unsanitary processing in the food-processing environment which had dangers effect on customers health [15]. In this study *Escherichia coli* had been detected in most sausage samples for four regions of study. The average contaminated samples with *Escherichia coli* were 81%. This result is similar to [1], who reported that the average of fresh sausage contaminated with *E. coli* was around 71.4%.

Detection of E. coli O157:H7

The percentages of *E. coli* O157:H7 were 48% of samples, and were conformity the results by biochemical test. The detection of *E. coli* O157:H7 in this study revealed that the local produced sausages could be considered as unconfirmed with [16] which determined, the *E. coli* O157:H7 should be zero in fresh uncooked sausages. The isolation of *E. coli* O157:H7 from meat and its products reported in many local studies like [17] who found 27.1% of uncooked beef burger contaminated with *E. coli* O157:H7, and [1] found 39.3% of fresh sausage samples contaminated with *E. coli* O157:H7 of samples which collected from some butchers in Tripoli city.

PCR test dna extraction for bacteria Escherichia coli O157:H7

During this study a chemical method for DNA extraction was employed with some modification [11] Extracted DNA samples were run on 2.0% Agarose in

TAE. Concentrations of extracting DNA were measured using spectrophotometerically.

Detection of shiga toxin genes

PCR technique performed on 48 biochemically confirmed *E. coli* O157:H7 isolated from sausages' samples for detected and amplified off *E. coli* O157:H7.

Virulence genes shiga toxin genes (stx).

The resulting amplified fragments were loaded on an agarose gel. A DNA marker was used to ensure that the amplified fragments were of the expected size. Primers were designed to be flanking. Amplified regions were approximately 513 bp of stx1 and 284bp of stx2 bp product. The DNA fragment was clearly shown after sinking the gel in Ethedium Bromide Fig: (3) and (4).

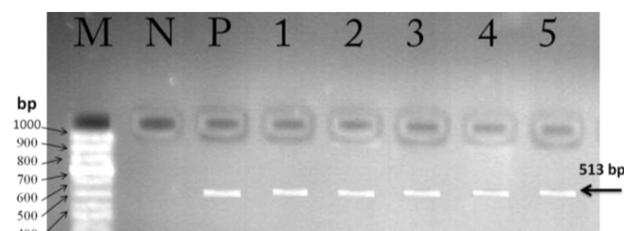

Fig. (3): *Detection of stx1 gene of E. coli O157:H7 that isolated from sausage samples, PCR products (513 bp) were separated on a 2.0% agarose gel . Lane M: contains 100 b p DNA size markers. Lane N: negative control (non-157:H7 E. coli) and lane P: positive control (E. coli O157:H7 with stx1 enes). Lanes 1, 2, 3,4 and 5 lanes are the E. coli O157:H7.*

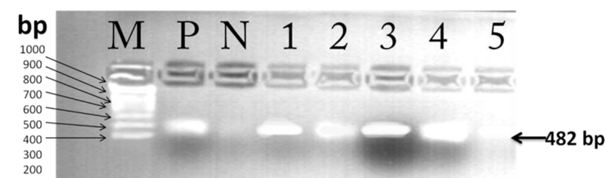

Fig. (4): *Detection of Stx2 gene of E. coli O157:H7 that isolated from sausage samples, PCR products (482 b p) were separated in a 2.0% agarose gel. Lane M: contains 100 b p DNA size markers. Lane N: negative control (non-157:H7 E. coli) and lane P: positive control (E. coliO157:H7 with stx1 genes). Lanes 1, 2, 3,4 and 5 lanes are the E. coli O157:H7*

CONCLUSION

The mean of total bacterial count for (Almargaz) sausage samples values for all regions of the study was 2.5 x 10^8 CFU/gram, and the range from (7 x 10^4 to 7.5 x 10^9CFU/g), there were no significant differences in level (P< 0.05) between the regions of study. The general mean of the most probable number of coliforms for all regions of the study was 1.4x10^3 Cell/g, and the range from (1.6 x 10^2 to 2.4 x 10^3Cell/g), there were significant differences at level (P< 0.01). The percentage of *Escherichia coli* that contaminated and isolated from the sausage samples in Tripoli meat shops, were 81%, with no significant differences at level (P< 0.01) between regions of study.

