Effect of radiation , hydrogen peroxide and chlorine on bacterial decontamination of broiler carcasses

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Abstract
A total of eighty broiler chicken carcasses samples were collected randomly from an automatic poultry slaughtering plant in Dakahlia Governorate just after washing in the chiller and divided into four groups each group consists of 20 chicken carcasses which were subdivided into two subgroups (ten carcasses for each subgroup).The1st group left as a control ,the 2nd group treated with 25 ppm chlorine (first subgroup), the 2nd subgroup of 2nd group treated with 50 ppm chlorine for three minutes where the reduction were 8,6,8 and 11,14,12% for APC,MPN of coliforms and *Staph. aureus* after 25 and 50 ppm chlorine respectively, the 3rd group treated with 1% hydrogen peroxide (first subgroup) and the 2nd subgroup treated with 2% hydrogen peroxide for two minutes where the reduction were 49,42,40 and 71,52,56% respectively and the 4th group treated with gamma rays first subgroup irradiated with 2 Kilo Gray (KGy) and the 2nd subgroup irradiated with 3KGy and the reduction were 84,87,80 and 89,ND,ND % respectively. Therefore this study was focused on the effect of Chlorine, Hydrogen peroxide and Irradiation on bacterial population, Coliforms and *Staph. aureus* in whole chicken carcasses from a local poultry plant with comparison between the three methods.

Key words: coliforms , *Staph. aureus*, gamma rays .

Introduction

Poultry meat is a nutritious food consumed all over the world because of its relatively low cost and low fat content, However it is highly perishable with a relatively short shelf ife even where it is kept under refrigeration. Although poultry conistitute an excellent source of high quality and easily digested protein it is liable to transmit different types of potentials to consumers **Zahra, (2001).** Many attempts were applied to reduce the microbial findings and level of poultry meat contamination through using variable chemicals like sodium hypochlorite which resulted in significant reduction in the number of microorganisms, **Ismail *et al*. (2001).** One of the newly emerging technologies to ensure microbiological safety of meat is radiation processing **Kanatt *et al*. (2005).** The safety and efficacy of irradiation in food preservation has been thoroughly demonstrated worldwide **Pelezar, *et al*. (1997)**. The radiation doses required to inactivate 90% of the common food borne pathogens associated with meat and poultry are in the range of 1-4 KGy, **Thayer *et al*. (1993).** The aerobic plate counts (APCs),Most Probable Number of Coliforms (MPN) and counts of *Staph. aureus* in chicken carcasses collected from different poultry shops were $3.38x10^6 \pm 1.02x10^6$, $2.58x10^4 \pm 0.9x10^4$and $9.95x10^3 \pm 1.56x10^3$cfu/gm respectively, **Morshdy *et al*., (2008).**
While the counts were 6.1±0.1, 2.7±0.1 and 3.7±0.1 log cfu/gm respectively in fresh whole chicken broiler carcasses, **Mira** and **Eskandar, (2007)** also **Mahmoud** and **Hammouda(2006**) examined thigh and breast and found that APCs and *Staph. aureus* count were $1.4x10^6 \pm 4x10^5$and $6x10^5 \pm 2x10^4$ while *Staph. aureus* count were $8.9x10^3 \pm 0.3x10^3$ and $2.7x10^3 \pm 1.7x10^4$ respectively. **May (1974)** mentioned that final rinsing of commercially slaughterd broilers with 25 ppm chlorine resulted in significant reduction in TVCs., **Whyte *et al.*, (2001)** proved that final rinsing of poultry carcasses with 25ppm chlorine reduce TVCs from 4.98±0.38 to4.52±0.24 and Enterobacteriaceae from3.37±0.31 to 3.16±0.16 log cfu/gm. Commercial chlorine chiller on poultry carcasses during processing reduce APCs, *Staph. aureus* from $6.2x10^4$and$1.4x10^4$ to$2.4x10^4$ and $6x10^3$cfu/gm respectively **Gelis and Kabul (2006)**, while

