PUBLIC HEALTH HAZARDS OF SOME BACTERIAL PATHOGENS ASSOCIATED WITH MEAT AND STUDYING THE MOST EFFECTIVE METHODS OF COOKING ON THEIR DESTRUCTION

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ABSTRACT

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The present investigation aimed to evaluate the prevalence of some microorganisms of public health importance (*Escherichia coli, Salmonella, Staphylococcus aureus* and *Listeria monocytogenes*) in fresh beef and study the growth and survival behavior of these pathogens when subjected to different types of cooking. Our findings showed that out of 100 fresh beef samples analyzed for microbial quality 90 (90%) were contaminated with different kinds of microorganisms namely *E.coli* (48%), *Salmonella* spp. (18%), *Staphylococcous* spp. (16%) and *Listeria* spp. (8%). The most *E.coli* isolated was *E.coli* O_{111}: H_4 (43.75%) followed by *E.coli* O_{126}:H_{11} (27.08%), *E.coli* O_{128}:H_{11} (22.92%) and *E.coli* O_{157}:H_7 (6.25%). Three species of *Salmonella* were isolated. The highest prevalence of them was *S.typhimurium* (44.4%), while *S.enetertiidis* and *S. anatum* rank as a second (27.8%) for each. *Staphylococcous* spp was isolated from (16%) of tested samples whereas (75%) of them recorded as *Staph. aureus* and (18.75%) recorded as *Staph. epidermis*, while (6.25%) recorded as *Staph. gallinarum*, all of them were coagulase–positive. Fifty percent of isolated *Listeria* spp. were characterized as *L. monocytogenes*, (25%) as *L.innocua* while the presence of *L.welshimeri* and *L.invanovii* was (12.5%) for each. Thermal inactivation of inoculated *E.coli* O_{157}:H_7, *S.enetertiidis*, *Staph. aureus* and *L.monocytogenes* inoculated in fresh beef were evaluated by boiling, frying and roasting treatments. At internal temperature of 65°C using boiling, the log cycles reduction were 1.3, 2.1, 2.2 and 2.2 for aforementioned microorganisms, respectively. By frying the reduction values were 1.5, 2.1, 2.3 and 2.1, respectively. The corresponding values by roasting were 2.6, 2, 2.3 and 1.4, respectively. *E.coli* O_{157}:H_7 couldn't be detected at internal temperature of 80, 83 and 74°C by boiling, frying and roasting, respectively. Both *S. enetertiidis* and *Staph aureus* couldn't be detected at internal temperature of 80, 80 ad 71°C by the treatments, respectively, while *L. monocytogenes* couldn't be detected at internal temperature of 80, 80 and 78°C respectively. The sensitivity of the isolated pathogens to heat inactivation was measured by assessing the *D*-values. These values were calculated from the survival curves. For *E.coli* O_{157}:H_7, they were 1.1, 1.1 and 1.2 minutes by boiling, frying and roasting treatments, respectively. Those recorded for *S. enteritidis* were 1.1, 1.0 and 1.2 minutes, respectively. In case of *Staph aureus* they were 1.1, 0.9 and 1.1 minutes, respectively while in case of *L. monocytogenes* the recorded values were 1.1, 0.8 and 1.1, minutes, respectively. Cooking fresh beef by boiling resulted in cooking weight loss (CWL) ranged from 8.1 to 17.47% according to time of exposure. By roasting the CWL ranged from 4.77 to 23.5% while by frying it was 15 to 23.53%. The increase in the pH value was directly proportional to time of exposure to boiling but not clearly demarked by other cooking methods. This study cleared that fresh beef from fresh beef shops at Assiut City, Egypt can acts as a source of major human pathogens. For safe consumption, such meat must cooked to internal temperature of 83°C when using traditional cooking methods. The *D*-values recorded in this study may be helpful guide for thermal processing of meat.

Key words: *Bacterial, Pathogens, Beef, Heat, Inactivation, D-value, Quality, Changes.*

INTRODUCTION

Meat is a major constituent of the human diet. It is an essential food item, (Rao *et al.,* 2009) and one of the main sources of protein, vitamins, minerals, lipids and savory sensation, (Zweifer *et al.,* 2008). Most meat has high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth, (Rao *et al.,* 2009). Meat is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically. Microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites, (Jackson *et al.,* 2001). Meat is a good material for bacterial growth; its quality depends on the initial bacterial contamination. This contamination causes meat deterioration, lowers quality and sometimes illness may be caused by bacterial pathogens or their toxins through meat and meat products.

In fact, tissue from healthy animal is sterile, (Lawrie, 1984) but the immune system are destroyed during the slaughter process, (Romans *et al.,* 2001). However, contamination of meat occur during slaughtering, preparation of carcasses, (Huffman, 2002) or from feces, soil, and water (Jay,1996), where microorganisms came chiefly from the exterior of the animal and its intestinal tract, and that more added from knives, clothes, air, carts and equipment in general, (Lawrie, 1984). Retail cut could also result in greater microbial load because of the large amount of exposed surface area, (Forest *et al*., 1985).

Food-borne pathogens of concern in beef carcass decontamination are *E. coli* O_{157}:H_7, *Salmonellae* and *Stapylococcus aureus*, (Huffman, 2002). Contaminated raw meat is one of the main sources of food-borne illnesses (Bhandare *et al.,* 2007 and Podpečan *et al.,* 2007) and death in developing countries costing billions of dollars in medical care (Fratamico *et al.,* 2005) and record of 3900 deaths each year, (Buzby *et al.,* 1997). Changes in eating habits, mass catering complex, lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors of illness and death, (Hedberg *et al.,* 1992).

The symptoms of food poisoning may vary depending on the type of bacteria causing the illness. Symptoms include nausea, stomach cramps, vomiting, diarrhea, fever and headache. Some food-borne pathogens cause other symptoms FAO (1999), for instance, pathogenic *Listeria* cause listeriosis In pregnant women and meningitis in Immuno-depress individuals while Salmonellosis is caused by *Salmonellae*, (Estes, 2003). One the other hand, *E. coli* O_{157}:H_7 attracted attention not only because food-borne transmission is more common, but also because it can cause life-threatening conditions such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), (Buchanan and Doyle, 1997)

The most used meat preservation step is thermal processing which is the application of heat to food in order to destroy pathogenic microorganisms below the concentration of their ability to produce disease, (Richardson, 2002). Heat treatments is influenced by many factors, some of them are due to inherent resistance of microorganisms (Brown, 1994), which include differences among species, strains, spores and vegetative cells of bacteria (Tomlins, 1976), while others are due to environmental influences (Brown, 1994) such as the composition of the heating menstruum (amount of carbohydrates, proteins, lipids, solutes.), water activity, pH, added preservatives and method of heating, (Smelt *et al.,* 1994). The optimum temperature for the multiplication of most food poisoning bacteria is between 5 - 63^{o}C, whilst, at temperatures over 70^{o}C most bacteria are killed and below 5^{o}C most food poisoning bacteria can only multiply slowly or not at all. Most cooking methods if performed properly will heat foods to over 70 ^{o}C, so applying such a temperature for a carefully calculated time period will prevent many food borne illnesses that would otherwise manifest if the raw food was eaten. The fundamental types of cooking are grilling, frying and boiling. Grilling is cooking of food using a direct dry heat, frying is the cooking of food in oil or fat while boiling is the cooking of foods in a liquid which is the common type of cooking, (EUFIC, 2010).