The percentage of *E. coli* O157:H7 that contaminated and isolated, from the sausage samples in Tripoli meat shops, was 48% with no significant differences at level (P< 0.05) between regions of study. PCR was used to detect and verify the existence of pathogenic Stx-encoding genes Stx1 and Stx2 in *E. coli* O157:H7. Specific primers for these genes were designed in order to detected and identify them. PCR products were later run through agarose gel Electrophoresis to verify the presence of those pathogenic genes. Comparing the length of the amplified fragments genes 513 bp Stx1 and 482 bp Stx2 with the negative control, positive control and marker, it was found out that the positive control was Stx1 and Stx2 genes of *E. coli* O157:H7. The statistical analysis of Stx results shows that there were no significant differences at level (P< 0.05) between regions of study. Stx1& Stx2 in all regions of study, which were detected means most samples of regions study were contaminated with stx- positive.

DISCLOSURE STATEMENT

Conflict of interest statement was not declared.

REFERNCES

1. Cocolin L et al., The ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods. (Appl. Environ. Microbiol, 2004), vol. 70 no. 4.
2. Alexandre, M et al., Detection of enterohemorrhagic *Escherichia coli*. (J. Vet. Med, 2001), 48: 321-33.
3. Suo B., He Y., Tu, S. I. and Shi X. A Multiplex real-time polymerase chain reaction for simultaneous detection of salmonella spp., *Escherichia coli* O157, and Listeria monocytogenes in meat products. (Foodborne Pathogen Disease2010). 7: 619-628.
4. Abomengeal W. "Microbiological Quality Study of Mergaze "(MSc dsis., Tripoli University. 2010.
5. Kuhnert P., Boerlin P. and Frey J. Target genes for virulence assessment of *Escherichia coli* isolates from

water, food and the environment. (FEMS Microbiol. 2000) Rev. 24, 107–117.

6. Bartlett. John, M. S., and Stirling D. A short history of the polymerase chain reaction. (J. Methods in Molecular Biology. 2003) 226:3-6.
7. Cheesbrough, M. Medical laboratory manual for tropical countries. 2nd. ed. (Cambridge University press. 1985) pp. 206-24. 18.
8. AOAC. Official Analytical Of Analysis. 6th Ed. Association of Official Analytical Chemists International Gaithersburg, (USA,1998) Volume 62, (15):451-465.
9. Collee, J. G. and Duguid, J. P. Practical medical microbiology. (Churchill Livingstone, Edinburgh, 1989). (13th ed).
10. Fode-Vaughan, et al., Direct PCR detection of Escherichia coli O157:H7. University of Wisconsin-Milwaukee, USA.J.(Letters in Applied Microbiology 2003).37, 239–243.
11. Karch H., and Meyer T., Single primer pair for amplifying segments of distinct shiga-like-toxin genes by PCR. (J. Clin. Microbiol. 1989). 27, 2751–275.
12. Alsahoky, M., and Waheeb, K. M. Applications in design and experiments analysis. Beat Alhekma for printing and publishing. (University of Baghdad, Iraq 1990). Pp. 74-78.
13. Libyan National Center for Standardization and Metrology (LNCSM) ,last modified May 31,2012.
14. Saudi Arabia standard no 1261 for sausage (1998), last modified 2016.
15. Bell, C., and Kyriakides, A. *E. coli*. A practical approach to the organism and its control in foods. First ed. Blackie Academic and Professional, (London. 1998), pp. 1-14.
16. Alexandre, M et al., Detection of enterohemorrhagic *Escherichia coli*. (J. Vet. Med, 2001), 48: 321-33.
17. Elshrek Y. et al. Microbiological studies of spiced beef burgers in Tripoli city, Libya. (Eastern Mediterranean Health J, 2008). 14(1):172-178.