Karen *et al.*,(2010)mentioned that significant reduction in APCs and Coliforms by 50 ppm chlorine rinsing for three minutes the reduction were 0.4and0.21 log cfu/gm respectively. Hydrogen peroxide is highly unstable and breaks down into water and single oxygen molecule which is strong oxidizing and disinfecting agent **Black *et al*.,(2008),** also several investigators concluded that chlorine and hydrogen peroxide were most frequently used in commercial poultry processing as antimicrobials due to their availability, low cost and efficacy **Bolder (1997), Hugas and Tsigarida (2008)**, **Northcutt and Jone (2004**), also **Mostafa (2010)** concluded that Hydrogen peroxide0.1% in chiller reduce APCs, Coliforms and *Staph. aureus* counts by 97.3%, 72.01% and 94.9 % respectively and similar results were recorded by **EL-said *et al.*(2002).Oliveira *et al.(*2009)** mentioned that gamma radiation 1.5and 3 KGy on chicken breast reduce APCs from 3.0± 0.01 to less than1.0 and total Coliforms reduced from 2.9±0.3 to 0.3±0.4 and *Staph. aureus* to less than 0.5 log cfu/gm respectively. Also **Min *et al.,(*2007)** reported that no viable cells were detected after exposure of inoculated chicken thigh and breast to 2KGy.Irradiation with air and vacuum reduce mesophiles from 4 and 3.8 to 3.3 and2.7 log cfu /gm at 2 KGy and 1.7 and1.6 log cfu/gm at 3 KGy while Coliforms reduced from2.2 and 3 log cfu/gm to 0.6 and 0.5 log cfu/gm by 2 KGy and not detected at 3 KGy respectively **(Mantilla *et al.*, 2011).**
The results recorded by **Mohamed *et al., (*2008)** on fish fillets exposed to radiation on APCs, Coliforms and *Staph. aureus* were in control samples 6.4,2.68 and 2.38 while at 2 KGy were 2.6, less than 3 and less than 2 and at 3KGy were2.1, less than 3 and less than 2 log cfu/gm respectively.

Materials and Methods

1-Sampling;

A total number of 80 broiler chicken carcasses from an automatic poultry slaughtering plant in Dakahlia, Egypt were collected after complete preparation (slaughtering, scalding, defeathering and evisceration), Just after washing in the chiller. They were divided into four groups, the first group [control group] were placed in a chiller containing cold water for 60 minutes and left as a control. The second group were divided into two subgroups (each one ten carcasses) one of them rinsed with 25ppm chlorine (sodium hypochlorite 14% vol./vol.) and the other one rinsed with 50 ppm chlorine for three minutes then sampling. The third group were also divided into two subgroup (each one ten carcasses) one of them rinsed with 1% hydrogen peroxide and the other one rinsed with 2% hydrogen peroxide for two minutes. The fourth group divided into two subgroups (each one ten carcasses), 250 gm from each carcass were packed in sterile polyethylene bags heat sealed then irradiated at National Center for Radiation Research and Technology (NCRRT) Nasr City, Cairo. The irradiation source was Cobalt 60 irradiation model ISS LEDDVAT-ED. The dose rate was established using alanine transfer dosimeter and variation in the absorption of irradiation dose was minimized by placing the samples within a uniform area of the irradiation field. One subgroup exposed to 2 KGy and the other subgroup exposed to 3 KGy of gamma rays. After irradiation, hydrogen peroxide and chlorine treatment 25gm of each exposed samples were homogenized with 225 ml of 0.1% peptone water in a stomacher for 2.5 minutes at 3000 rpm followed by ten fold serial dilution in 0.1% peptone water. Each of the prepared samples were examined for enumeration of its microorganisms content as follows;

2-Total Colony Count;

