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EFFECT OF THERMAL PROCESSING
ON COLIFORMS IN MINCED BEEF AND THEIR
PUBLIC HEALTH SIGNIFICANCE
IN PORT-SAID CITY

(With 4 Tables and One Figure)

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تأثير المعاملات الحرارية علي ميكروبات الكولي فورم المتواجدة في اللحم
البقري المفروم وعلاقتها بالصحة العامة في مدينة بورسعيد

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في دراسة لتحديد مدي تأثير المعاملات الحرارية علي ميكروبات الكولي فورم المتواجدة في اللحم البقري المفروم تم فحص خمسون عينة صالحة ظاهريا والتي تم جمعها عشوائيا من محلات الجزارة بمدينة بورسعيد بهدف عد وعزل وتصنيف ميكروبات الكولي فورم المتواجدة في هذه العينات قبل و بعد المعاملات الحرارية بالاضافة لمناقشة اثر هذه الميكروبات علي الصحة العامة. وأظهرت النتائج ان نسبة العينات الايجابية لميكروبات الكولي فورم الكلية ، الكولي فورم البرازية و ميكروب الاشريشيا كولاي كانت ١٠٠٪ (٥٠)، ١٠٠ ٪ (٥٠) و ٦٦ ٪ (٣٣) في اللحم البقري المفروم الطازج علي التوالي بينما كانت ٨٢٪ (٤١)، ٧٤ ٪ (٣٧) و ٥٠ ٪ (٢٥) في العينات المشوية و ٦٤ ٪ (٣٢)، ٦٠ ٪ (٣٠) و ٣٤ ٪ (١٧) في العينات المقلية علي التوالي. وكان متوسط العدد الكلي لميكروبات الكولي فورم الكلية 4.6×10^3 ، 1.5×10^3 و 5.8×10^2 خلية/جرام ومتوسط العدد الكلي لميكروبات الكولي فورم البرازية كان 1.1×10^3 ، 5.1×10^2 و 2.6×10^2 خلية/ جرام بينما كان متوسط العدد الكلي لميكروب الاشريشيا كولاي 5.8×10^2 ، 2.1×10^2 و 4.8×10^1 خلية/ جرام وذلك في عينات اللحم البقري المفروم الطازج و المشوي و المقلي على التوالي. وتم عد و تصنيف العترات المعزولة فوجد أن عدد عترات ميكروبات الكولي فورم الكلية ٨٨، ٥٤ و ٣٢ بينما كانت عترات ميكروبات الكولي فورم البرازية ٦٣، ٤٤ و ٣١ وكان عدد عترات ميكروب الاشريشيا كولاي ٣٣، ٢٥ و ١٧ وذلك في عينات اللحم البقري المفروم الطازج والمشوي والمقلي على التوالي. وبتصنيف الانواع المعزولة من العينات موضع الدراسة وجد ان العترات المعزولة كانت انواع من الاشريشيا كولاي، انتيروبكتر كلواكا، انتيروبكتر ايروجينز، ستروباكتر فرونداي، ستروباكتر كوزيراي، سيريتيا مارسيسينز. وتم مناقشة تأثير المعاملات الحرارية علي ميكروبات الكولي فورم المتواجدة في عينات اللحم البقري المفروم موضع الدراسة.

SUMMARY

Fifty samples of apparently healthy minced beef were randomly purchased from butcher's shops of Port-Said city. The samples were examined for enumeration, isolation and identification of total coliforms, fecal coliforms and *Escherichia coli* before and after thermal treatment. The incidence of positive fresh minced beef samples for total coliform, fecal coliform and *Escherichia coli* were 100% (50), 100% (50) and 66% (33), while that of roasting samples were 82% (41), 74% (37) and 50% (25) but the incidence of pan frying minced beef samples were 64% (32), 60% (30) and 34% (17) respectively. The mean values of the total coliform counts were 4.6×10^3, 1.5×10^3 and 5.8×10^2 MPN/g and that of fecal coliform counts were 1.1×10^3, 5.1×10^2 and 2.6×10^2 MPN/g in fresh, roasting and pan frying minced beef respectively. Meanwhile, the mean values of the *Escherichia coli* counts were 5.8×10^2, 2.1×10^2 and 4.8×10^1 MPN/g in fresh, roasting and pan frying minced beef respectively. The numbers of total coliform isolates were 88, 54 and 32 and that of fecal coliform were 63, 44 and 31 and the number of *Escherichia coli* isolates were 33, 25 and 17 for fresh,

roasting and pan frying minced beef samples respectively. The bacterial isolates in the examined samples were identified as *Escherichia coli, Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, Citrobacter koseri and Serratia marcescens*. The effect of thermal processing on total and fecal coliforms and *Escherichia coli* in the examined samples were discussed.

