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ASSESSMENT AND IMPROVEMENT OF HYGIENIC STATUS OF CHICKEN FILLET FROM SLAUGHTERHOUSES USING ORGANIC ACIDS FROM NATURAL SOURCES

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ABSTRACT

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This study was conducted firstly to investigate the bacteriological status of chicken fillet produced in poultry slaughterhouses and secondly to improve its safety at the home level during preparation for cooking using vinegar or lemon juice as a natural source for organic acids. Sixty samples of chicken fillet, (30 each of breast and thigh) were collected from slaughterhouses. Mean aerobic plate and coliforms counts for thigh samples (4.41 and 1.83 log cfu/g) were significantly higher ($P<0.01$) than that of breast samples (3.89 and 1.42 log cfu/g). Each of *Salmonella* Typhimurium and *S. aureus* were isolated from 3.3% of samples, meanwhile, *E. coli* was detected in 30% and 10% of thigh and breast samples, respectively. Accordingly, 36.7% and 80.0% of thigh and breast samples, respectively were compatible with the Egyptian standards. Dipping of chicken fillet in vinegar or lemon juice (2% acetic or citric acids) for 25 min reduced the aerobic plate count by one log cfu/g and *S. aureus* by 2 log cfu/g without significance difference ($P>0.05$) between them. On the other hand, lemon juice significantly reduced salmonella (2 log cfu/g) and *E. coli* (3 log cfu/g) counts one log more than vinegar (1 and 2 log cfu/g for each of them, respectively).

***Key words**: Chicken fillet, E. coli, S. aureus, Salmonella, vinegar, lemon juice*

INTRODUCTION

Food-borne diseases, caused by agents that enter the body through the intake of contaminated food materials are one of the primary public health concerns (Tan *et al*., 2013). Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning (Mulder, 1999). Poultry and poultry products rank first or second in foods associated with disease in most of the countries all over the world (Bean and Griffin, 1990). Unhygienic practices, use of contaminated instruments and materials in food processing are mainly associated with food-borne diseases (Wilfred *et al*., 2012). An effective way of preventing food-borne human diseases is to monitor the microbiological quality of poultry meat and meat products during production, storage and distribution. Monitoring of foodborne pathogens in food products are the only means to cope with the problem promptly (Chang *et al*., 2013). Microflora of raw chicken meat is heterogeneous and originates from slaughtering premises, operators' hands, equipment and outfit, and water and air (Fries, 2002). In addition to pathogenic bacteria, special attention in the hygienic production and storage of chicken meat is paid also to total count of aerobic mesophilic bacteria, enterobacteria and *Escherichia coli*. These bacteria are considered indicators of microbiological quality (Capita *et al*., 2002), which give an idea about the hygienic measures during further processing and help in assessing the keeping quality of further processed chicken meat products (Aberle *et al*., 2001).

Foodborne Salmonellosis is important public health problem in many parts of the world, causing gastrointestinal illness, substantial morbidity, and hospitalization and economic burden worldwide (Fearnley *et al*., 2011). The Salmonella serovars most frequently isolated from humans are *Salmonella* Typhimurium and *Salmonella* Enteritidis, the last is the most prevalent global serovar of Salmonella (Hassanein *et al*., 2011). The primary reservoir of Salmonella is the intestinal tract of animals and birds, which contaminate the muscles and organs during slaughtering (Paiao *et al*., 2013). Poultry and poultry products are the most potential source of Salmonella food poisoning in man (Lynch *et al*., 2006), that can be transmitted to humans through the handling of raw products, or through consumption of undercooked poultry meat (Kimura *et al*., 2004).

E. coli is responsible for 25% of the infant diarrhoea in developing countries (WHO, 2000). Shiga toxin producing *E. coli* (STEC) was first recognized as a human pathogen in 1982 in the USA when strains of serotype O157:H7 caused two outbreaks of hemorrhagic colitis (Wells *et al.*, 1983). Its presence in food materials are considered to be an indicator for the presence of other pathogenic bacteria in the respective food items (Shar *et al*., 2010).