Although cooking, moreover, improves the hygienic quality of the food by inactivation of pathogenic microorganisms (Bognár, 1998), it also, causes a complex series of physical and chemical changes to occur. These changes vary depending on the type of food being cooked and the method used to cook it. The changes may be advantageous e.g., improving the flavour, texture and colour of the food, or they may be disadvantageous e.g., reducing the nutrient value of the food, or the generation of undesirable compounds (EUFIC, 2010).

So, it is important to select the proper cooking method and the degree of cooking which employed further effects on the number and the types of microorganisms. Since, consumption of healthy food is one of the significant factors affecting the health; such studies are extremely important and will be helpful in supervision and control of quality of meat.

The present investigation aimed to evaluate the prevalence of some microorganisms of public health importance (*Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Listeria monocytogenes*) in fresh beef and studying the growth and survival

behavior of these pathogens when subjected different types of cooking.

MATERIALS and METHODS

Collection of samples:
A total of 100 random samples (500g each) of fresh beef were collected from butcher shops with different sanitation levels in Assiut city. The samples were transferred separately and aseptically in an ice box without delay to the laboratory where they were examined.

Isolation procedures:
1- Isolation of *Staph*. *aureus*: Feingold and Martin (1982).
2- Isolation of *Salmonella spp*: According to the method recorded by APHA (1992).
3- Isolation of *Listeria monocytogenes*: Oxoid Manual (1990).
4- Isolation of *E. coli*: AOAC (1990).

In each case enrichment procedures was applied using 25g sample followed by selective plating as recommended by the corresponding reference. All isolates were identified morphologically using staining reaction (APHA, 1992) and motility test (Baron *et al.,* 1994), as well as, biochemically using catalase, coagulase, triple sugar iron (TSI) agar test (Baron *et al.,* 1994), citrate utilization, indole production, methyl red, urease, voges-Proskauer tests (Koneman *et al.,* 1992), nitrate reduction test (Cowan and Steel, 1974), sugar fermentation reaction (APHA, 1992), and Christine-Alkine- Munch- Peterson (CAMP) test (Herrera, 2001).

E .coli isolates were serologically identified according to Kok *et al.* (1996) by using rapid diagnostic *E.coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the enteropathogenic types. Serological identification of Salmonellae was carried out according to Kauffman – White scheme (Kauffman, 1974).

For further confirmation of *L. monocytogenes* isolates were inoculated into 10% aqueous stock solution of mannitol, rhammose and xylose, (Collee and Miles, 1989). While Further Identification of *Staphylococcus aureus* is done by Thermostable nuclease test "D-Nase activity", (Lachia *et al.,* 1971).

Heat- inactivation experiment:
Preparation of cultures: Murphy *et al*. (2000)

For each trial, a 24h culture was prepared individually for *E. coli* O_{157}:H_7, *Salmonella enteritidis*, *L. monocytogenes* and *Staph. aureus* by culturing in Tryptic Soya Broth and incubation at 37°C. The count for each /ml was determined by surface plating decimal dilutions on MacConkeys sorbitol gar, MacConkeys agar, Paird-parker agar and PALCAM agar (Oxoid, Basingstoke, UK), respectively. Just prior to the thermal treatment calculated broth portions were mixed to form a mixed cocktail of test strains.

Meat samples: (Patel *et al*., 2004).

Fresh boneless strip lions (longissimus muscle) were purchased from a local retailer. After removing the fat from the surface, the strip lions were sliced into 25 g portions (3 cm thickness x 2cm x 4cm) using sterile knife. The inoculation was applied by injecting 1.5 ml of the mixed strain cocktail (10^6 CFU of individual strain /g) in the center of each meat sample. The inoculated samples were placed onto a sterile tray covered with aluminum foil and then stored at 4°C for 30 minutes to permit bacterial attachment to meat and heat equilibrium.

Thermal treatment:
The target cooking internal temperature of the samples was the temperature recommended by (FAO, 2007) for intact beef (65°C, medium-rare cooking) and then for an excess of 6 minutes using three different heating methods. For heating, one single inoculated refrigerated food item was placed in boiling water (100°C) , heated oil (150°C) or heated oven (180°C) and then heated for times ranging from 0 (not heated) to 15°min.

For the boiling-inactivation test, a cooking pan (24°cm diameter) with water (2.5 L) was brought to the boil. The water was constantly heated and as the weight of the added matrices was small (25°g) compared to that of the water (2.5L), the temperature profile of the water was constant at 100°C, (de Jong *et al*., 2012). In roasting, the samples in a metal baking tray lined with aluminum foil were placed on an oven rack in the center of electric oven at 180°C for the specified time, (Jefferies, 2011). For frying, the samples were fried in a common fryer at 150°C (oil temperature) for the specified time, (Miller *et al*., 2011). Due to variations in initial temperature between trials, a standardized start time of when samples were 20°C employed to determine cooking time. The temperature of water, oil or oven was monitored to be constant along the heating times using digital thermometer (thermometer ST-131 waterproof digital).

Enumeration of survivors and analysis for quality changes:
Just after removal of the sample from the heating medium, the internal temperature of the sample was determined by inserting the actual sensor in the last two centimeters of the temperature probe (thermometer ST-131 waterproof digital) to the geometric center of the sample. The sample was then placed in sterilized plastic bag and immersed in a mixture of ice-water. Testing of survivors and

analysis for quality changes were carried out when samples cooled to room temperature.

Enumeration of survivors: Murphy *et al.* (2000)
Prior to thermal treatment, 25g sample each of inoculated and non-inoculated samples was combined with 225 mL of sterile peptone solution (0.1%) in a sterile stomacher bag and blended in a stomacher for 2 minutes. Counting of test microorganisms was carried out by spread-plating decimal dilutions on appropriate media. Cultivability of the inoculated bacteria in the meat after heat-treatment was determined by preparing sample suspensions and spread plating appropriate dilutions on appropriate media. The plates were incubated at 37°C for72h for all the test microorganisms except listeria where they were incubated for 144 h. The plates were counted each 24h until the number of colony formation unit no longer increased. Suspected colonies of the test microorganisms were confirmed by biochemical and serological methods. Average values of bacterial counts, from duplicate plate samples, were converted to log for each bacterium. In order to validate complete destruction of tested microorganisms, sample enrichment were performed on samples that contained no growth at the experimental detection limit of 1 log CFU/g. Ten ml of each sample time that produced no growth were diluted with 90 ml of Tryptic Soya Broth. Each enrichment solution was incubated at 37°C for 24 h. After incubation, the solutions were streak plated onto appropriate media and incubated at 37°C for 48h.