Key words: *Meat products, minced beef, coliforms, thermal processing*

INTRODUCTION

Minced beef is the largest selling and popular beef items in most markets. Besides to their palatability it has very high value protein and valuable quantities of B vitamins (Kalalou *et al.,* 2004).

Minced meat provides a favorable environment for the growth of bacteria which normally found on the meat surface. These bacteria were distributed throughout the entire product during grinding and mixing processes used in fabrication of minced meat. Also minced meat may be subjected to the contamination directly by coliforms group from the faecal contamination which may be occur during slaughtering, evisceration and transportation (Emswiler *et al.,* 1976; Hamdy *et al.,* 1989; Rice *et al.,* 1997).

Coliform is a group of bacteria inhabit the intestinal tract of worm blooded animals including man. Some species of them are opportunistic pathogens responsible for a wide range of infections while many other species of them are normally free living saprophytic. These bacteria are Gram-negative, aerobic and facultatively anaerobic bacteria, non spore forming and rod shaped, motile by flagella except *Klebsiella* (non motile), capable of fermenting lactose with the production of acid and gas includes *Escherichia coli* and species from the genera *Escherichia, Klebsiella, Enterobacter, Serratia and Citrobacter* (Greenberg and Hunt, 1985; FAO, 1992; Hitchins *et al.,* 1998; FDA 2002).

Due to the heating process was not enough to ensure the safe eating of minced meat (Bayhan *et al.,* 1990), so a lack of good sanitary practices in the production of minced meat may results in a meat of less keeping quality, increase the possibility of spoilage and causing a major health problem (Emswiler *et al.,* 1976; Lochowich, 1978).

The health problem of coliform group depends upon the production of different toxin and colonization factors. The enterotoxigenic strains of *Klebsiella, Escherichia, Enterobacter,*

Serratia and *Citrobacter* have been isolated from infants and children with acute gastroenteritis. These toxins are heat labile and heat stable types and have other properties in common with *Escherichia coli* toxins. The endotoxins of most coliforms show a bacteraemia in human characterized by fever, vital organs hypoperfusion, depletion of complement, hypotension, irreversible shock and death. Coliforms rarely cause extra-intestinal diseases as bacteraemia and meningitis unless host defense are compromised (Guentzel, 1982; APHA, 1984).

The most virulent and major enteric pathogenic group particularly in developing countries was Enterovirulent *Escherichia coli* group (EEC) which implicated in food born illness and includes enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *Escherichia coli* (EHEC), enteroinvasive *Escherichia coli* (EIEC) (Hitchins *et al.,* 1998; FDA, 2002). These groups have been incriminated in many cases of food borne disease outbreaks, travelers' diarrhea, infantile diarrhea and colibacillosis in adults. There are two forms of the disease which differ clinically from each other to some degree. The first form caused by enteropathogenic strain, is characterized by excessive loss of fluid from profuse diarrhea (Cholera like syndrome) (Pickering, 1986; Nataro and Kaper, 1998). The second form tend to reflect toxins, heat stable toxin (ST) or heat labile toxin (LT) leading to food poisoning, possible accompanied by nausea, fever, abdominal cramps, vomiting, watery or bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenic purpura (Karmali, 1989; Reed, 1994; Acheson *et al.,* 1996; Ward *et al.,* 1997).

Thus the main purpose of this work was undertaken to evaluate the effect of thermal processing on coliforms in minced beef besides the study of their public health significance in Port-Said city.