Staphylococcus aureus is a significant cause of avian disease and may thus contaminate foods as a result of processed carcasses (Mead and Dodd, 1990). Enterotoxin-producing *S. aureus* is the most common cause of food-borne human illness throughout the world (Do Carmo *et al*., 2004). The foods that most frequently cause this type of poisoning are red meat and poultry and their products (Kitai, 2005). While staphylococci commonly occur on the skin and nasopharynx of healthy poultry (Mead and Dodd, 1990), it is primarily *S. aureus* which can survive, colonize, and persist at various processing stages in commercial poultry processing plants due to the expression of various key properties, including adhesion and chlorine resistance (Huys, 2005). Monitoring of *S. aureus* is important for both of the evaluation of safety and hygienic quality of chicken meat, and also in the aetiology of food poisoning (Jablonski and Bohach, 1997).

Chemical decontamination was first used in the 1960s and contributed to the control of food pathogens (Acuff, 2005). There is an increasing interest in applying natural antimicrobial compounds in the food industry as consumers are increasingly avoiding the consumption of foods treated with chemicals. This creates new challenges in providing efficient food preservation, especially in the area of microbial safety (Suppakul *et al*., 2003). Organic acids are popular because of the lack of toxicological implications when applied at the prescribed concentrations. U.S. Department of Agriculture (2008) states that acetic and citric acids are generally recognized as safe substances (GRAS) and is allowed in or on processed products labeled as organic. Application of organic acids on meat surfaces is a common procedure; acid treatments are cheap, simple and fast, and have shown clear efficiency (Hinton and Corry, 1999).

Citric and acetic acids have been used for years for decontamination of bacteria on beef, pork, and poultry (Mani-Lopez *et al.*, 2012). Using of lemon juice or vinegar in food (as salads) provide a harsh environment for foodborne pathogens such as Salmonella and *E. coli* to survive because of the acetic or citric acids (Beuchat *et al.*, 2006). Acetic acid is the active ingredient of house-hold vinegar has been tested and approved as dipping or spraying treatments. Normal white household vinegar consists of a concentration of approximately 5% acetic acid. When this diluted to at least 2% it is actually recommended as a preservative (Mani-Lopez *et al.*, 2012). However, the use of acetic acid might be limited due to their flavor and taste, diluted solutions of organic acids (1-3%) are generally without effect on the desirable sensory properties of meat (Min *et al*., 2007).

After appearance of avian influenza and as a preventive measure the government restricted transmission of life chicken between governorates and encouraged establishment of poultry slaughterhouses, consequently many new slaughterhouses appeared. Therefore, this study aimed to investigate the bacteriological status of chicken fillet produced in slaughterhouses and the use of vinegar and lemon juice as natural sources of acetic and citric acids to improve its safety at the home level during preparation for cooking.

MATERIALS and METHODS

First part: Survey of chicken fillet from slaughterhouses

Sample Collection: A total of 60 samples of chicken skin less fillet from slaughterhouses, 30 each of breast and thigh meat, were collected and transported to the laboratory in ice box without due delay to be examined bacteriologically. Homogenate of each sample (10^{-1}) was prepared by buffered peptone to perform aerobic plate count (APC) and coliform count cfu/g, in addition, detection of *Escherichia coli*, *Staphylococcus aureus* and Salmonella species were performed according to APHA (2001).