0.1 ml of each sample was evenly spread over the dry surface of standard plate count agar using a sterile bent glass spreader only plates contain 30-300 colonies were counted as the total colony per gram of samples according to **APHA(1985).**

3-*Coliform Count;*

It was carried out by using most probable number technique (MPN)according to **ICMSF (1978).**

4-*Staph.aureuscount;*

Using Baired Parker agar medium with egg yolk tellurite emulsion **APHA(1992).**

Biochemical Identification of *Staph. aureus*; Identification of *Staph. aureus* according to **APHA (1992);** films were prepared from the pure culture of isolated organisms stained with Gram's stain and examined microscopically for G+ve cocci. The tests for identification were motility, catalase test, mannitol test and coagulase test (tube method).

Results

Table (1). Statistical analytical results for effect of chlorine on decontamination process with chlorine.

Microbial count mean±S.E.	control	before decontamination	after decontamination with 25 ppm chlorine	Red. %	before decontamination	after decontamination with 50 ppm chlorine	Red. %
APC	$5.4x10^5 \pm 1.7x10^3$	$5.6x10^5 \pm 1.4x10^3$	$5.2x10^5 \pm 1.2x10^3$	7.14	$4.7x10^5 \pm 2.5x10^3$	$4.2x10^5 \pm 3.8x10^3$	11
MPN	$5.5x10^3 \pm 2.8x10$	$3.4x10^3 \pm 2.9x10$	$3.2x10^3 \pm 1.2x10$	6	$4.2x10^3 \pm 3.4x10$	$3.6x10^3 \pm 2.8x10$	14
Staph.aureus	$4.2x10^3 \pm 3.9x10$	$4x10^3 \pm 2.6 x10$	$3.7x10^3 \pm 1.5x10$	8	$5x10^3 \pm 1.7x10$	$3.7x10^3 \pm 1.4x10$	26

APC=aerobic plate count & MPN=most probable number of coliforms&*Staph.aureus*=staphylococcus aureus count, Red. = reduction

Table (2). Statistical analytical results of examined whole chicken carcasses before and after decontamination process with hydrogen peroxide.

Microbial count mean±S.E.	control	before decontamination	after decontamination with 1 % H_2O_2	Red. %	before decontamination	after decontamination with 2 % H_2O_2	Red. %
APC	$5.4x10^5 \pm 1.7x10^3$	$4.3x10^5 \pm 1.8x10^3$	$2.2x10^5 \pm 0.1x10^3$	49	$5.4x10^5 \pm 2.6x10^3$	$1.6x10^5 \pm 0.3x10^3$	71
MPN	$5.5x10^3 \pm 2.8x10$	$5.8x10^3 \pm 2.9x10$	$3.4x10^3 \pm 2.9x10$	42	$4.1x10^3 \pm 2.6x10$	$2x10^3 \pm 1.1x10$	52
Staph. aureus	$4.2x10^3 \pm 3.9x10$	$4.6x10^3 \pm 1.8x10$	$2.8x10^3 \pm 1.x10$	40	$4.5x10^3 \pm 2.9x10$	$2x10^3 \pm 1.2x10$	56

Table (3). Statistical analytical results of examined whole chicken carcasses before and after decontamination process with gamma rays irradiation.

Microbial count mean±S.E.	Control	before irradiation	after irradiation with 2KGy	Red. %	before irradiation	after irradiation with 3KGy	Red. %
APC	$5.4x10^5 \pm 1.7x10^3$	$5.3x10^5 \pm 1.4x10^3$	$8.4x10^4 \pm 1.3x10^2$	84	$5.5x10^5 \pm 3.1x10^3$	$6.2x10^4 \pm 0.1x10^2$	89
MPN	$5.5x10^3 \pm 2.8x10$	$5.6x10^3 \pm 1.7x10$	$7.5x10^2 \pm 0.01x10$	87	$4.6x10^3 \pm 2.2x10$	ND	
Staph.aureus	$4.2x10^3 \pm 3.9x10$	$4.2x10^3 \pm 2x10$	$8.6x10^2 \pm 0.09x10$	80	$3.8x10^3 \pm 2.7x10$	ND	