MATERIALS and METHODS

1: Samples collection:

A total of 50 apparently healthy samples of minced beef were randomly collected from butcher's shops from Port-Said city. Each individual sample (250 g) was placed separately into sealed sterile plastic bag, thoroughly identified and delivered to the laboratory in a refrigerated container at 4°C. All specimens were prepared, treated and examined within 4 hours of collection. The samples were divided into 2 parts; the 1st part weight 50 g directed to counting, isolation and identification of coliforms and *Escherichia coli.* The 2nd part (200 g) was

thermally treated according to the approved processing technique before the bacteriological examination.

2: Bacteriological examination:

2-1: Preparation of the samples:

A representative 50 g sample of minced beef (fresh or thermally treated) were taken aseptically and homogenized in 450 ml bufferfield's phosphate buffer and blend for 2 min. at 10000-12000 rpm. Then tenfold serial dilution was prepared using bufferfield's phosphate buffer till dilution 10^5 according to FAO (1992).

2-2: Presumptive test for coliforms:

Three tubes each containing 10 ml of sterile lauryl tryptose broth (LT) with Durham's tubes, were inoculated with 1 ml from each of the original dilution. Inoculated tubes were thoroughly mixed before being incubated in a thermostatically controlled incubator at 35°C for 48 hours. The inspection was done after 24 and 48 hours incubation for positive gas production (FAO, 1992).

2-3: Probable number of total coliforms:

Gently agitate each positive LT tube and transfer loopful of suspension to tube of brilliant green bile broth 2% (BGB) containing inverted Durham's tube. All broth tubes were incubated at 35°C for 48 ± 2 hr and examined for gas production. Enumeration of total coliforms was applied by using table of most probable number (MPN) based on combination of confirmed gassing LT tubes for 3 consecutive dilutions.

2-4: Isolation of total coliforms:

Streak loopful of suspension from gassing LT tube to xylose lysine desoxycholate agar (XLD) and incubate at 35°C for 18-24 hr according to FAO (1992).

2-5: Probable number of fecal coliforms:

Gently agitate each gassing LT tube and transfer loopful of suspension to tube of EC medium containing inverted Durham's tube. All broth tubes were incubated at $45.5 \pm 0.2^\circ$C for 48 ± 2 hr. The tubes were examined at 24 hr for positive gas production and re-incubate the negative tube for additional 24 hr and examined 2nd time for gas production. Enumeration of fecal coliforms was applied by using table of most probable number (MPN) based on proportion of confirmed gassing EC medium tubes for 3 consecutive dilutions.

2-6: Isolation of fecal coliforms:

Streak loopful of suspension from gassing EC medium tubes to xylose lysine desoxycholate agar (XLD) and incubate at 35°C for 18-24 hr according to FAO (1992).

2-7: Enumeration of *Escherichia coli*:

Streak loopful of suspension from gassing EC medium tubes to L-EMB (Levine's Eosin-Methylene Blue) agar plate and incubate at 35°C for 18-24 hr. Then examined the plate for the typical *Escherichia coli* (dark center with or without metallic sheen). Enumeration of *Escherichia coli* were applied by using table of MPN based on proportion of EC medium tubes in 3 consecutive dilutions which shown to contain *Escherichia coli* according to FAO (1992).

2-8: Biochemical identification of the isolates:

Pick three typical colonies from each agar plate and transfer to plate count agar (PCA) slants tube and incubate at 35°C for 18-24 hr according to FAO (1992). All PCA slants were directed to morphological and biochemical identification according to Brenner (1984); Holt *et al.* (1994) and Farmer (1995).

3: Experimental thermal processing:

The 2nd part (200 g) was divided into 2 parts (100 g for each part), one part for the conventional roasting technique and the other for pan frying technique.

4- Statistical methods

Minimum, maximum, mean, standard deviation and standard error of mean and frequency distribution were used to describe data. T-test was used to evaluate the significant effect of the different methods of thermal processing on the total and fecal coliforms and *Escherichia coli*. P value was considered significant if less than 0.05 and 0.01 at 95% and 99% respectively. These tests were analyzed using the Statistical Package for Social Scientists (SPSS) for windows 12.0 (SPSS Inc., Chicago, IL, and USA).

RESULTS

Table 1: Statistical analytical results of the total coliform, fecal coliform and *Escherichia coli* counts (MPN/g) recovered from fresh and thermal processed minced beef.