Second part: Decontamination using lemon juice and vinegar

S. aureus (ATCC 29213), *Salmonella* Typhimurium (ATCC 14028) and *Escherichia coli* (ATCC 8739) strains (acquired from the Department of Food hygiene, Animal Health Research Institute, Dokki, Giza) from frozen cultures were activated with two successive passes in 9 ml of tryptic soy broth (TSB) (Oxoid) and incubated at 37^{o}C for 18 h. For each individual strain, 1 ml of the stock inoculum was added to 100 ml of TSB and incubated with shaking at 37^{o}C for 18 - 24 h, then further diluted to reach a final concentration of approximately 5 log cfu/mL (determined by plating on specific media). Then, 2.5 ml of the stock inoculum was added to 250 ml of sterilized saline to give final concentration of approximately 3 to 4 log CFU/mL in the dipping solution. Chicken fillet (previously tested to be free of concerned microorganisms) were inoculated by being placed for 20 s in the dipping solution followed by drying under a hood at least 20 min to allow attachment of bacteria (Corry *et al*., 2007).

Acetic and citric acids 2% from vinegar (5% acetic acid) and lemon juice (4.5% citric acid), respectively, were prepared for acid treatments. Each one of the inoculated chicken fillet was placed separately in the dipping solution (at ambient temperature) for 5, 10, 15, 20 or 25 min.

Bacterial count: The acid-treated and non-treated chicken fillet were counted on selective media for each strain (Baird Parker for *S. aureus*, XLD for *S*. Typhimurium and EMB, for *E. coli*) in duplicate to determine the initial count before treatment and after treatment with the organic acids. Twenty-five grams of chicken fillet were placed in a stomacher bag with 225 ml of 0.1% peptone water and stomached for 1 min. Serial dilutions were prepared, spread plated in duplicate on selective media for each strain and incubated at 35^{o}C for 24-48 h. Colonies were enumerated, and the cfu/g was calculated.

Chicken fillet samples (from first part) proved to be exceeding the permissible limit of aerobic plate count (8 samples) according to the Egyptian standards were treated and recounted as inoculated microorganisms.

Statistical analysis: Data were analyzed by using mixed procedure from SPSS software (release 20, IBM CO) after logarithmic transformation for bacteriological count. A completely randomized design was selected in the second part. The experiment was conducted in three repetitions. Means were separated by T-test, and significance was tested at $\alpha = 0.05$.

RESULTS

Part I: survey of chicken fillet from slaughterhouse

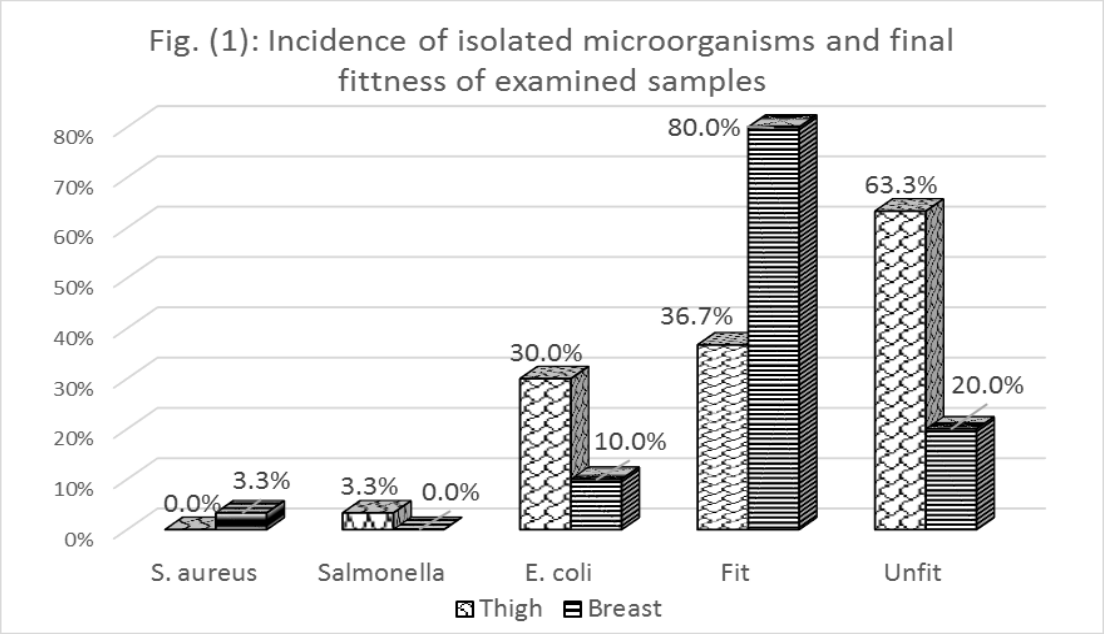
Table 1: Statistical analysis of bacterial counts (log cfu/g) in examined samples