Discussion

The sanitary evaluation of slaughtered birds is based mainly on its microbial findings and level of contamination. Many investigators proved that more than 70 % of poultry carcasses are contaminated and the microbial count being more than $3x10^5$cfu/ml, **Tamblyn *et al*.(1997).**

Table (1) shows that the mean values of APCs in examined control samples. Samples before and after decontamination with 25ppm and 50ppm chlorine were $5.4x10^5 \pm 1.7x10^3$,$5.6x10^5 \pm 1.4x10^3$, $5.2x10^5 \pm 1.2x10^3$, $4.7x10^5 \pm 2.5x10^3$ and $4.2x10^5 \pm 3.8x\ 10^3$ cfu/ gm with reduction percent 7.14% and 11% after decontamination with 25 and 50 ppm chlorine respectively. While MPN count were $5.5x10^3 \pm 2.8x10$, $3.4x10^3 \pm 2.9x10$, $3.2x10^3 \pm 1.2x10$, $4.2x10^3 \pm 3.4x10$ and $3.6x10^3 \pm 2.8x10$ cfu/gm with reduction percent 6% and 14% after decontamination with 25 and 50 ppm chlorine respectively and the counts of *Staph. aureus* were $4.2x10^3 \pm 3.9x10$, $4x10^3 \pm 2.6x10$, $3.7x10^3 \pm 1.5x10$, $5x10^3 \pm 1.7x10$and $3.7x10^3 \pm 1.4x10$ cfu/gm with reduction percent 8% and 26% after decontamination with 25 and50 ppm chlorine respectively. The results in control samples nearly in accordance with **Tamblyn *et al.*(1997), Mahmoud** and **Hammouda (2006), Mira and Eskandar(2007)**; **Morshdy *et al.* (2008).** The higher microbial load could be attributed to the technique of evisceration and poor hygienic levels. The reduction percent were more than those reported by **Whyte *et al.* (2001)** and similar to **Gelis** and **Kabul(2006), Stopforth *et al.(*2007)**; **Karen *et al.(*2010)** the difference in percent of reduction may be due to the final processing and rinsing before decontamination process.

Table(2); the results of decontamination with hydrogen peroxide (H_2O_2) declared that. the APCs were $5.4x10^5 \pm`1.7x10^3$, $4.3x10^5 \pm 1.8x10^3$, $2.2x10^5 \pm 0.1x10^3$, $5.4x10^5 \pm 2.6x10^3$ and $1.6x10^5 \pm 0.3x\ 10^3$cfu/gm with reduction percent 49% and71%after decontamination with hydrogen peroxide1% and 2% respectively while MPN count were $5.5x10^3 \pm 2.8x10$,$5.8x10^3 \pm 2.9x10$, $3.4x10^3 \pm 2.9x10$, $4.1x10^3 \pm 2.6x10$ and $2x10^3 \pm 1.1x10$ cfu/gm with reduction percent42% and 52% after decontamination with 1% and 2% hydrogen peroxide respectively and the counts of *Staph.aureus* were $4.2x10^3 \pm 3.9x10$, $4.6x10^3 \pm 1.8x10$, $2.8x10^3 \pm 1x10$, $4.5x10^3 \pm 2.9x10$ and $2x10^3 \pm 1.2x10$ cfu/gm with reduction percent 40% and56%respectively similarly with those recorded by **Bolder(1997), Northcutt** and **Jones(2004),Black *et al.(*2008)**; **Huga and Tsigarida(2008)** higher results recorded by **EL -Said *et al.*(2002)**; **Mostafa (2010),**while the reduction percent after 2%H_2O_2 were similar to **Northcutt** and **Jones (2004*)*** and lower than the results recorded by **Mostafa (2010).**