			Total coliform in minced beef			Fecal coliform in minced beef			*Escherichia coli* in minced beef		
			Fresh	Roasting	Pan frying	Fresh	Roasting	Pan frying	Fresh	Roasting	Pan frying
Samples	Total	No.	50	50	50	50	50	50	50	50	50
		%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	(ND)	No.	0.00	9	18	0.00	13	20	17	25	33
		%	0.00	18.00	36.00	0.00	26.00	40.00	34.00	50.00	66.00
	(D)	No.	50	41	32	50	37	30	33	25	17
		%	100	82.00	64.00	100	74.00	60.00	66.00	50.00	34.00

Statistic for the counts of (D) samples										
	Min.	4.0×10^1	3.0×10^1	3.0×10^1	3.0×10^1	3.0×10^1	3.0×10^1	4.0×10^1	3.0×10^1	3.0×10^1
	Max.	4.6×10^4	4.6×10^3	2.4×10^3	4.6×10^3	1.5×10^3	7.5×10^2	2.4×10^3	1.1×10^3	1.1×10^2
	Mean	4.6×10^3	1.5×10^3	5.8×10^2	1.1×10^3	5.1×10^2	2.6×10^2	5.8×10^2	2.1×10^2	4.8×10^1
	S.E.	1.1×10^3	2.7×10^2	1.0×10^2	1.8×10^2	7.9×10^1	4.2×10^1	9.8×10^1	5.0×10^1	0.6×10^1
	S.D.	7.4×10^3	1.8×10^3	5.9×10^2	1.3×10^3	4.8×10^2	2.3×10^2	5.7×10^2	2.5×10^2	2.7×10^1

ND= Non-Detectable (<30).   D= Detectable (>30).   MPN = Most Probable Number
Min. = Minimum.   Max. = Maximum.   SE = Standard Error   SD = Standard Deviation.

Table 2: Frequency distribution of the examined fresh and thermal processed minced beef based on their total coliform, fecal coliform and *Escherichia coli* counts (n=50 of each).

Count range	Total coliform in minced beef						Fecal coliform in minced beef						*Escherichia coli* in minced beef					
	Fresh		Roasting		Pan frying		Fresh		Roasting		Pan frying		Fresh		Roasting		Pan frying	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<30	0	0	9	18	18	36	0	0	13	26	20	40	17	34	25	50	33	66
30 - <10²	6	12	8	16	7	14	9	18	9	18	10	20	2	4	10	20	15	30
10² - <10³	16	32	16	32	18	36	18	36	24	48	20	40	26	52	14	28	2	4
10³ - <10⁴	15	30	17	34	7	14	23	46	4	8	0	0	5	10	1	2	0	0
≥10⁴	13	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100

< 30 = Non detectable level.