Bacterial count	APC				Coliforms			
Samples	Thigh		Breast		Thigh		Breast	
Minimum	3.30		2.95		1.00		0.56	
Maximum	5.90		5.00		3.38		3.04	
Mean ± SE	4.41 ± 0.16^{A}		3.89 ± 0.12^{a}		1.83 ± 0.12^{B}		1.42 ± 0.13^{b}	
Compatibility	No.	%	No.	%	No.	%	No.	%
Less than limit	22	73%	30	100%	19	63%	26	87%
More than limit	8	27%	0	0%	11	37%	4	13%

There are significance differences between means have same capital and small litter (P<0.01) for the same count.

Table (1) revealed the statistical analysis of bacterial counts of examined samples. Mean aerobic plate count for thigh samples (4.41 log cfu/g) was significantly higher (P<0.01) than breast samples (3.89 log cfu/g). Also coliforms count for thigh samples (1.83 log cfu/g) was significantly higher (P<0.01) than breast samples (1.42 log cfu/g). Concerning the compatibility with the Egyptian standards (2005), 27% of thigh samples was more than the aerobic count stated by the standard (5 log cfu/g); meanwhile all breast samples were within this limit. On the other hand, for coliforms count, 37% and 13% of thigh and breast samples respectively were more than the accepted limit stated in the standard (2 log cfu/g).

Fig. (1) illustrate the incidence of isolation of *S. aureus*, Salmonella and *E. coli* and final fitness of samples according to bacterial counts and isolation comparing to the Egyptian standards. *S. aureus* was isolated from one sample only (3.3%) of breast but failed to be detected from any sample of thigh. On the contrary, Salmonella was isolated from one sample only from thigh (3.3%) (*Salmonella* Typhimurium) but failed to be detected from any sample of breast. On the other hand, *E. coli* was detected in 9 samples of thigh (30%) and 3 samples of breast (10%).