The results in ***table(3)*** declared that the APCs were $5.4x10^5 \pm 1.7x10^3$, $5.3x10^5 \pm 1.4x10^3$, $8.4x10^4 \pm 1.3x10^2$, $5.5x10^5 \pm 2.6x10^3$ and $6.2x10^4 \pm 0.1x10^2$cfu/gm with reduction percent of 84% and 89% after irradiation with 2 KGy and 3 KGy respectively while MPN count were $5.5x10^3 \pm 2.8x10$, $5.6x10^3 \pm 1.7x10$, $7.5x10^2 \pm 0.01x10$, $4.6x10^3 \pm 2.2x10$cfu/gm respectively with reduction percent 87% after irradiation with 2KGy and not detected after irradiation with 3KGy and the counts of *Staph .aureus* were $4.2x10^3 \pm 3.9x10$, $4.2x10^3 \pm 2x10$, $8.6x10^2 \pm 0.09x10$, $3.8x10^3 \pm 2.7x10$ cfu/gm respectively with reduction percent 80% after irradiation with 2 Kgy and not detected after irradiation with 3 KGy the reduction percent after irradiation nearly similar to **Mohamed *et al* . *(*2008),Oliveira *et al. (*2009); Mantilla *et al.* (2011**), while higher reduction recorded by **Min *et al. (*2007)** and the reduction percent after irradiation with 3 KGy were 89% for APC while Coliforms and *Staph. aureus* were not detected similarly to **Mohamed *etal .(*2008), Oliveira *et al. (*2009); Mantilla *et al.(*2011).**

In conclusion from the aforementioned results it is declared that chlorine is a bactericidal, viricidal and fungicidal agent can be successfully applied in the food industry and may constitute an important factor in reducing losses resulting from food spoilage and human infection. The use of hydrogen peroxide in chiller used for chicken chilling prior to its packaging, greatly reduce its bacterial load. It is a power-

ful sanitizer for poultry carcasses, it enhances its meat quality. Radiation did not affect the sensory character of chicken carcasses and produces no apparent changes in the organoleptic characteristics, it inhibit microbial growth and so extends the shelf life of the product. From the results it was obvious that the microbial reduction was greater after radiation than after hydrogen peroxide and after hydrogen peroxide than after chlorine decontamination.

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تأثير الإشعاع وماء الأكسجين والكلور على إزالة التلوث البكتيري لذبائح بدارى التسمين

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الملخص العربى

اشتملت هذه الدراسة على عدد ثمانون عينة لذبائح بدارى التسمين تم تجميعها من احدى المجازرالألية بمحافظة الدقهلية بعد انتهاء مراحل الذبح والتنظيف والتجويف والغسيل بالماء البارد مباشرة حيث تم تقسيمها إلى أربعة مجموعات : الأولى : لم يتم معالجتها واستخدمت كدليل عددها عشرون عينة ، الثانية : تم تقسيمها إلى مجموعتين كل منها عشرة عينات حيث تم غسل المجموعة الأولى منها بمحلول الكلور 25 جزء في المليون والمجموعة الثانية تم غسلها بمحلول الكلور 50 جزء في المليون لمدة ثلاث دقائق ، الثالثة : تم تقسيمها إلى مجموعتين كل منها عشرة عينات حيث تم غسل الاولى بمحلول ماء الأكسجين 1 % والثانية بمحلول ماء الأكسجين 2 % لمدة دقيقتين ، الرابعة : تم تعريضها لأشعة جاما بعد تقسيمها إلى مجموعتين كل مجموعة عشرة عينات حيث تم تعريض المجموعة الأولى إلى أشعة جاما 2 كيلو جراي والمجموعة الثانية تم تعريضها لأشعة جاما 3 كيلو جراي ولذلك تركز هذه الدراسة على تأثير الكلور وماء الأكسجين وأشعة جاما على إزالة التلوث البكتيري في لحوم بدارى التسمين .