Table 3: Correlation between total coliform, fecal coliform and *Escherichia coli* counts based on the examined fresh and thermal processed minced beef (n=50 of each).
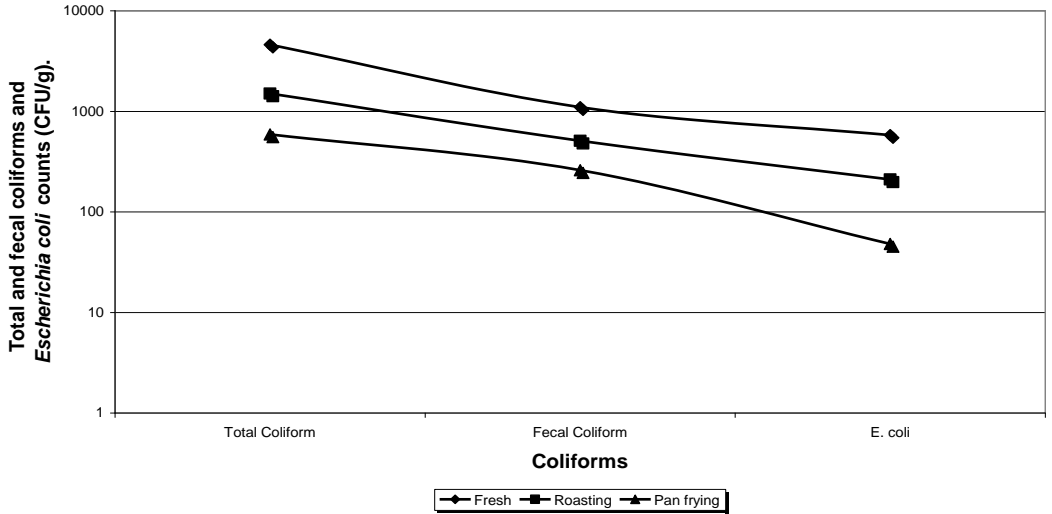
				Minced beef								
				Fresh			Roasting			Pan frying		
				Coliform		*E. coli*	Coliform		*E. coli*	Coliform		*E. coli*
				Total	Fecal		Total	Fecal		Total	Fecal	
Minced meat	Fresh	Coliform	Total	------	------	------	0.044 (*)	------	------	0.001 (**)	------	------
			Fecal	------	------	------	------	0.043 (*)	------	------	0.001 (**)	------
		E. coli		------	------	------	------	------	0.040 (*)	------	------	0.000 (**)
	Roasting	Coliform	Total	0.044 (*)	------	------	------	------	------	0.031 (*)	------	------
			Fecal	------	0.043 (*)	------	------	------	------	------	0.039 (*)	------
		E. coli		------	------	0.040 (*)	------	------	------	------	------	0.044 (*)
	Pan frying	Coliform	Total	0.001 (**)	------	------	0.031 (*)	------	------	------	------	------
			Fecal	------	0.001 (**)	------	------	0.039 (*)	------	------	------	------
		E. coli		------	------	0.000 (**)	------	------	0.044 (*)	------	------	------

(**) = Highly significant correlation at <0.01 (99% confidence) by using t-test (Paired system test).
(*) = Significant correlation at <0.05 (95% confidence) by using t-test (Paired system test).
E. coli = Escherichia coli

Fig. (1): Effect of thermal processing on total coliform, fecal coliform and *Escherichia coli* counts (MPN/g) recovered from minced beef.

Table 4: Incidence of coliforms isolates recovered from fresh and thermal processed minced beef.

Incidence of coliforms organisms			Minced beef								
			Total coliform			Fecal coliform			*Escherichia coli*		
			Fresh	Roasting	Pan frying	Fresh	Roasting	Pan frying	Fresh	Roasting	Pan frying
Examined samples		No.	50	50	50	50	50	50	50	50	50
		%	100	100	100	100	100	100	100	100	100
Positive samples		No.	50	41	32	50	37	30	33	25	17
		%	100	82.00	64.00	100	74.00	60.00	66.00	50.00	34.00
Total isolates		No.	88	54	32	63	44	31	33	25	17
		%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Isolates type	1-*Escherichia coli*	No.	33	25	17	33	25	17	33	25	17
		%	37.5	46.30	53.13	52.38	56.82	54.84	100.00	100.00	100.00
	2-*Enterobacter cloacae*	No.	19	11	9	19	11	9	------	------	------
		%	21.59	20.37	28.13	30.16	25.00	29.03	------	------	------
	3-*Enterobacter aerogenes*	No.	11	8	5	11	8	5	------	------	------
		%	12.5	14.81	15.63	17.46	18.18	16.13	------	------	------
	4-*Citrobacter freundii*	No.	12	5	1	------	------	------	------	------	------
		%	13.64	9.26	3.13	------	------	------	------	------	------
	5-*Citrobacter koseri*	No.	8	3	0	------	------	------	------	------	------
		%	9.09	5.56	0.00	------	------	------	------	------	------
	6-*Serratia marcescens*	No.	5	2	0	------	------	------	------	------	------
		%	5.68	3.70	0.00	------	------	------	------	------	------

DISCUSSION

Members of the coliform groups are referred as general indicator microorganisms to measure the potential presence of enteric pathogens (for example *Escherichia coli*) in foods, besides the measuring of fecal contamination of the food products and the sanitary condition in the food-processing environment (Greenberg and Hunt, 1985; APHA, 1992; FAO, 1992). The possibility of food health problem by coliforms depending upon the number, types and the virulent of these pathogens whereas, the higher the number, the greater the possibility of pathogenic organisms being present (FAO, 1992).