Analysis for quality changes: Nikmaram *et al.* (2011)

Cooking losses were determined by measuring the difference in sample's weight before cooking and then after cooking when samples cooled to room temperature.

$$\text{Cook loss \%} = \frac{\text{Weight of raw sample} - \text{Weight of cooked sample}}{\text{Weight of raw sample}} \times 100$$

The pH of meat homogenate was measured after microbial analysis using digital pH meter (Gallenhamp No.101284).

Calculation of D-values**:** Juneja *et al.* (2001)

D-values (time to inactivate 90% of the population) were calculated from the straight portion of the survival curves by plotting the log of survival counts compared with their corresponding heating times, using SPSS (2007).

RESULTS

Table 1: Incidence of bacterial pathogens in raw beef samples.

Types of microorganisms	Positive samples	
	N0.	%
***Staph.* spp**	**16**	**16**
Staph .aureus	12	75
Staph . epidermidis	3	18.75
Staph . gallinarum	1	6.25
***Salmonellae* spp.**	**18**	**18**
S. typhimurium	8	44.44
S. enteritidis	5	27.78
S. anatum	5	27.78
***Listeria* spp.**	**8**	**8**
L. monocytogenes	4	50
L. welshimeri	1	12.5
L.innocua	2	25
L.ivanovii	1	12.5
E. coli	**48**	**48**
E.coli O_{157} : H_7	3	6.25
E.coli O_{111} : H_4	21	43.75
E.coli O_{128} : H_{11}	11	22.92
E.coli O_{126} : H_{11}	13	27.08

Table 2: Effect of cooking methods on *E. coli* O_{157}: H_7 and *Salmonella entertidis*.

Treat. time (minutes)	Stats. parameters	*E. coli* O157 :H7			*Salmonella enteritidis*		
		Boiling	Frying	Roasting	Boiling	Frying	Roasting
o	Int. temp. °C	20	20	20	20	20	20
	Mean survivors log 10 CFU/g	5.80	5.80	5.80	5.3	5.3	5.3
	S E	0.00058	0.00058	0.00058	0.00058	0.00058	0.00058
	Significance	a	a	a	a	a	a
1	Int. temp. °C	39	50	34	39	50	34
	Mean survivors log 10 CFU/g	5.40	5.30	5.30	4.4	3.5	4.33
	S E	0.05774	0.05774	0.05774	0.057	0.05774	0.033
	Significance	a	a	a	a	b	a
2	Int. temp.	54	65	46	54	65	46
	Mean survivors log 10 CFU/g	5.10	4.30	4.50	4.2	3.266667	4.1
	S E	0.05774	0.05774	0.05774	0.057	0.03333	0.057
	Significance	a	b	b	a	b	a
3	Int. temp.	65	72	65	65	72	65
	Mean survivors log 10 CFU/g	4.50	3.33	3.20	3.2	3.3	3.266
	S E	0.05774	0.06667	0.05774	0.057	0.115	0.033
	Significance	a	b	b	a	a	a
4	Int. temp.	70	80	67	70	80	67
	Mean survivors log 10 CFU/g	3.30	3.10	3.13	2.5	UD	3.033
	S E	0.05774	0.05774	0.06667	0.05774		0.08819
	Significance	a	a	a	a		c
5	Int. temp.	80	83	71	80	83	71
	Mean	UD	UD	2.13	UD		UD
	S E			0.08819			
	Significance			b			
8	Int. temp.	82	86	74			
	Mean survivors log 10 CFU/g			UD			
	S E						
	Significance						

U D: Under detectable level (less than 1 log 10 CFU/g)
a, b, c: data with different litters are significantly different at $P< 0.05$

Table 3: Effect of cooking methods on *Listeria monocytogenes* and *Staph. aureus*

Duration of treatment (minutes)	Stats. parameters	*Listeria monocytogenes*			*Staph aureus*		
		Boiling	Frying	Roasting	Boiling	Frying	Roasting
o	Int. temp. °C	20	20	20	20	20	20
	Mean survivors log 10 CFU/g	5.6	5.6	5.6	5.4	5.4	5.4
	S E	0.000577	0.000577	0.000577	0.0006	0.0006	0.0006
	Significance	a	a	a	a	a	a
1	Int. temp.	39	50	34	39	50	34
	Mean survivors log 10 CFU/g	5.3	4.2	5.366667	4.4	3.866667	4.2
	S E	0.057735	0.057735	0.033333	0.0577	0.3844	0.0577
	Significance	a	b	a	a	b	a
2	Int. temp.	54	65	46	54	65	46
	Mean survivors log 10 CFU/g	4.3	3.466667	4.533333	3.5	3.1	3.4
	S E	0.057735	0.066667	0.033333	0.0577	0.0577	0.0577
	Significance	a	b	a	a	b	a
3	Int. temp.	65	72	65	65	72	65
	Mean survivors log 10 CFU/g	3.4	2.1	4.2	3.2	2.033333	3.1
	S E	0.057735	0.057735	0.11547	0.0577	0.0333	0.0577
	Significance	a	b	c	a	b	a
4	Int. temp.	70	80	67	70	80	67
	Mean survivors log 10 CFU/g	3.1	UD	3.7	2.1	UD	2.333333
	S E	0.057735		0.057735	0.0577		0.1202
	Significance	a		b	a		a
5	Int. temp.	80	83	71	80		71
	Mean	UD		3.3	UD		UD
	S E			0.057735			
	Signif			b			
8	Int. temp.	82	86	74	82	86	74
	Mean survivors log 10 CFU/g			2.1			
	S E			0.057735			
	Significance			b			
9	Int. temp.	85	90	78			
	Mean survivors log 10 CFU/g			UD			

UD: Under detectable level (less than 1 log 10 CFU/g)
a, b, c: data with different litters are significantly different at P< 0.05

Table 4: Effect of cooking methods on weight loss % and pH values.

