Significance of application of HACCP system on the bacteriological quality of chicken meat marketed in Giza governorate, Egypt

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ABSTRACT

Fifteen samples from each of chilled whole chicken carcasses, cut-up parts (breast & thigh) and frozen mechanically separated chicken meat (MSC) were collected from traditional shops not applying HACCP system (group A).The same type and numbers of samples produced by poultry processing plant applying HACCP system (group B) were also collected from supermarkets in Giza governorate. The total numbers of samples (one hundred and twenty samples) were subjected to determination of deterioration criteria, isolation and identification of spoilage and pathogenic microorganisms. The isolated pathogenic strains were subjected to serological investigation and antibiotics sensitivity tests. The obtained results revealed that the mean pH values and TVBN of MSC samples collected from A were significantly higher than those collected from B. Moreover, mean TBA values of all samples collected from A were significantly higher than those collected from B except whole chicken carcass samples which were non significantly differ. It is obvious from these findings that MSC collected from A and B were nearly deteriorated while other samples still acceptable. Data of bacteriological examination showed that all investigated bacterial count (total mesophilic, total psychrotrophic, total anaerobic, Coliforms and Fecal Coliforms bacterial counts) of whole chicken carcasses, cut-up parts (breast & thigh) samples of A were significantly higher than that obtained from B. It was also clear that most of investigated bacterial counts of whole chicken carcass and cut-up parts collected from the source A exceeded the limit described by E.S (1651/2005). Moreover, the bacterial counts of MSC collected from both A and B were higher than the permissible limit described by E.S (4178/2005). *E. coli* poly II O_{127}:K_{63} was isolated from whole chicken carcasses and thigh samples, while, *E. coli* poly I O_{26}:K_{60} was isolated from MSC collected from the source A with an incidence rate 13.33, 13.33 and 20%, respectively. At the same time, *S.* Infan*tis* was isolated only from MSC collected from the source A with an incidence rate of 20%. However, *E. coli poly I and II* and *S.* Infantis failed to be isolated from samples collected from the source B. This indicates that the production of chicken meat samples collected from the source B were prepared under good sanitary measures and the application of HACCP system was very effective and produce highly safe products. It is of significance to emphasize that the isolated *E. coli* poly I and II strains were sensitive to amoxicillin-clavulenic, piperecillin-tazobactam, ceftriaxone, ceftazidme, cefepime, imipenem, aztreonam, gentamycin and amikacin and resistant to ciprofloxacin, co-trimoxazole, chloramphenicol and tetracycline. Meanwhile, the isolated *S.* Infantis strain was sensitive to cefotaxime and ciprofloxacin and resistant to ampicillin, co-trimoxazole and chloramphenicol.

Keywords: Chicken carcass, cut-up parts, MSC, HACCP, pH, TVBN, TBA, *E. coli* and *S.* Infantis

1- Introduction

Poultry production and processing involve a series of interrelated steps designed to convert domestic birds into ready-to-cook broiler whole carcasses, cut-up carcass parts, or various forms of deboned meat products. The increasing consumption of chicken meat can be attributed to low production costs, rapid growth rates, high nutritional values, and the increased production of further processed products ***(Barbut, 2002)***. Moreover, poultry meat has received much of its good reputation on the basis of its high protein, low fat contents and relatively high concentrations of polyunsaturated fatty acids which are considered to be positive and healthy for consumers ***(Bonoli et al., 2007).*** In the late 1950s and early 1960s, the poultry industry began

marketing more cut-up and further processed poultry meat products. Cutting a carcass into parts is probably the simplest example of value-added processing for poultry where, value-added processing is making some change in the product to increase its appeal to the consumer and consequently reflected in an increase in price ***(Sams, 2001)***. Moreover, ***Froning and McKee (2001)*** pointed out that as the popularity of the consumer choices grew along with the increased consumption of poultry meat, more parts such as frames, backs, necks, drumsticks, wings, etc. became available for production of mechanical separation chicken meat (MSC). More than 95% of all food-borne illnesses are the result of activities occurring after the product has left the plant; that is, illness is generally the result of temperature abuse and improper handling or preparation ***(Ayres et al., 1980)***.

It is necessary to prolong the shelf life of chilled poultry meat, its cut-up parts and frozen MSC meat. All available technological tools can be used to comply with strict government regulations to avoid microbiological contamination, mainly pathogens, from slaughter to the consumer. The two overall strategies used by poultry processors to control pathogens in the plant are Good Manufacturing Practices (GMPs) and Hazard Analysis and Critical Control Points (HACCP). The main goal of HACCP is food protection, moreover, it provides other benefits such as increase customer and consumer confidence, gives market protection, reduce costs through reduction of product losses, increase focus and ownership of food safety, simplify inspections because of record keeping and documentation, provide consistent quality product and demonstrates conformance to the product requirements and regulations ***(McNamara, 1997; Conner et al., 2001)***.Therefore, the present study was designed to compare between the deterioration criteria and bacteriological quality of chilled whole chicken carcasses, cut-up parts (Breast & Thigh) and frozen mechanically separated chicken meat (MSC) collected from traditional shops not applying HACCP system (group A) with those from supermarkets marketing products of poultry processing plant applying HACCP system (group B).