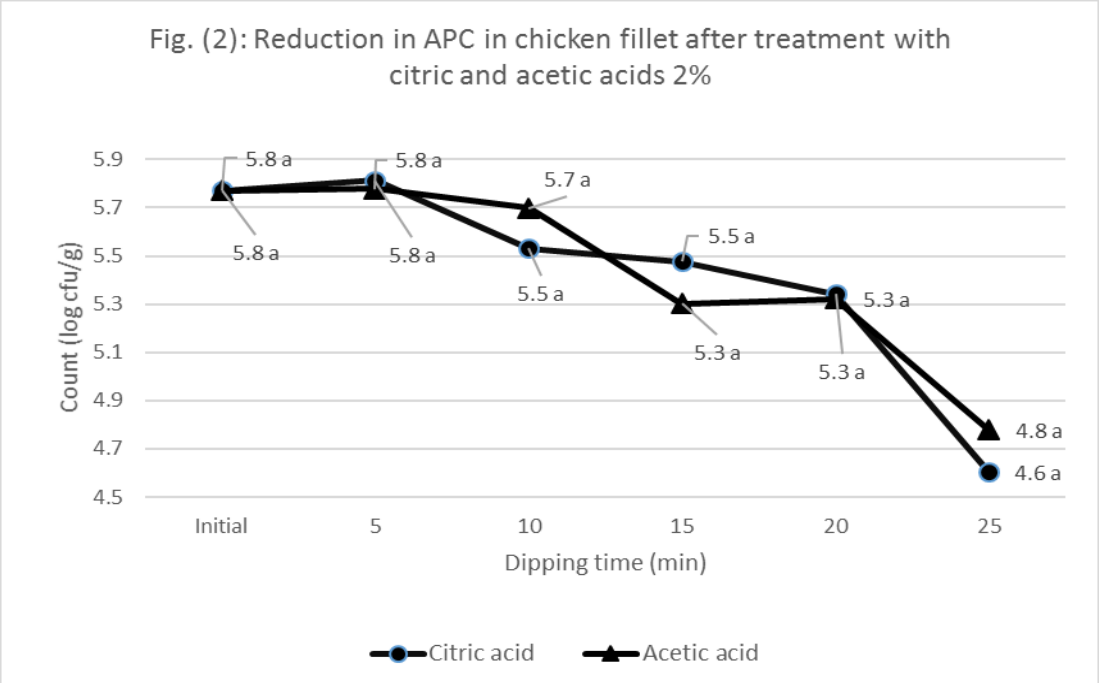


The overall fitness of samples according to microbial counts and isolation of food poisoning microorganisms in competence with the Egyptian standards was 11 samples (36.7%) in thigh and 24 samples (80.0%) in breast.

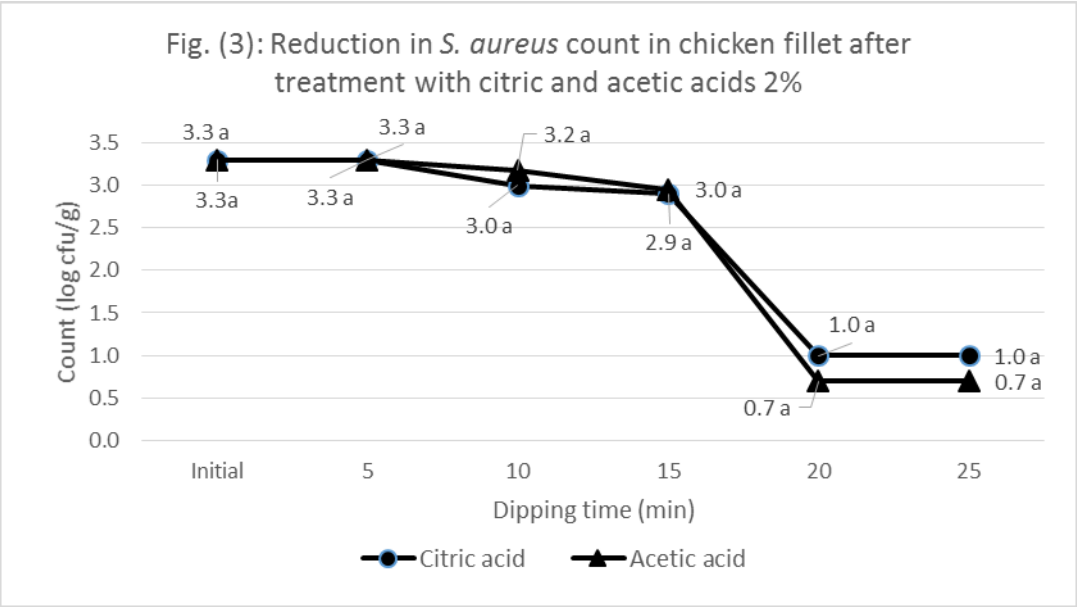
Part II: effect of citric and acetic acids on improvement of chicken fillet

Prior to treatment with organic acids, the mean initial APC was 5.8 log cfu/g (Fig. 2), which slowly decreased after dipping in each of the two treatments. The reduction reached 0.5 log cfu/g after dipping for 20 min, but after 25 min, the count decreased by one log. There was no significance difference (P>0.05) between the two treatments at the same dipping time.