Duration of treat. (minutes)	Stats. parameters	weight loss %			pH		
		Boiling	Frying	Roasting	Boiling	Frying	Roasting
0	Mean	0	0	0	5.1	5.1	5.1
	S E	0	0	0	0.0006	0.0006	0.0006
	Significance	a	a	a	a	a	a
1	Mean	0	15	0	5.233333	5.1	4.133333
	S E	0.0000	0.5774	0.0000	0.0333	0.0577	0.0882
	Significance	a	b	a	a	a	b
2	Mean	8.10	16.47	4.77	5.466667	4.6	4.633333
	S E	0.0577	0.0333	0.1453	0.0333	0.0577	0.0882
	Significance	a	b	c	a	b	b
3	Mean	9.00	10.83	9.83	5.7	4.4	4.2
	S E	0.5774	0.4410	0.4410	0.0577	0.0577	0.0577
	Significance	a	a	a	a	b	b
4	Mean	10.00	15.53	15.53	6.5	4.6	4.7
	S E	0.5774	0.0882	0.0882	0.0577	0.0577	0.0577
	Significance	a	b	b	a	b	b
5	Mean	14.33	21.00	21.07	6.733333	4.1	4.4
	S E	0.6009	0.5774	0.6360	0.0333	0.0577	0.0577
	Significance	a	b	b	a	b	b
8	Mean	16.20	21.83	21.83	7	4.9	4.733333
	S E	0.1528	0.9280	0.9280	0.0577	0.0577	0.0882
	Significance	a	b	b	a	b	b
9	Mean	17.47	23.53	23.50	7.1	5.1	4.9
	S E	0.3180	0.0333	0.0577	0.0577	0.0577	0.0577
	Significance	a	b	b	a	b	b