The given results in Table (1) revealed that the incidences of positive fresh minced beef samples for total and fecal coliforms and *Escherichia coli* were 100% (50), 100% (50) and 66% (33) respectively. The incidence of positive samples for coliforms was higher than the results recorded by (Kaloianov *et al.,* 1987; Willshaw *et al.,* 1993; Heredia *et al.,* 2001; Tanios *et al.,* 2002; Cohen *et al.,* 2006) but that of *Escherichia coli* was lower than those recorded by (Heredia *et al.,* 2001). The high incidence of positive samples may be attributed to the unsanitary conditions during the production and manufactured of minced beef (Wilson, 2001). The mean values of the total and fecal coliforms and *Escherichia coli* counts in fresh minced meat were 4.6×10^3, 1.1×10^3 and 5.8×10^2 (MPN/g) in the examined fresh minced beef respectively (Table 1). These results agreed with the results recorded by (Rogers and McCleskey, 1957; Küpeli, 1996) but lower than the coliforms results recorded by (Yilmaz *et al.,* 2002a). On the other hand our results were higher than the results recorded by (Kaloianov *et al.,* 1987; Yilmaz *et al.,* 2002; Firstenberg *et al.,* 2004). The high figures of our results may be attributed to contamination of meat during slaughtering, evisceration and transportation (Hamdy *et al.,* 1989), bad microbiological quality and heavy load coliform of trimming and cuts of beef (Emswiler *et al.,* 1976; Bayhan *et al.,* 1990), unsanitary conditions during the manufacture (APHA 1992; Wilson, 2001) and after manufacture as bad handling and packaging of minced beef (Yilmaz *et al.,* 2005; Cohen *et al.,* 2006), besides long chilling storage or holding of the minced beef at an inappropriate temperature (Kandeepan and Biswas, 2005; Yilmaz *et al.,* 2005). Meanwhile the higher counts of fecal coliform and *Escherichia coli* may be attributed to recent fecal contamination (Caplenas and Kanarek, 1984; Greenberg and Hunt, 1985; APHA, 1992; FDA, 2002) from contaminated or infected cattle (Rice *et al.,* 1997) with the upper mention causes.

The presented results in Table (2) showed that the frequency distribution of the total and fecal coliforms and *Escherichia coli* within the range 30 - $<10^4$ were 74% (37), 100% (50) and 66% (33) respectively but 26% (13) of the fresh minced beef showed the total coliforms with the range $\geq 10^4$ MPN/g and 34% (17) of the examined fresh minced beef had non detectable levels (< 30 MPN/g) for *Escherichia coli.* These results were higher than the results recoded by (Surkiewicz *et al.,* 1975; Firstenberg *et al.,* 2004). Higher results may be attributed to heavy load and high initial counts of coliforms and *Escherichia coli* (Bayhan *et al.,* 1990) and unsanitary conditions during the manufacture of minced beef with long chilling storage (Wilson, 2001).

Regarding to the effect of thermal processing on total and fecal coliforms and *Escherichia coli* presented in Table (1) It is evident that the incidences of positive roasting minced beef samples for total and fecal coliforms and *Escherichia coli* were 82% (41), 74% (37) and 50% (25) respectively while in case of the examined pan frying minced beef the incidence were decreased to 64% (32), 30% (30) and 34% (17) respectively. On the other hand the mean values of the total and fecal coliforms and *Escherichia coli* counts were 1.5 X 10^3, 5.1 X 10^2 and 2.1 X 10^2 MPN/g for roasting minced beef respectively while that of pan frying minced beef were reduced to 5.8 X 10^2, 2.6 X 10^2 and 4.8 X 10^1 MPN/g under effect of roasting technique respectively.