2- Materials and methods

Collection of samples

A total one hundred and twenty samples of chilled whole chicken carcasses, cut-up parts (breast & thigh) and frozen mechanically separated chicken meat (MSC) (Fifteen samples for each) were collected from traditional shops not applying HACCP system (sixty samples, group A) and from supermarkets marketing products of poultry processing plant applying HACCP system (sixty samples, group B). Chilled chicken carcasses and cut-up parts (breast & thigh) were obtained within one day from their slaughtering while, frozen MSC samples were obtained within one month after processing. Samples were transferred to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Cairo University and kept chilled at 4°C for whole chicken carcasses and cut-up parts and stored frozen at -18 °C for frozen MSC until the time of investigation. All samples were subjected to determination of deterioration criteria, isolation and identification of spoilage and pathogenic microorganisms. The isolated pathogenic strains were subjected to serological investigation and antibiotics sensitivity tests.

Investigations

1. Deterioration criteria

1.1. pH value

Five grams from each chicken carcass, cut-up parts and MSC were homogenized with 20 ml distilled water for 10-15 seconds ***(Kandeepan et al., 2009)***. The pH was measured using pH

meter (LovibondSenso Direct) with a probe type electrode (Senso Direct Type 330) where three reading for each sample were obtained and the average was calculated. The pH meter was calibrated every two samples using two pH buffers 7.0 and 4.0.

1.2. Total Volatile Basic Nitrogen

Ten grams of chicken carcass, cut-up parts and MSC were macerated with 100 ml distilled water and washed into a distilling flask with 200 ml distilled water, then 2 grams magnesium oxide were added. A macro-Kjeldahl distillation apparatus was connected to the distillation flask containing 25 ml of 2% boric acid solution and few drops of methyl-red indicator (0.016 g methyl red, 0.083 g bromocresol green per 100 ethanol) with the receiving tube was dipped below the liquid, with distillation continued till collection of 200 ml. The condenser was then washed with distilled water and the distillate was titrated with 0.05 M (0.1N) sulphuric acid. The Total Volatile Base Nitrogen (mg/100 gram sample) was calculated as the titration multiply by 14 ***(Kearsley et al., 1983)***.

1.3. Thiobarbituric Acid value

Five grams from each chicken carcass, cut-up parts and MSC were homogenized with 15 ml deionized distilled water using a stomacher (Lab blender 400) for 10 seconds at the highest speed. One milliliter of the homogenate was mixed with 50 µl butylated hydroxyl anisole (7.2%) and one ml each of 15mM 2-thiobarbituric acid and 15% trichloroacetic acid. The mixture was vortexed, incubated in a boiling water bath for 15 minutes to develop color, then cooled under running water for 10 minutes, vortexed again, and centrifuged for 15 min at 2500 rpm. The absorbance of the resulting supernatant was measured at 531 nm using Unico 1200 (USA) series spectrophotometer against a blank containing 1 ml of deionized water and 2 ml of 2-thiobarbituric acid-trichloroacetic acid solution. The reading was multiplied by 7.8 to obtain the value of thiobarbituric acid expressed as milligrams of malonaldehyde per kilogram of sample ***(Du and Ahn, 2002)***.

3. Bacterial examination

The spreading technique and the standard plate count agar were used for enumeration of aerobic mesophilic count ***(Swanson et al., 1992)***, and aerobic psychrotrophic count ***(Cousin et al., 1992)***. While, reinforced clostridial agar and the anaerobic system were performed for enumeration of the anaerobic spore formers ***(Brewer and Allgeier, 1966).*** Coliforms were enumerated "MPN" using lauryl sulfate tryptose broth according to ***Hitchins et al. (1992)***, Fecal Coliforms "MPN" were calculated according to ***FAO (1992)*** and *E. coli* was isolated by inoculation on EMB according to ***Krieg and Holt (1984)***. For Salmonella isolation, the technique adopted by ***FAO (1992)*** was used by pre-enrichment in buffered peptone water 1% followed by selective enrichment in Rappaport medium and selective plating on both S.S. and XLD agar. The isolated *E. coli* and Salmonella were serologically identified according to ***Edwards and Ewing (1972)*** and ***Kauffman (1974)***, respectively in the department of clinical microbiology, Central Health Laboratories, Ministry of Health and Population.