a, b, c: data with different litters are significantly different at P< 0.05

Fig. 1: The rate of heating in different cooking methods

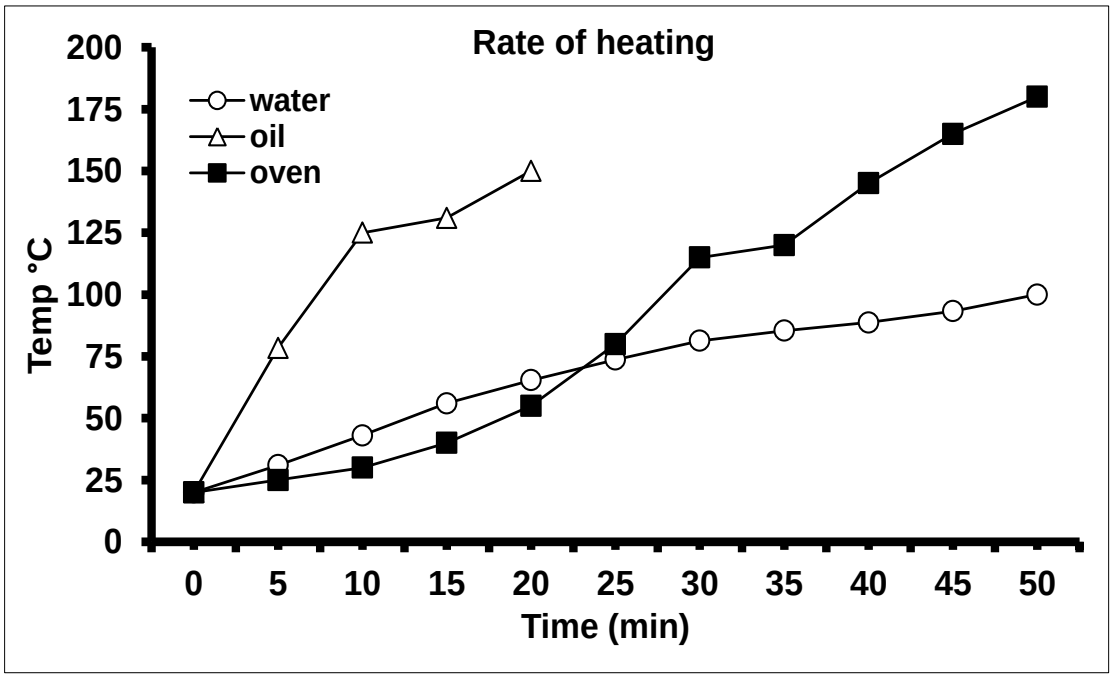


Fig. 2: Effect of cooking methods on *E. coli* O_{157}: H_7

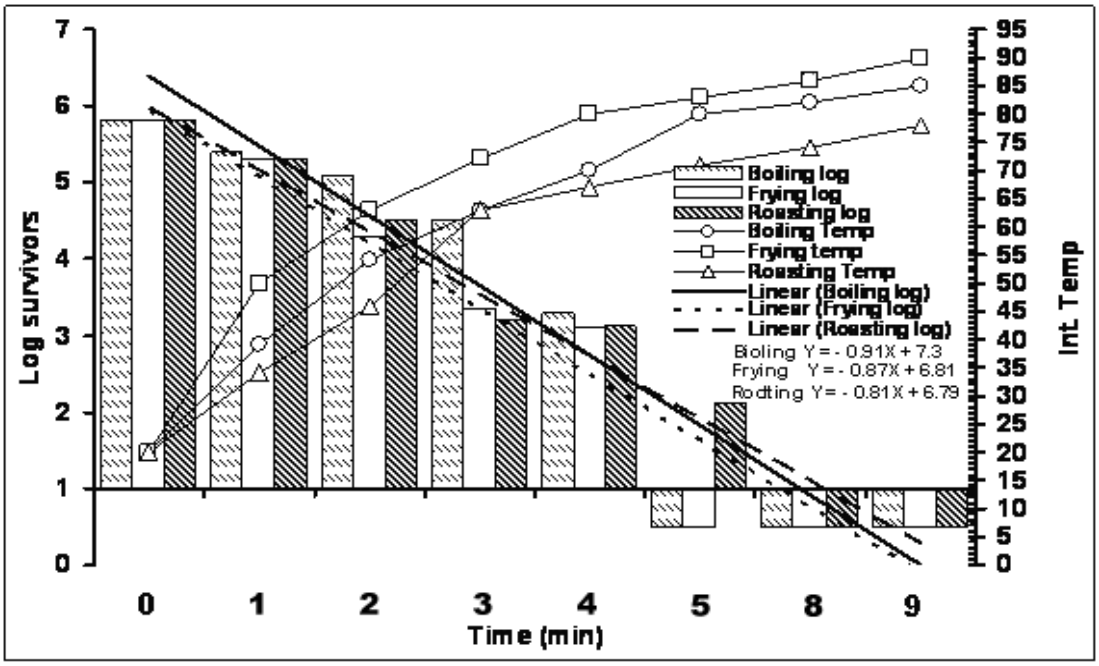


Fig. 3: Effect of cooking methods on *Salmonella enteritidis*

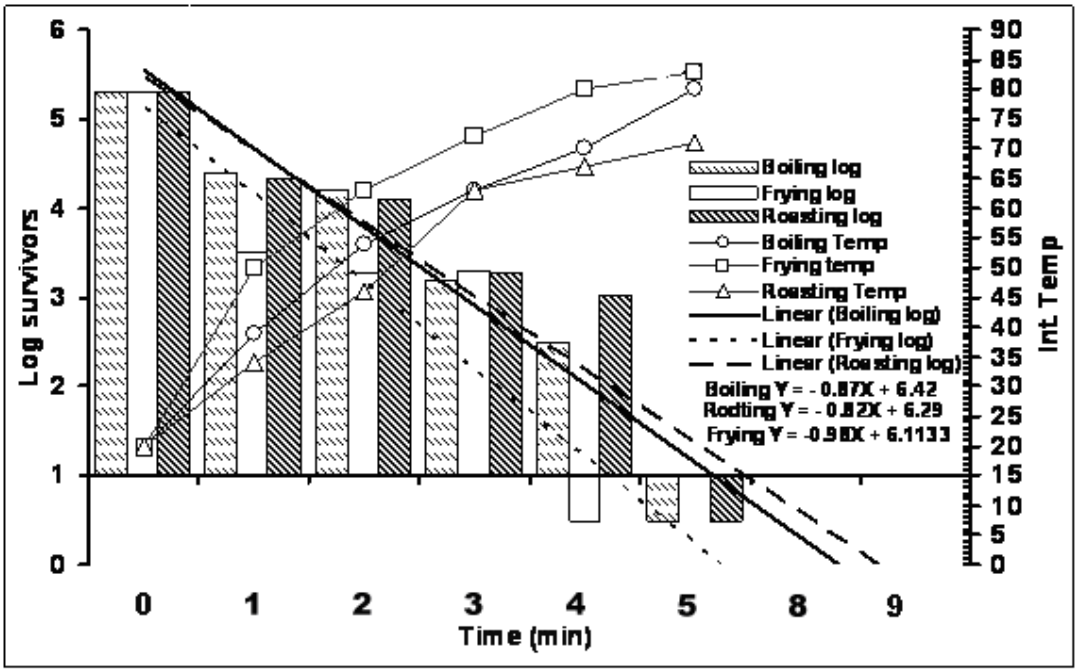


Fig. 4: Effect of cooking methods on *Staph. Aureus*

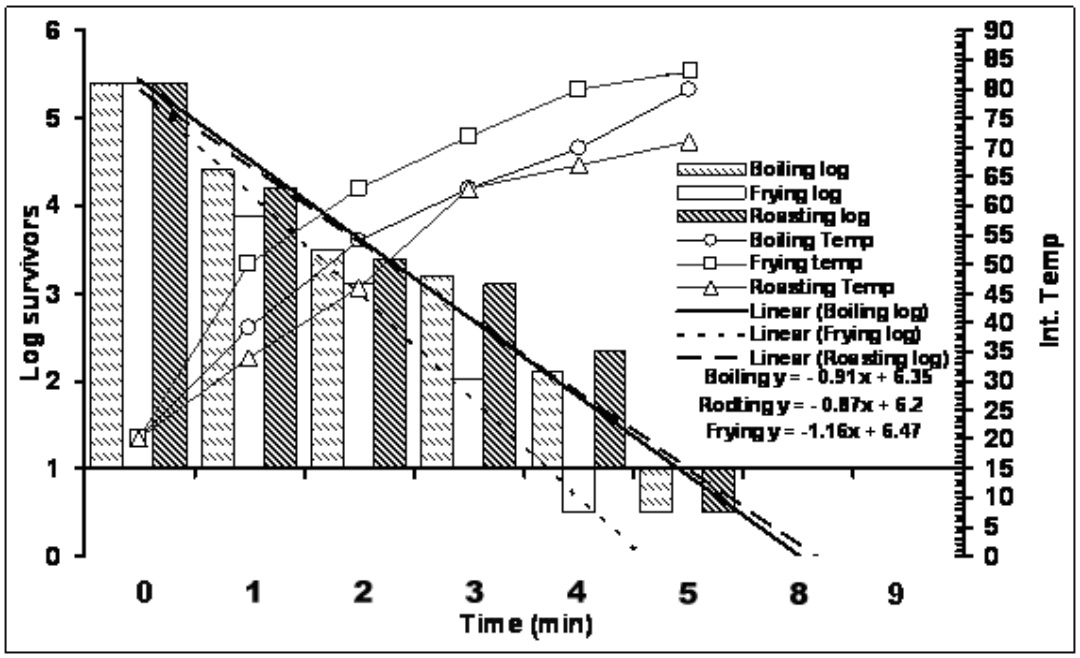


Fig. 5: Effect of cooking methods on *L. monocytogenes*

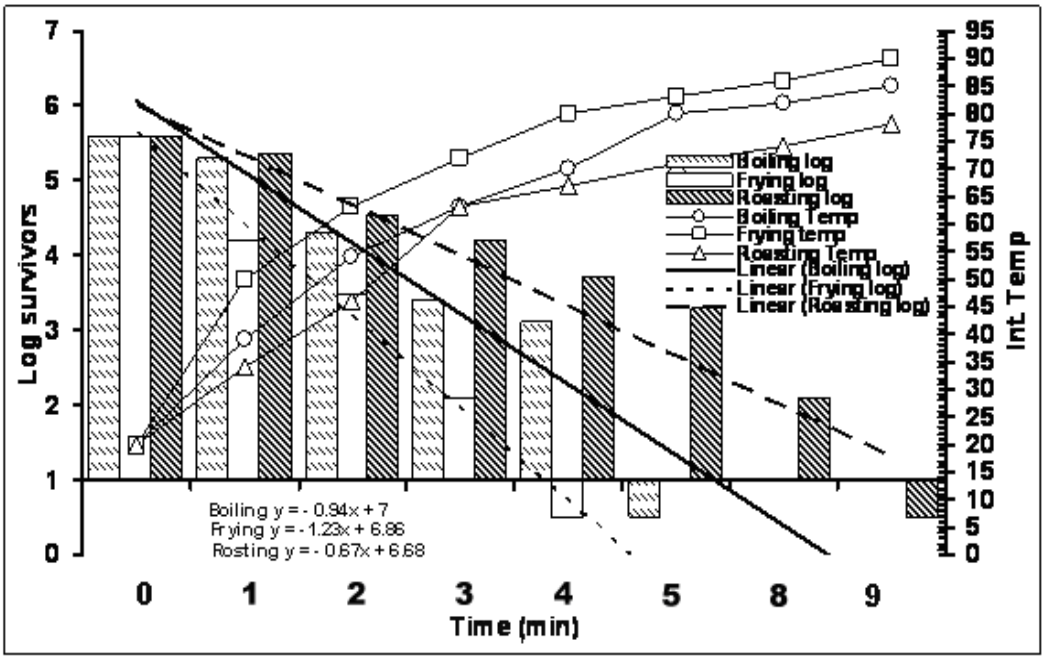


Fig. 6: Effect of cooking methods on weight loss %

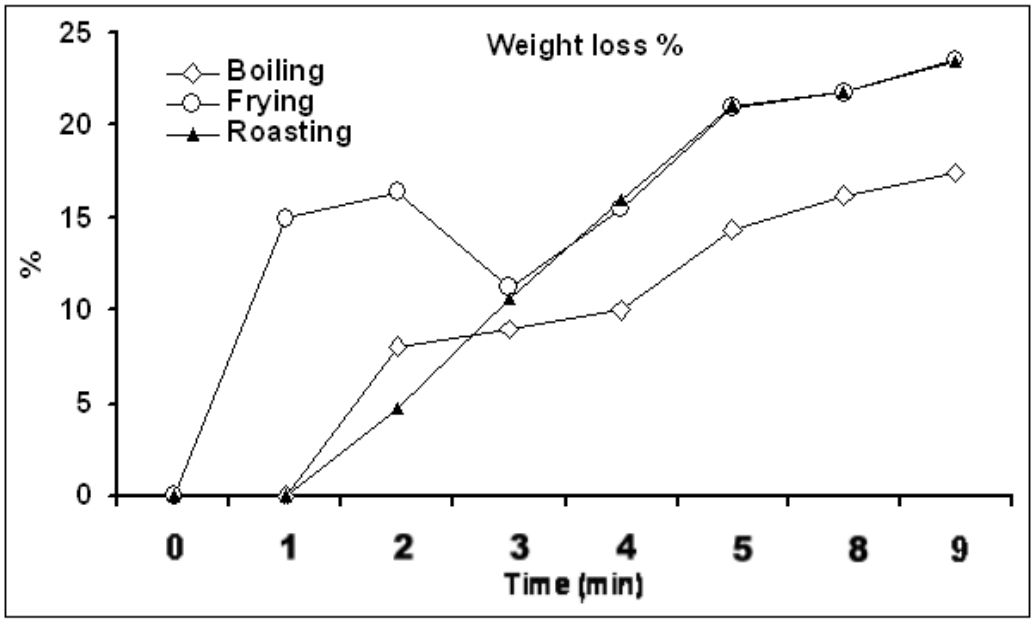
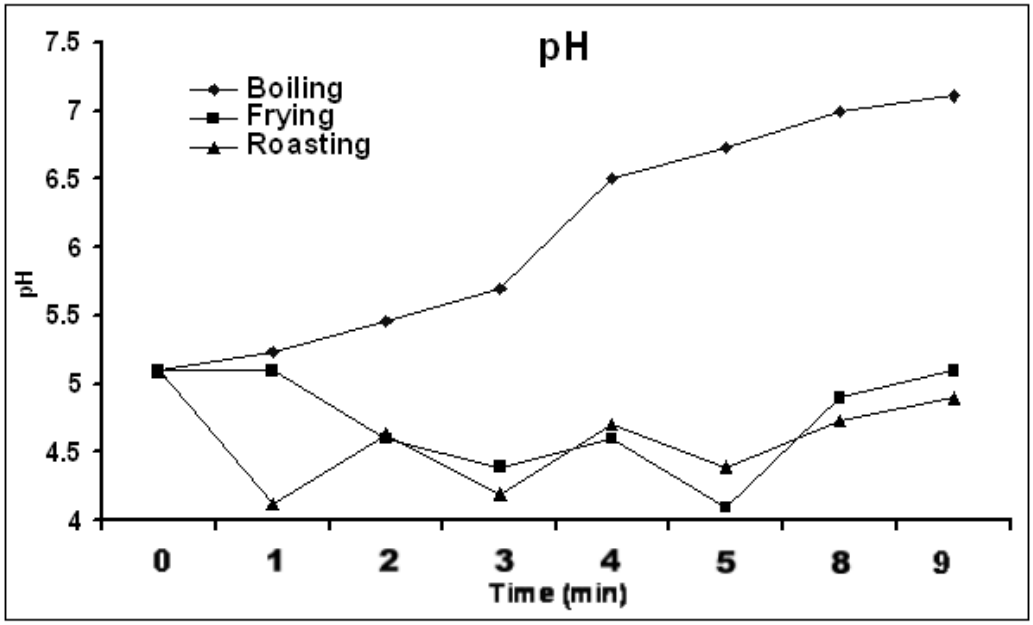


Fig. 7: Effect of cooking methods on pH values



DISCUSSION

The present study evaluated the microbial quality of raw meat sold in butcher shops in Assiut City, Egypt. Our findings showed that out of 100 meat samples analyzed for microbial quality 90 (90%) were contaminated with different kinds of microorganisms namely *E. coli* 48 (48%), *Salmonellae* spp. 18 (18%), *Staphylococcus* spp. 16 (16%), and *listeria* spp. 8 (8%) as showed in Table (1). Our result indicated the predominance of Gram-negative organisms such as *E.coli* and *Salmonella* as reported by (zakpaa *et al.,* 2009) and (Iroha *et al.*, 2011).

The distribution of the isolated pathogens were listed in the same table these results show that most of *E.coli* isolates were *E.coli* O_{111}: H_4 21(43.75%) followed by *E.coli* O_{126}: H_{11} 13 (27.08%) then *E.coli* O_{128}: H_{11} 11(22.92%) and *E.coli* O_{157}: H_7 3 (6.25%).