The initial count of *S. aureus* inoculated on the chicken fillet (Fig. 3), was 3.3 log cfu/g, which didn't reduced even after dipping in acid solutions for 5 min. After 10 min of dipping of the inoculated fillet, the count begin to be reduced slowly. On the contrary, the count was sharply reduced by 2 logs after 20 min of treatment without significance difference (P>0.05) between the two treatments.



There are significance differences between means have same capital and small litter (P<0.01) for the same time.



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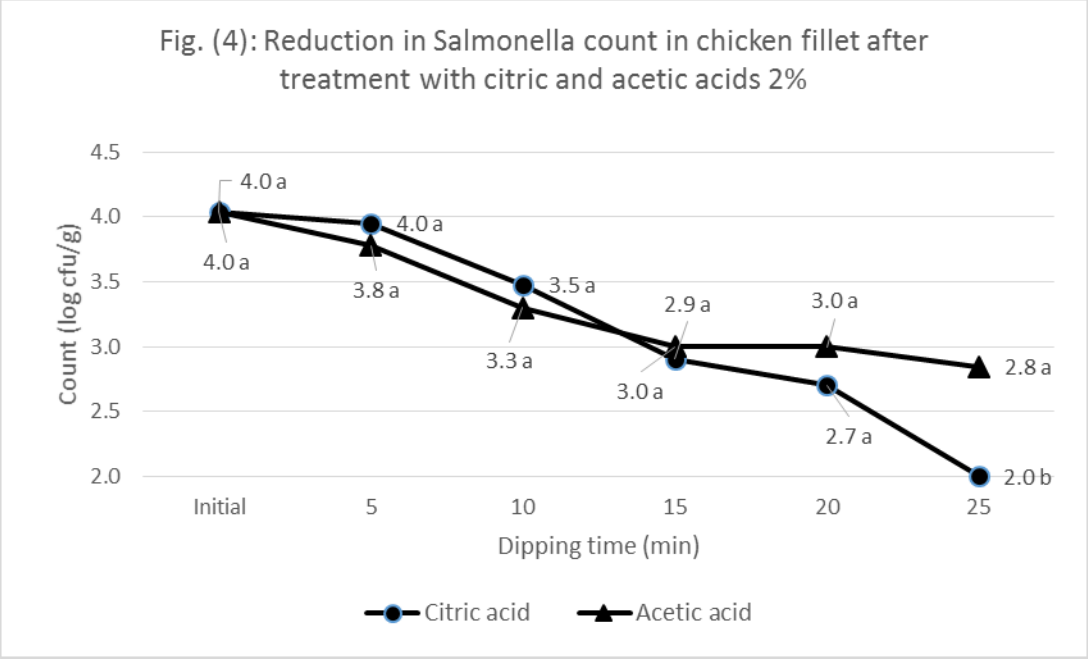
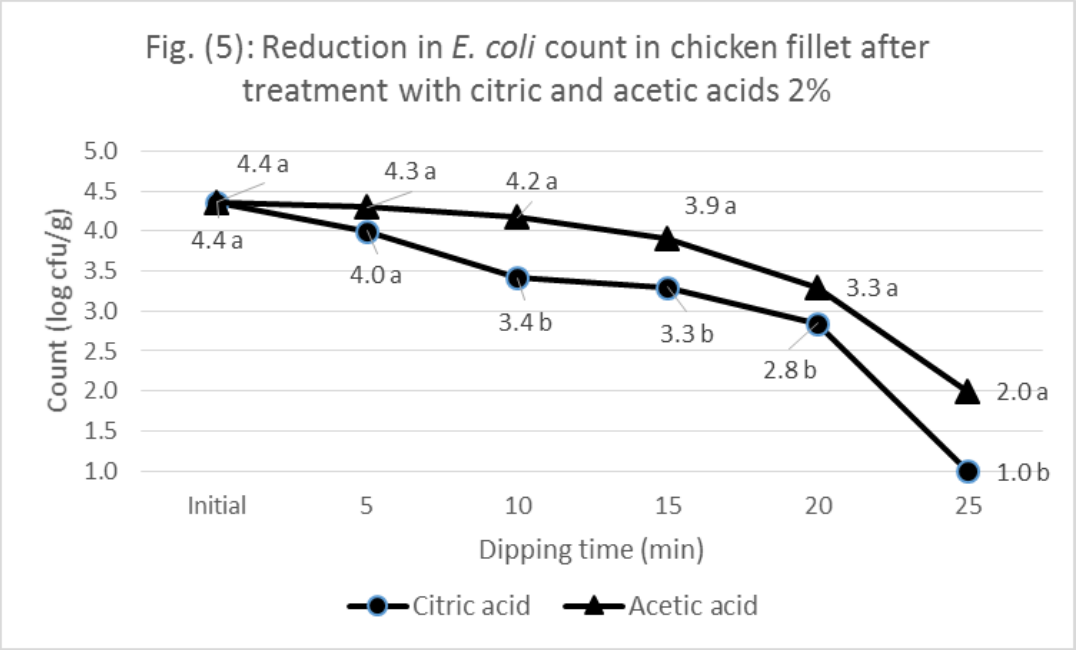