4. Antibiotics sensitivity test

Antibiotics susceptibility tests were performed on Mueller-Hinton (Oxoid, UK) using disc diffusion technique ***(Bauer et al., 1996)*** in the department of clinical microbiology, Central Health Laboratories, Ministry of Health and Population.

Statistical analysis

Data of all samples were subjected to analysis of variance test (ANOVA) and results were recorded as Mean±SE. These means were compared by protected least significant difference (PLSD) test at 0.05 significant levels ***(Steel and Torrie, 1980)***.

3- Results and discussion

Table (1): Deterioration criteria of whole chicken carcasses, cut-up parts (Breast &Thigh) and mechanically separated chicken meat (MSC) collected from Traditional shops (A) and from supermarkets marketing products of poultry processing plant applying HACCP system (B) (n=15)

Samples from	Traditional shops (A)				supermarkets marketing products of poultry processing plant applying HACCP system (B)			
	Whole chicken carcasses	Breast	Thigh	MSC	Whole chicken carcasses	Breast	Thigh	MSC
	Mean± SE							
pH	$6.08^{a}\pm0.12$	$5.79^{a}\pm0.06$	$6.54^{b}\pm0.06$	$8.83^{c}\pm0.22$	$5.82^{a}\pm0.09$	$5.82^{a}\pm0.06$	$6.47^{b}\pm0.06$	$7.36^{d}\pm0.16$
TVBN	$10.25^{a,c}\pm0.31$	$9.95^{a}\pm0.41$	$10.95^{c}\pm0.28$	$12.79^{b}\pm0.32$	$7.50^{d}\pm0.23$	$9.47^{a}\pm0.38$	$8.43^{e}\pm0.17$	$9.63^{a}\pm0.42$
TBA	$0.22^{a,d}\pm0.02$	$0.27^{a}\pm0.05$	$0.53^{b}\pm0.07$	$0.85^{c}\pm0.02$	$0.21^{a,d}\pm0.02$	$0.14^{d}\pm0.03$	$0.18^{a,d}\pm0.02$	$0.62^{b}\pm0.05$

***: Means with different superscripts differ significantly at P<0.05.**
TVBN expressed as mg/100g sample.
TBA expressed as milligrams of malonaldehyde/kg
MSC= mechanically separated chicken meat.

Table (2): Bacterial load (log_{10} CFU/g) of whole chicken carcasses, cut-up parts (Breast &Thigh) and mechanically separated chicken meat (MSC) collected from Traditional shops (A) and from supermarkets marketing products of poultry processing plant applying HACCP system (B) (n=15)

Samples from	Traditional shops (A)				supermarkets marketing products of poultry processing plant applying HACCP system (B)			
	Whole chicken carcasses	Breast	Thigh	MSC	Whole chicken carcasses	Breast	Thigh	MSC
	Mean± SE							
Total mesophilic bacterial count	$5.86^{a}\pm0.24$	$6.26^{a,b}\pm0.16$	$6.37^{b}\pm0.14$	$7.87^{c}\pm0.14$	$4.57^{d}\pm0.14$	$4.66^{d}\pm0.08$	$4.93^{d}\pm0.12$	$5.86^{a}\pm0.24$
Total psychrotrophic bacterial count	$5.29^{a}\pm0.21$	$5.84^{b}\pm0.16$	$5.96^{b}\pm0.16$	$6.88^{c}\pm0.24$	$3.83^{d}\pm0.11$	$3.83^{d}\pm0.14$	$4.56^{e}\pm0.07$	$5.28^{a}\pm0.21$

Total anaerobic bacterial count	$5.28^{a}\pm0.21$	$6.02^{b}\pm0.15$	$6.17^{b}\pm0.17$	$6.88^{c}\pm0.21$	$3.98^{d}\pm0.11$	$3.96^{d}\pm0.14$	$4.16^{d}\pm0.11$	$5.29^{a}\pm0.21$
Coliforms count	$2.53^{a}\pm0.14$	$2.65^{a}\pm0.14$	$3.12^{b}\pm0.12$	$3.30^{b}\pm0.12$	$1.19^{c,d}\pm0.13$	$0.79^{c}\pm0.22$	$1.24^{c}\pm0.23$	$2.53^{a}\pm0.14$
Fecal Coliforms	$2.35^{a}\pm0.17$	$2.52^{a}\pm0.15$	$2.45^{a}\pm0.21$	$2.64^{a}\pm0.09$	$0.84^{b}\pm0.11$	$0.75^{b}\pm0.13$	$0.54^{b}\pm0.14$	$2.35^{a}\pm0.17$