Also the thermal processing effect presented in Table (2) revealed that the percentage of the examined samples showed non detectable levels (< 30 MPN/g) for total coliform, fecal coliform and *Escherichia coli* under effect of roasting technique were 18% (9), 26% (13) and 50% (25) respectively, while under the effect of pan frying technique the percentage of the examined samples showed non detectable levels (< 30 MPN/g) for total coliform, fecal coliform and *Escherichia coli* were increased to 36% (18), 40% (20) and 66% (33) respectively. On the other hand most of the examined roasting minced beef samples [82% (41), 74% (37) and 50% (25)] had a rang of 30 - $<10^4$ MPN/g for total and fecal coliforms and *Escherichia coli* while that of pan frying minced beef samples [50% (25), 60% (30) and 34% (17)] were reduced to 30 - $<10^3$ MPN/g respectively. These results with Fig (1) showed that the heat processing either roasting or pan frying technique reduce the total and fecal coliforms and *Escherichia coli* counts to a different levels. This reduction effect agrees with the result recorded by (Delchev *et al.,* 1978; Jay, 1978; Zahra *et al.,* 1985;

Desmarchelier and Grau, 1997; Yilmaz *et al.,* 2005) but this reduction was impossible to eliminate all forms of coliforms (Jay, 1978).

Statistically by using t-test, showed a highly significant relationship between the counts of each of total and fecal coliforms and *Escherichia coli* recovered from fresh minced beef samples with that of the pan frying samples but roasting technique showed a significant relationship between them (Table 3). On the other hand the relationship between pan frying and roasting technique showed a significant relationship. This mean pan frying technique was more effective and more efficient than roasting technique in reduction effect (Yilmaz *et al.,* 2002).

The obtained results in Table (4) showed that the number of total and fecal coliforms and *Escherichia coli* isolates of fresh minced beef were 88, 63 and 33 isolates respectively. In case of roasting technique, these isolates were reduced to 54, 44 and 25 for total and fecal coliforms and *Escherichia coli* respectively while in case of pan frying minced beef samples the isolates were more reduced to 32, 31 and 17 isolates respectively. The coliforms organisms recovered from the examined samples were identified as *Escherichia coli, Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, Citrobacter koseri* and *Serratia marcescens.* The main and important isolate found in fresh and treated samples was *Escherichia coli.* On the other hand *Citrobacter freundii, Citrobacter koseri* and *Serratia marcescens* found only in total coliform and this may be attributed to that these group of microorganisms can not tolerate high temperature but fecal coliform and *Escherichia coli* can tolerate and considered thermotolerant organisms (Caplenas and Kanarek, 1984). The reduction in the number of isolates was more in pan frying than in roasting technique and this agrees with the result recorded by (Zahra *et al.,* 1985) who showed that coliforms were easily killed (Jay, 1978) and decreased markedly after heat treatment but heating process was not enough to ensure the safe eating quality (Bayhan *et al.,* 1990) and impossible to eliminates all forms of coliforms (Jay, 1978). The variation between our result and the other results recorded by (Yilmaz *et al.,* 2005) may attributed to the variation in season and locality (Cohen *et al.,* 2006) and the high initial counts of coliforms found in minced beef (Zahra *et al.,* 1985).

The safety of minced meat associated with the keeping quality of meat cuts, sanitary condition during handling, fabrication, packaging, equipment and utensils used and time/temperature of storage (Emswiler *et al.,* 1976; APHA, 1992). The endotoxins of coliform groups, heat labile and heat stable types specially that of *Escherichia coli* toxins

(Pickering, 1986; Hitchins *et al.,* 1998) with the undercooked or improperly cooked minced beef and cross and post cooking contamination by these microorganisms (Guentzel, 1982; Ward *et al.,* 1997; Cohen *et al.,* 2006) results in a major health problem to the consumers. Consequently the presence of large number of coliforms in foods is highly undesirable but it would be almost impossible to eliminate all forms of coliform (Jay, 1978). In conclusion, to increase the shelf life and the safety of minced meat and decrease their coliforms loads and prevention the infections by these microorganisms to the consumers, a groups of measurement should be attempt such as good and strictly hygienic measurement pre and during slaughtering, evisceration and transportation with the avoidances of the contamination of meat from other infected or contaminated animals. Also personal hygiene and removal the source of pollution as fecal contamination and sewage water with good sanitary condition during handling, grinding and mixing processes besides the equipment and utensils used in fabrication produce a minced beef of good keeping quality. The refrigeration (below 4°C) and good time/temperature storage with sufficiently cooking of minced meat and prevent the post cooking cross contamination and prohibiting people who are ill from working in food operations and strictly prevention the consumption of insufficient cooked minced meat specially in compromised hosts reduce the consumers health problem.

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