There are significance differences between means have same capital and small litter (P<0.01) for the same time.



There are significance differences between means have same capital and small litter (P<0.01) for the same time.

Regarding salmonella inoculated chicken fillet (Fig. 4), the initial count before treatment was 4 log cfu/g, which begin to reduce after treatment for 10 min. the reduction reached one log cfu/g after 15 min without significance difference (P>0.05) between the two treatments. On the other hand, after dipping for 25 min, citric acid treatment significantly (P<0.05) produced more reduction in salmonella count than acetic acid to reach 2 log reduction than the initial count comparing to 1.2 log for acetic acid.

For *E. coli* inoculated chicken fillet (Fig. 5), the initial count before treatment was 4.4 log cfu/g. Citric acid treatment reduced the count significantly (P<0.05) more than acetic acid beginning from 10 min dipping time as it reduced the count by one log cfu/g after 10 min. After 25 min of dipping, citric acid reduced *E. coli* count by 3.3 log cfu/g, while acetic acid reduced the count to a lesser extent (P<0.05) (2.4 log cfu/g).

DISCUSSION

The initial microbial load depends on the physiological status of the animal at slaughter, the spread of contamination into slaughterhouses and during processing, while temperature and other conditions of storage during distribution can also influence the rate of spoilage (Nychas *et al*., 2008). Concerning the bacterial count nearly similar results were obtained by Daoud *et al*. (2012) for coliforms count (1.7 log cfu/g); Kozačinski (2006) and Odwar *et al*. (2014) for breast meat and a little bit higher results were recorded by Shawish (2011) (5 log cfu/g). Meanwhile, lower results were recorded by Haleem *et al*. (2013) (3.45 log cfu/g in thigh and 2.33 cfu/g in breast meat), Odwar *et al*. (2014) and Daoud *et al*. (2012) 3.3 log cfu/g for APC. On the other hand, higher results were recorded for APC by Al-Dughaym and Altabari (2010), Azab (2013) (7.33 log cfu/g) and Ibrahim *et al*. (2014) (6.7 log cfu/g) and for coliform Haleem *et al*. (2013) (2.3 log cfu/g in thigh and 3.1 cfu/g in breast meat).

High levels of bacteria and microorganism in food products can potentially generate undesirable deteriorations in flavor, odor, color, sensory, and textural properties and may even become harmful to human health (Raouche *et al*., 2011). The higher content of microbial flora in thigh than breast may be attributed to high content of fat in thigh as compared with breast (Haleem *et al*., 2013). Also thigh need more hand work than breast which lead to more contamination from the work environment and workers' hands.