***: Means with different superscripts differ significantly at P<0.05** **MSC= Mechanically separated chicken meat**

Table (3): Incidence of pathogens in of whole chicken carcasses, cut-up parts (Breast &Thigh) and mechanically separated chicken meat (MSC) collected from Traditional shops (A) and from supermarkets marketing products of poultry processing plant applying HACCP system (B) (n=15)

Organisms	***Samples from Traditional shops (A)***								***supermarkets marketing products of poultry processing plant applying HACCP system (B)***							
	Whole chicken carcasses		***Breast***		***Thigh***		***MSC***		***Whole chicken carcasses***		***Breast***		***Thigh***		***MSC***	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
E. coli ****E. coli* poly II O_{127}:K_{63}** *****E. coli* poly I O_{26}:K_{60}**	*2	13.33	0	0	*2	13.33	**3	20	0	0	0	0	0	0	0	0
Salmonella ***S.* Infantis $O:_{6,7,14}$ $H_{;r,1,5}$**	0	0	0	0	0	0	3	20	0	0	0	0	0	0	0	0

Data in Table 1 summarized results of deterioration criteria of whole chicken carcasses, cut-up parts (Breast & Thigh) and mechanically separated chicken meat (MSC) collected from Traditional shops not applying HACCP system A and from supermarkets marketing products of poultry processing plant applying HACCP system B. Mean pH values of whole chicken carcasses, breast and thigh muscle samples (Table 1) collected from A were not significantly differ from that obtained from B and still within the acceptable limit (breast, 5.7-5.9 and thigh, 6.4-6.7) that described by ***E.S (1651/2005)*** for chilled breast and thigh muscle. The pH values of chicken breast and thigh collected from both the sources (A) and (B) (Table.1) are nearly similar and coordinated with those of ***(ICMSF 2005, AL-Dughaym et al. 2010 and Bhaisare et al. 2014)*** who observed that pH of breast muscle ranged from 5.6 to 5.9, while for leg muscles varied from 6.4 to 6.7. On the other hand, ***Edris et al. (2012)*** reported lower pH value for thigh muscles (5.77). There are two main kinds of poultry muscles, white (breast) and red (leg) which have structural and physiological differences, as well as different pH value where, 5.6 – 5.8 for breast muscle and 6.1- 6-4 for leg muscle ***(Mead, 2000)***. The significantly ($P< 0.05$) higher alkaline pH of thigh than breast samples collected from both A and B (Table 1) may be attributed to the lower glycogen content in poultry leg ***(Newton and Gill, 1981)***. Besides, higher pH values of thigh meat compared to breast meat may be due to increase of lactic acid concentration via anaerobic metabolism in breast meat ***(Hassanine and Hassan, 2003)***.Concerning pH values of MSC samples (Table 1), the mean pH values of MSC samples collected from A (8.83) were significantly ($P< 0.05$) higher than that obtained from B (7.36) and in general all MSC samples had high pH values (>6.40).Our results of pH values of MSC obtained from B were in agreement with of ***Field (1988)*** and ***Kolsarıcı and Candoğan (2002)*** who achieved a pH value of ranged from 6.8 to 7.4. Lower pH value (6.46 and 6.54) was recorded by ***Smyth and O'Neill (1997)*** and ***Ozkececi et al. (2008).*** Although the higher recorded pH values in the present study, the application of HACCP system slightly decrease the pH values which may be attributed to the lower bacterial loads (Table 2).

It was clearly that the mean TVBN values of whole chicken carcasses and thigh muscle samples collected from B were significantly ($P< 0.05$) lower and mean TVBN values of MSC collected from A were significantly ($P< 0.05$) higher than other samples (Table 1). In general TVBN values of all samples collected from both A and B were within the permissible limit (20 mg/100g) described by ***E.S (1651/ 2005)*** for chilled chicken and described by ***E.S (4178/ 2005)*** for MSC. The aforementioned results clearly indicated that the application of HACCP system significantly ($P< 0.05$) decreased TVBN values of most examined samples which may be attributed to the effective HACCP system application lead to decrease the spoilage microorganisms which destruct the meat protein. The higher TVBN value of breast samples than thigh samples which collected from B were explained by ***Hassan (2001)*** and attributed that to the higher protein content in breast as compared with thigh. However, lower TVBN value of breast samples than thigh samples which collected from A was observed in the present study and this may be due to the higher bacterial load in thigh than breast (Table 2). In this regard, ***Edris et al. (2012)*** and ***Saad et al. (2013)*** reported higher mean value (11.29 mg/100gm) for chicken breast than that for breast samples collected from both (A) and (B) obtained during this study (9.95 and 9.47 mg/100 gm respectively). On the other hand, ***Edris et al. (2012)*** recorded lower level of TVBN for thigh muscles (8.1mg/100 gm) than that obtained during the present study (10.95 and 8.43 mg/100 gm) for (A) and (B) samples respectively.