In 2013 (Ahmad *et al.*, 2013 and Archana *et al*., 2013) reported the presence of high percent of *E.coli* in beef samples 75% and 65% respectively, also in 2014 (Sami *et al.*, 2014) could isolate (60%) of *E.coli* from beef samples while (zhao *et al.*, 2001 and Iroha *et al.*, 2011) could isolate lower percent (19%) of this organism than that reported in our study.

Ebrahim *et al.* (2012) reported that 8.2% of beef samples were *E.coli* O_{157} positive where 1.2% of them was *E.coli* O_{157}: H_7, while (Mboto *et al*., 2012) could not detect *E.coli* O_{157}: H_7 in any of the fresh meat samples examined.

E.coli O_{157}: H_7 is an enteric organism associated with animal and slaughter hygiene, it may be present in the feces and intestines of healthy bovines (Mcevoy *et al.*, 2004). Therefore, meat can be contaminated during slaughter operation. The severity of the illness and the low infective dose (<100) make this organism among the most serious food borne pathogens (Meng *et al.*, 2007).

Three species of the *Salmonella* genus were isolated from tested samples, the highest prevalence of them was *Salmonella typhimurium* (44.4%), while *Salmonella enteritidis* and *Salmonella anatum* rank as a second (27.8%) for each.

The percent of *Salmonella* spp. Isolated by (Ahmad *et al.*, 2013, Archana *et al.*, 2013 and Sami *et al.*, 2014) from beef samples was higher than that obtained in our study, their percentages were 35%, 45% and 26.6% respectively, while the number of *Salmonella* spp. isolated by (zhao *et al.*, 2001 and Iroha *et al.*, 2011) was lower than that obtained in our result, their percentages were 1.9% and 4% respectively.