Results in Table 1, clearly revealed that there was no significant difference between mean TBA value of whole chicken carcasses collected from both the sources (A) (0.22 mg/kg) and (B) (0.21mg/kg). The mean TBA value of cut-up parts (breast, thigh) and MSC samples 0.27, 0.53 and 0.85 mg/kg collected from (A) were significantly higher ($P< 0.05$) than those obtained from

(B) (0.14, 0.18and 0.62 mg/kg). It is of significance to emphasize that the TBA values of chilled whole chicken carcasses, breast and thigh samples were within the acceptable limit (0.9 mg malonaldehyde/kg sample) established by ***E.S (1651/ 2005).*** However, it is of significance to denote that ***E.S (4178/ 2005)*** didn't state the limit of TBA for MSC. Several authors attributed the higher values of TBA to the high bacterial load ***(Lai et al., 1991),*** the release of haem, oxidative enzymes and incorporation of oxygen into the product during mechanical deboning ***(Parry, 1995, Rhee et al., 1996, Pettersen et al., 2004)***; presence of unsaturated lipids, contact with metals and temperature rise during mechanical separation ***(Parry, 1995, Gray et al., 1996, Abdullah and Al-Najdawi, 2005)*** ; prolonged storage periods ***(Ertaş, 1998, Mulla, 2002).*** The effective application of HACCP system could be retard the rancidity through controlling the bacterial loads and the elevated temperature, therefore these may explain the lower TBA values of all samples obtained from B than those obtained from A.

Data of bacteriological examination (Table. 2) showed that all investigated bacterial count (total mesophilic, total psychrotrophic, total anaerobic bacterial count, Coliforms and Fecal Coliforms) of all samples collected from A were significantly ($P< 0.05$) higher than that obtained from B except Fecal Coliforms of MSC samples which is non significantly differ. It was also clear that most of investigated bacterial counts of whole chicken carcass and cut-up parts collected from A exceeded the limit described by ***E.S (1651/2005)*** and bacterial counts of MSC collected from both A and B were higher than the permissible limit described by ***E.S (4178/2005)***. In general all investigated bacterial counts were higher in MSC followed by thigh and finally breast and whole chicken carcasses and these results substantiated the data of deterioration criteria with nearly the same sequence. The higher contamination rate of thigh muscle as compared to that of breast muscle might be due to during evisceration process the thigh or leg muscle are highly prone for contamination from the gut content during evisceration in case of improper procedure ***(Eyigor et al., 2005).*** At the same time, ***ICMSF, (2005); Bhaisare et al., (2014)*** emphasized the significance of the higher alkaline PH (6.4 to 6.7) of thigh muscles as compared to that of breast muscles (5.7 to 5.9) which is more favorable media for growth of microorganism. Moreover, whole chicken carcasses was less contaminated than the cut-up parts which may attribute to the higher contamination rate during handling and cutting of whole chicken into cut-up parts.

Considering the total mesophilic bacterial counts of whole chicken carcass samples collected from the source (A), nearly similar results were obtained by ***Fliss et al. (1991)*** who reported that the mean contamination level of APC for poultry carcasses collected from the Tunisian markets was 6.01 $\log_{10}$ CFU/gm. On the other hand, higher level of APC (7.24 $\log_{10}$ CFU/g) was published by ***Barbuddhe et al. (2003)*** for poultry meat in India. However, lower contamination level of contamination of fresh chicken carcasses in the United States (2 to 4 $\log_{10}$ CFU/cm2) was reported by ***Johnston and Tompkin (1992).*** Regarding the cross contamination of thigh samples collected from the source (A) with mesophilic bacteria, ***AL-Dughaym et al. (2010), Saikia and Joshi (2010) and Hemmat et al. (2014)*** mentioned nearly similar results in the following order 5.1×10^6, 1.07×10^6 and 1.87×10^6 CFU/g, respectively. While lower result (2.18×10^5) was recorded by ***Ruban and Fairoze (2011)***. The high psychrophilic bacterial count of chicken meat was recorded by ***Mukhopadhyay et al. (2004).*** At the same time, ***Chaiba et al. (2007)*** recorded higher results (4.48 and 4.36 $\log_{10}$ CFU/g) for samples collected from Morocco popular market and artisanal slaughterhouses. It was also clear that lower anaerobic bacteria count ($2.2x10^4$ CFU/ g).of chicken meat samples was recorded by ***(Fahim et al. 2015)*** than those collected from A (Table 2).

Coliforms bacteria usually gain access to chicken meat during evisceration, as they constitute part of the normal intestinal flora of poultry ***(Notermans et al., 1980)***. Nearly similar result (2.73 $\log_{10}$ MPN/g) was recorded by ***Capita, et al. (2002)*** in Spain. However, higher results (3.95, 4.60 and 5.85 $\log_{10}$ MPN/g) were recorded by ***Fliss et al. (1991), Mukhopadhyay et al. (2004)*** and ***Chaiba et al. (2007)*** in Tunisia, India and Morocco, respectively for total coliforms of chicken carcasses.

The obtained results of total aerobic bacterial count of MSC collected from A and B were lower than those recorded by ***Yuste et al. (2002)*** and ***Emara (2005).***However lower result of total aerobic mesophilic bacterial count for MSC collected from A and nearly similar result of total aerobic psychrotrophic bacterial count was recorded by ***(Mohamed et al., 2011)***. It was clear that detectable organoleptic spoilage of mechanically separated meat is a result of decomposition and the formation of metabolites caused by the growth of Gram negative microorganisms especially psychrotrophic bacteria ***(Lee et al., 1997).*** Similar findings regarding Coliforms and Fecal Coliforms of MSC collected from A were reported by ***(Mohamed et al., 2011)***.

Results in Table 3 clearly revealed that *E. coli* poly II O_{127}:K_{63} was isolated from whole chicken carcasses and thigh muscle samples and *E. coli* poly I O_{26}:K_{60} was isolated from MSC collected from A with an incidence rate of 13.33, 13.33 and 20%, respectively. While *S.* Infan*tis* was isolated only from MSC collected from A with an incidence rate of 20%. The obtained results also indicated that *S.* Infantis failed to be isolated from whole chicken carcasses and cut-up parts (breast and thigh) collected from the source (A) and all samples collected from the source (B), while *E. coli* failed to be isolated from breast samples of the source (A). It is important to point out that chilled chicken ***(E.S., 1651/2005)*** and MSC ***(E.S., 4178/2005)*** should be free from *E. coli* and Salmonellae. The isolated *E. coli* strains are sensitive to amoxacillinclavulenic, piperecillin-tazobactam, ceftriaxone, ceftazidme, cefepime, imipenem, aztreonam, gentamycin and amikacin and resistant to ciprofloxacin, co-trimoxazole, chloramphenicol and tetracycline. Meanwhile, the isolated *S.* Infantis is sensitive to cefotaxime and ciprofloxacin and resistant to ampicillin, co-trimoxazole and chloramphenicol.

Concerning the results of isolation of *E. coli* from whole chicken carcass samples collected from the source (A), higher contamination rates (100, 38.7, 11.4, 34.2, 48.4, 70 and 78%) were recorded by ***Bhattacharjee et al. (1996); Zhao et al. (2001); Northcutt et al. (2003); Khalifa and Abd El-Shaheed (2005); Cohen et al. (2007); AL-Dughaym et al. (2010) and Odwar et al. (2014)***, respectively. However, ***Akbar et al. (2014)*** recorded a lower contamination rate (2%) in samples collected from Indonesia. Regarding the failing of isolation of *E. coli* from breast samples collected from the source (A), similar result was recorded by ***Edris et al. (1992).*** However, higher results (14.28%, 10 % and 33.33%) were recorded by ***Ebeid, (1996), El-Sbagh, (2010) and Hemmat et al. (2014)*** respectively. Furthermore, ***Alvarez-Astorga et al. (2002)*** found that *E. coli* in chicken parts in Spain was higher than the maximum limits established in the guidelines for poultry meat. It was also clear that lower incidence rate (5.71%) than our finding regarding *E. coli* in MSC was observed by ***Mohamed et al. (2011).***

Our obtained data concerning Salmonella free cut-up parts (breast and thigh) samples which collected from A was in agreement with ***Edris et al. (1992)*** and ***Ebeid (1996)***. Variable incidence rates for Salmonellae were reported by different authors, where high incidence rates were recorded by ***Ahmed (1995); Harrison et al. (2001); Živković (2001)*** and ***Bao et al. (2006)*** and 25% to 65% were recorded by ***Ramya et al. (2012)*** and ***Ruban et al. (2012)***. Meanwhile, lower incidence rate of 1.6- 4.2 % from retail fresh chicken were recorded by ***Zhao et al. (2001);***

Corry et al. (2002); Cohen et al. (2007) and ***Ulloa et al. (2010)***. Moreover, incidence rates of 17.4 % in Austria, 8.4 % in Spain, 2.4% in Greece and 7.7 % in Italy from retail fresh chicken carcass were reported by ***EC (2002)***. ***Saikia and Joshi (2010)*** isolated *S.*Typhi by 20 % from chicken meat. Furthermore, incidence rates of 10 % and 15 % were obtained in Egypt ***(Rabie et al., 2012; El-Safey, 2013).***