Also the same table showed that 16% of tested samples was *staphylococcus* spp. whereas 12 (75%) of them recorded as *Staphylococcus aureus* and 3 (18.75%) recorded as *Staphylococcus epidermidis*, while 1(6.25%) recorded as *Staphylcococcus gallinarum*, all of them were coagulase positive and this was disagreement with (Goja *et al.*, 2013) who reported that most of *Staphylococci* isolates from fresh beef samples were coagulase negative.

The isolated spp. of *Staphylococci* in our study were considered to be well known pathogens to humans and animals, specially *Staph aureus*, their presence could be due to the insanitary condition of the butcher and absence of the health services in butcheries.

The number of *Staph aureus* 2 (2%) isolated by (Iroha *et al.*, 2011) was lower than that obtained in our result, while (Ahmad *et al*., 2013) and (Sami *et al.*, 2014) could isolate higher percent of the same spp. than ours, their percentages were 70% and 46% respectively.

Four (50%) of isolated *listeria* spp. were characterized as *Listeria monocytogens*, and 2(25%) of them were characterized as *Listeria innocua* while the percent of *Listeria welshimeri* and *Listeria invanovii* was 1 (12.5%) for each.

The presence of zoonotic bacteria in meat indicates poor anti- mortem inspection of the animals as well as unhygienic meat processing (Barros *et al.*, 2007).

The inactivation of infectious pathogens using a heat treatment is a critical control point in the safe preparation of meat, the major benefit of thermal processing is the overall improvement of product quality and safety. There are three main thermal processing methods to treat meat (boiling, frying and roasting).

The objectives of this part of the study were to evaluate the thermal inactivation of inculcated *E.coli* O_{157}: H_7, *Salmonella enteritidis*, *Staph aureus* and *listeria monocytogenes* in fresh meat using boiling, frying and roasting treatments and to compare between the thermal lethality kinetics of these pathogens after using the three methods of inactivation.

Heating rate was presented in (Fig.1), it was 2°C /min by boiling, 7.5°C /min by frying, while it was 3.6°C /min at roasting. When the data were fitted to relate log of survivors to time in each of the experiments resulted a linear model. The form of this model was: $Y = aX + E$

Where, Y: is log CFU (log of colony forming per gram), **a**: represents the slope of the model for log CFU / g vs. time, X: represents time in minutes and E: represents error. Estimates of a could be used to then estimate D values. The D values for the individual experiments were obtained as the inverse negative of the slope (**a**) of the linear regression line.

The experimental inactivation of inoculated *E.coli* O_{157}: H_7 in fresh meat and fitted curves are included in (Fig.2). The target internal Temp. (65°C) of all treated samples (with selected organisms) was attained in 3, 2 and 3 min (come up time) of boiling, frying and roasting treatments, respectively. Table (2) showed that at this Temp, the log cycles reductions of *E.coli* O_{157}: H_7 were 1.3, 1.5 and 2.6 at boiling, frying and roasting treatments respectively compared with the control. Statistical analysis revealed that at the third minute of each treatment there was significant difference between boiling and frying on reduction of *E.coli* O_{157}: H_7 count, also between boiling and roasting, while there was no significant difference between frying and roasting on reducing the count of the same organism.

By boiling, the count of the organism reached to undetectable level (inverted columns, Fig. 2) at internal Temp. 80°C.This cooking end point of boiled samples was achieve with holding time 2 min,(time to reach internal Temp. of 65°C and excess 2 min exposure). By frying, an internal Temp. of 83°C

(cooking end point) resulted after holding time of 3 min, while the count reached to undetectable level in roasted samples at internal Temp. 74°C with holding time 5 min.

D-values are used in the food industry to determine the effectiveness of the heat inactivation process, these values were calculated for *E.coli* O_{157}: H_7 from the survival curves (Fig. 2), they were 1.1, 1.1 and 1.2 minutes at boiling, frying and roasting treatments respectively.

Kawang (2014), reported that pathogenic cells like *E.coli* O_{157}: H_7 on the meat surface may be translocate and trapped in sterile internal tissues, protecting themselves from thermal destruction if the meat is undercooked.

The survivor curves of *Salmonella enteritidis* were constructed by plotting recovered CFU/g of sample versus heating time (Fig. 3). As expected, as heating temperature increased, survival of *S. enteritidis* decreased.

At the time of internal Temp. 65°C and depending on the method of heat treatment (as shown in Table 2 and Fig.3), *S. enteritidis* decreased 2 Log cycle for all heat treatments compared with the control, and the statistical analysis revealed that there was no significant difference between these treatments on reducing the count of the organism at the third minute, also this Table showed that, the count of *S.enteritidis* reached to undetectable level at internal Temp 80°C, 80°C and 71°C at the cooking end point of boiling, frying and roasting treatments respectively, each with a holding time 2 minutes.

D-values were calculated in meat samples inoculated with *S. enteritidis* and obtained D- values of 1.1, 1.0 and 1.2 minutes at boiling frying and roasting treatments, respectively.

When the different heat treatments were applied on inculcated meat samples with *Staph aureus* and at internal Temp. 65°C, nearly results were observed, *Staph aureus* decreased 2.2, 2.3 and 2.3 1og cycles at boiling frying and roasting treatments, respectively (Table 3 and Fig. 4). The statistical analysis at the third minute of these treatments revealed that there was significant difference, between boiling and frying, also between frying and roasting, while no significant difference detected between boiling and roasting, and the count of the organism reached to undetectable level at internal Temp. 80°C, 80°C and 71°C at the cooking and point of boiling, frying and roasting treatments respectively, each with a holding time 2 minutes (Table 3 and Fig.4).

D- Values of *Staph aureus* in inoculated meat were calculated from survival curves, (Fig.4) they were 1.1, 0.9 and 1.1 minutes at boiling, frying and roasting treatments respectively.

The survival of *L. moncytogenes* at different cooking methods is given in (Table 3 and Fig. 5), *L. monocytogenes* suffered 2.2, 2.1 and 1.4 1og cycles reduction when the internal Temp. of inculcated meat samples reached 65°C of boiling, frying and roasting treatments respectively, the results of statistical analysis indicated that there were significant differences between the three treatments at the third minute.

The same table showed that the count of *L. monocytogenes* reached to undetectable limit at internal Temp. 80°C, 80°C and 78°C at the cooking end point of boiling, frying and roasting treatments receptively with holding time 2 min for each of boiling and frying treatments, while this time was 6 min for roasting treatment.

D- Values of *L.monocytogens* in inculcated meat samples were detected to be, 1.1, 0.8 and 1.1 minutes at boiling, frying and roasting treatments, respectively (Fig. 5).

The high temperature used in thermal processing destroys microbial cells by destabilizing the structural and functional integrity of the cytoblasmic membrane, (Hoover, 1993).

The results of different studies indicate the existence of considerable variation among the reports on the heat resistance of these inoculated organisms, there are several factors altering the level of heat resistance of these organisms, such as differences among the strains, inoculum level, preparation of the product, substrate specific effects, experimental condition, protocols, recovery media and methods. Thus, direct comparisons between the studies are difficult, although it reasonable to accept that at least some of these factors underlie the observed variations in the heat resistance.