The prevalence of Salmonella spp. was higher in thigh meat (31.99 %) as compared to breast muscles (24.88%) ***(Ruban et al., 2010)***.However, ***Gad (2004)*** and ***Shawish (2011)*** found that the prevalence of Salmonella spp. was higher in breast meat (8.6-12.5 %) than thigh muscles (2.5-5.8%). ***Saad et al., (2011)*** reported that the prevalence of Salmonella spp. (16%) was equally in chicken breast and thigh. Other authors recorded that the incidence of Salmonella spp was 11.11**%** ***(Nawar, 2007)***, 33.33%. ***(Hemmat et al., 2014)*** in thigh muscle and was 48% in breast muscle ***(Vural et al., 2006)***. A significant lower contamination rate of Salmonella for poultry cuts without skin compared to poultry cuts with skin was noticed by ***Uyttendaele et al. (1999)***. Meanwhile, Salmonella spp. was found in 15.39% of chicken breast fillets and in 9.52% of chicken breasts with skin ***(Kozačinski et al., 2006).*** Finally, ***Mohamed et al. (2011)*** isolated Salmonella from MSC with percentage of 2.83%. Variations of isolation rates of Salmonella incidence between authors may be attributed to the country where the study was carried out, the sampling plan and the detection limit of the methodology as well as the initial contamination rates during transportation through bird cages. Furthermore, during defeathering process, Salmonella enters the feather follicle and this act as a kind of protection to the microorganism and may be one of limitation during Salmonella isolation ***(Rasschaert et al., 2007)***.

Our obtained results regarding *E. coli* and Salmonella free samples collected from B may be attributed to the effective HACCP system application. Raw poultry is often highly contaminated and it recognizes the challenge in reducing the microbial contamination of such products ***(Schlosser et al., 2000)***. Therefore, the Pathogen Reduction, Hazard Analysis and Critical Control Point (PRHACCP) Systems rule, published in 1996, requires poultry plants to develop and implement HACCP plans and sanitation standard operating procedures. All plants must test for generic *E.coli* as an indicator of fecal contamination and must ensure Salmonella rates are below standards set in the rule ***(USDA-FSIS, 1996)***. Moreover, ***USDA-FSIS (2000b)*** found that testing for generic *E. coli* is required by the PR-HACCP systems rule for all poultry slaughter plants as an indication of proper processing and sanitation procedures**.** ***USDA-FSIS (2000a)*** reported that only 10.9% of the 5,697 rinse samples taken from broiler carcasses collected from 124 large processing plants during the first year of HACCP implementation were Salmonella positive. The incidence of Salmonella species prior to implementation of the PR-HACCP rule was15.9% and 11.9% in chicken carcasses and turkey carcasses, respectively, where, *S.* Heidelberg and *S.* Hadar were two of the most common species detected in both chicken and turkey carcasses ***(Schlosser et al., 2000)***. Moreover, ***Rose et al. (2002)*** found that Salmonella prevalence in poultry meat collected at federally inspected establishments in the United States was lower after the implementation of PR/HACCP than in pre-PR/HACCP baseline studies and surveys conducted by the FSIS. However, ***USDA/FSIS (2010)*** observed that Salmonella isolated from FSIS / HACCP verification sampling of broilers and the most common serotype isolated was *S.* Enterica serovar Kentucky; this has been the case each year over the past decade. The prevalence of Salmonella was higher in traditional shops with minimal facilities and poor hygiene ***(Nawar, 2007; Ruban and Fairoze, 2011)***.

E. coli poly II O_{127}:K_{63} which isolated from whole chicken carcasses and thigh muscle samples collected from the source (A) are classified as enteropathogenic (EPEC) *E. coli* ***(Whittam and McGraw, 1996; Hernandes et al., 2009)***. EPEC is one of the oldest recognized

diarrheagenic *E.coli*, which cause either a watery or bloody diarrhea ***(Benenson, 2005).*** Moreover, it has been well known to be highly related to infant diarrhea in developing countries through the pioneering work of ***(Bray, 1945)*** who established the importance of EPEC as a cause of outbreaks of infantile gastroenteritis in the UK in the 1940s**.** These continued until the early 1970s, but since then outbreaks caused by 'classical' EPEC strains have become very rare ***(Smith et al., 2004).*** *E. coli* poly I O_{26}:K_{60} which isolated from MSC samples collected from the source (A) classified as one of Shiga toxin-producing *Escherichia coli* (STEC). ***Maysa et al., (2014)*** mentioned that certain strains of STEC are frequently identified as causative agents of life-threatening diseases in humans, such as hemorrhagic colitis (HC) and hemolytic uraemic syndrome (HUS). *S.* Enterica subspecies Enteric serovar Infantis was confirmed as the cause of human salmonellosis in several countries and is the third most frequently isolated serovar of Salmonella (1.1%) after *S.* Enteritidis and *S.* Typhimurium ***(Ishihara et al., 2009).*** Poultry, especially from layer and broiler farms, as well as pigs are the main animal reservoirs for *S. serovar* Infantis ***(Nógrády et al., 2012).*** This serovar is also dominant in broiler meat, accounting for 35.9% of all Salmonella isolates reported from European countries in 2014 ***(EFSA and ECDC, 2016).*** Moreover, *S.* Infantis belong to the 10 main Salmonella serotypes isolated ***(Weill and Grimont, 2005)*** that causes gastroenteritis in human. A report from UK has mentioned 0.3% deaths from salmonellosis caused by *S.* Infantis during 1996-2006 ***(Jones et al., 2008).***

4. Conclusions

This study has shown that the deterioration criteria of whole chicken carcasses, cut-up parts and MSC collected from markets were higher than those obtained from supermarkets marketing products of poultry processing plant applying HACCP system. Moreover, most of investigated bacterial counts of all samples collected from markets were significantly higher than those obtained from supermarkets marketing products of poultry processing plant applying HACCP system and did not copy with the Egyptian standard specifications. In addition to, two strains of *E. coli* were isolated from whole chicken carcasses, thigh samples and from MSC collected from markets, while *S.* Infantis was isolated only from MSC collected from markets. However, these strains failed to be isolated from samples collected from supermarkets marketing products of poultry processing plant applying HACCP system. Therefore, based on this evidence, all samples which collected from supermarkets marketing products of poultry processing plant applying HACCP system prepared under good sanitary measures and the HACCP system application was very effective and produces highly safe products.

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الملخص

تم جمع عدد مائه وعشرون عينه من ذبائح الدجاج وأجزائها (صدور وافخاذ) ولحم الدجاج المنزوع ميكانيكيا من الاسواق (أ) ومن مجازر الدجاج التى تطبق نظام الهاسيب (ب).واجريت على هذه العينات فحوصات دلالات الفساد, الفحص الميكروبيولوجى لتحديد مدى جودتها وسلامتها وايضا للمقارنه بين العينات التى تم جمعها من أ و ب بالاضافه الى اختبار السلالات المعزوله لحساسيتها للمضادات الحيويه. فقد دلت فحوصات دلالات الفساد على إرتفاع درجه الأس الهيدروجيني وحامض الثيوباربتيورك لعينات لحم الدجاج المنزوع ميكانيكيا التى تم جمعها من أ

و ب وارتفاع دلالات الفساد لعينات ذبائح الدجاج واجزائها التى تم جمعها من أ عن العينات التى تم جمعها من ب ولكنها ما زالت مقبوله . كما أشارت نتائج الفحص الميكروبيولوجى ان هناك إرتفاعاً جوهرياً في أعداد الميكروبات التى تم فحصها (الميكروبات الهوائية المحبة للحرارة وللبروده, واللاهوائيه والميكروب القولوني) لجميع العينات التى جمعت من أ عن ب وما زالت هذه الاعداد (أ) تفوق الحد المسموح به فى المواصفه القياسيه المصريه للدجاج المبرد (2005/1651) واللحم المنزوع ميكانيكيا (2005/4178). وتم عزل ميكروب الايشيريشياكولاى من ذبائح الدجاج, افخاذ الدجاج ولحم الدجاج المنزوع ميكانيكيا التى تم جمعها من أ بنسب 13.33, 13.33 و 20% على التوالى وعزل ميكروب السالمونيلا من لحم الدجاج المنزوع ميكانيكيا التى تم جمعها من أ بنسبه 20% بينما لم يتم عزل كلا من الايشيريشياكولاى و السالمونيلا من العينات التى جمعت من ب. وذلك يؤكد على مدى كفاءه نظام الهاسيب المستخدم فى تلك المجازر وان هذه العينات يتم تحضيرها تحت ممارسات تصنعيه جيده والتى تجعل تلك المنتجات غذاء امن للمستهلك. وباختبار حساسيه سلالات الايشيريشياكولاى و السالمونيلا المعزوله فى تلك الدراسه فقد وجد ان سلالات الايشيريشياكولاى حساسه لمعظم المضادات الحيويه ولكنهم يقاوموا السيبروفلوكساسين, الكو-تريمكزول, كلورمفنيكول والتيتراسيكلين. بينما سلاله السالمونيلا المعزوله حساسه للسيفوتاكسيم والسيبروفلوكساسين وتقاوم الامبيسيلن, الكو-تريمكزول والكلورمفنيكول.