In general the thermal resistance by inoculated organisms is variable, and semi logarithmic survivor curves showed a linear decline in population over heating time.

Some changes occur during thermal processing of meat leading to weight loss and change in pH. Cooking losses were determined by measuring the difference in meat samples weigh before cooking, and then after cooking when samples were cooled to room temperature, this recorded in (Table 4) in which boiling caused weight losses ranging from 8.1% to 17.47%, frying caused losses ranging from 15% to 23.53%, while the range of weight losses in roasting treatment was 4.77% to 23.5%.

Statistical analysis revealed that at the cooking end point of the three treatments there was significant difference between boiling and frying, also between boiling and roasting, while no significant difference appeared between frying and roasting this difference is most likely due to different cooking methods.

As expected, the higher cooked internal temperature resulted higher cooking losses (Table 4 and Fig. 6). Prolonged cooking time causing extra moisture loss via evaporation and the release of excess juice inside the meat samples. There by boiling may be the suitable cooking method for meat due to reducing cooking loss.

Sun (2006) observed an increase in cooking loses with an increasing internal Temp. of meat with greatest increments in cooking loses observed within Temp. range 50°C - 70°C, also they reported that denaturation of proteins during thermal processing can cause loss of up to 20% - 40%, mainly in the form of moisture and fat. Also, Sun (2006) observed that the low-steam cooking condition significantly increased cooking yield and the low-steam cooked samples were significantly different form high-steam samples producing a higher cooking yield.

The pH of control samples was 5.1 (Table 4 and Fig. 7). Boiling treatment caused increase in pH, resulted in pH values ranging from 5.1-7.1, but this correlation not clear in both frying and roasting treatments. The increase in pH for cooked meat is due to the reduction of free acidic groups as meat temperature increased during heating process, (Li, 2014). Doyle and Mazzotta (2000) reported that bacteria are more resistant to heat at pH 7.0 or higher.

Statistical analysis revealed that there was significant difference between boiling and frying treatments also between boiling and roasting treatments, while no significant difference appeared between frying and roasting treatments.

Conclusions:

The presence of food borne pathogens such as *E.coli*, *Salmonella*, *Staphylococcus* and *Listeria* in raw meat indicate poor ante-mortem inspection of the animals as well as unhygienic meat processing, it can be concluded that the cooking process carried out at an internal temperature of 65°C is not sufficient for eliminating the high contamination (10^6 CFU/g) of *E.coli* O_{157}: H_7, *S. enteritides*, *Staph. aureus* and *L. monocylogenes*, and the survival of these pathogens after this temperature indicate the possibility of a public health hazard. Results of this work emphasis the necessity for cooking meat at an internal temperature of 83ºC, that as cooking time and temperature increase, levels of microbial destruction increase, also higher cooked internal temperature resulted higher cooking losses and changes in pH.

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المخاطر الصحية لبعض البكتريا الممرضة المصاحبة لاستهلاك اللحوم مع دراسة افضل طرق الطهى للقضاء عليها

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تهدف هذه الدراسة لإستبيان مدي تواجد ميكروبات الايشيريكية القولونية الممرضة ، السالمونيلا ، المكور العنقودي ، الليستيريا في اللحم البقري الطازج المعروض بأسواق مدينة أسيوط. بالتحليل الميكروبيولوجي لمائة عينة تبين تواجد الميكوبات السالفة الذكر بنسب ٤٨ % ، ١٨% ، ١٦% و ٨% علي التوالي. وبإجراء الإختبارات البيوكيميائية والسيرولوجية للعترات المعزولة تبين تواجد ميكروب الايشيريكية القولونية عترة O157:H7 بنسبة ٦.٢٥% ، السالمونيلا تيفي ميوريم نسبة 44.4% ، السالمونيلا انترتيدز (27.78%) ، المكور العنقودي الذهبي (١٨.٧٥ %) والليستيريامونوسيتوجينز بنسبة (٢٥%). كما تناولت الدراسة تأثير المعاملة الحرارية بإستخدام طرق الطهي المختلفة (الغلي ، القلي ، الشي) علي ميكروبات الايشيريكية القولونية عترة O157:H7 ، السالمونيلا انترتيدز ، المكور العنقودي الذهبي والليستيريامونوسيتوجينز وكذلك تحديد زمن الخفض العشري (D- value) لتلك الميكروبات. أسفر هذا الجانب من الدراسة علي أنه لم يتم عزل ميكروب الايشيريكية القولونية عترةO157:H7 في اللحوم المعاملة بالغلي ، القلي أو الشي عندما سجلت درجة حرارة مركز العينات ٨٠، ٨٣ و٧٤ درجة مئوية لطرق الطهي السابقة علي التوالي بينما لم يتم عزل ميكروب السالمونيلا انترتيدز عندما سجلت درجة حرارة مركز العينات المعاملة ٨٠ ، ٨٠ و ٧١ علي التوالي ، تم الحصول علي نفس النتيجة بالنسبة ميكروب المكور العنقودي الذهبي (٨٠ ، ٨٠ و ٧٠ درجة مئوية) وكانت درجات الحرارة المقابلة لقتل ميكروب الليستيريامونوسيتوجينز هي ٨٠ ، ٨٠ و ٧٨ علي التوالي. نتج عن الطهي بطريقة القلي اعلي نسبة للفقد في الوزن نتيجة الطهي (٢٣.٥٣ %) بينما كانت النسب ١٧.٤٧ % و ٢٣.٥٣ % للشي والغلي علي التوالي حيث سجلت هذه النسب عند معاملة العينات لدرجة حرارة كافية للقضاء علي الميكروبات المذكورة تم دراسة التغيرات الكيميائية للعينات المعاملة حرارياً بالطرق المختلفة وذلك بتعيين الأس الهيدروجيني لتلك العينات وتبين إرتفاع قيمة الأس الهيدروجيني في العينات المعاملة حرارياً بالغلي بإرتفاع درجة حرارة مركز العينة وكانت التناسب طردياً. تؤكد الدراسة علي أن اللحم البقري الطازج المسوق بمدينة أسيوط من الممكن أن يمثل مصدراً لبعض الميكروبات التي تؤثر علي صحة الإنسان وأن معاملة تلك اللحوم بأي من طرق الطهي (الغلي والقلي والشي) حتي تصل درجة حرارة اللحم المطهي إلي ٨٣ درجة مئوية هو صمام أمان للمستهلك بالنسبة لميكروبات سالفة الذكر كما توضح الدراسة أن قيم زمن الخفض العشري التي أسفرت عنها الدراسة تعتبر دليل لحساب الوقت اللازم لقتل الميكروبات عند المعاملة الحرارية للحوم.