Concerning Salmonella isolation, nearly similar results were obtained by Anju *et al*. (2014) (4.44 %) and Shawish (2011) (4.3%), while Haleem *et al*. (2013) didn't isolated any salmonella strains from both thigh and breast meat. On the other hand, higher incidence were recorded by Kozačinski (2006) (10.60%); Freitas *et al*. (2010) (10%); Thai *et al*. (2012) (38.8%) and Saeed *et al*. (2013) (22%).

Regarding *E. coli*, nearly similar results were recorded by Suthienkul *et al*. (1990) (9%) for breast, Schaumburg *et al*. (2014) (23%) and Akbar *et al*. (2014) (25%) for thigh, but somewhat higher results were recorded by Zhao *et al*. (2001) (38%) and Bhattacharjee *et al*. (1996) (41%). On the other hand

very higher results were recorded by Hossain *et al*. (2008) (60%) and Odwar *et al*. (2014) (78%).

Concerning *S. aureus*, nearly the same results were recorded by Schaumburg *et al*. (2014) (3%), while lower results were recorded by Lin *et al*. (2009) (0.3% and 0.4%). On the other hand, higher and very higher results were obtained by Hanson *et al*. (2011) (17.8%), Shawish (2011) (21.4%), Kozačinski (2006) (30.30%), Martins *et al*. (2013) (62%) and Kitai *et al*. (2005) (65.8%).

Not only can *S. aureus* enter the process on raw materials, but it can also be introduced into foods during processing from unclean hands and unsanitary utensils and equipment. The hazard develops into toxin formation when raw materials and products are exposed to temperatures between 10°C and 21.1°C for more than 12 h or to temperatures greater than 21.1°C for more than 3 h (FDA, 2001).

Concerning the overall fitness Odwar *et al*. (2014) found that 76% of chicken meat samples fall under the unacceptable coliforms count limit. On the contrary of our results, Shawish (2011) and Azab (2013) didn't find any significant difference between thigh and breast.

Concerning the reduction in APC and pathogenic microorganisms, similar results were obtained by Min *et al.* (2007) and Frederick *et al*. (1994) who used 2% acetic acid to reduce APC and coliforms count by about 1 log cfu/g. Meanwhile higher reduction rates were recorded by Hamby *et al*. (1987) (1.8 to 4.3 log/cm^2), Min *et al.* (2007) who reported 3 log cfu/g reduction using citric acid and Menconi *et al*. (2013) (more than 6 log/ section).

Similarly, Tamblyn and Conner (1997) recorded 1.9 log reduction in *S.* Typhimurium count using citric acid (4%). Meanwhile, 2% acetic acid significantly reduced Salmonella according to Frederick *et al*. (1994) this reduction was 0.5 to 0.8 log CFU/cm^2 according to Dickson (1992). Menconi *et al*. (2013) reported a significant reduction in *S.* Typhimurium and *E. coli* O157:H7 (3.8 and 3.2 log cfu/g) using 0.8% organic acid combination.

Both of citric and acetic acids 2% proved to be effective as decontaminant in chicken fillet against *S. aureus* and *E. coli* by reducing more than 2 log cfu/g of count. Meanwhile, citric acid was effective in reducing salmonella by 2 log cfu/g, acetic acid reduced 1.2 log cfu/g. both acids reduced the APC by only one log cfu/g. According to Jetton *et al*. (1992) carcass rinse applications that decrease count by 2 log are considered effective.

In comparison between acetic and citric acids in the same concentration, there was no significance difference between them in reduction of APC and *S. aureus*, but the later was more effective ($P<0.05$) in controlling both of *E. coli* and salmonella. These results agree with that obtained by Parveen *et al*. (2007) who found that lactic and citric acids at concentrations of 1 to 3% have been shown to reduce *E. coli* O157:H7, and Salmonella serotypes when sprayed on beef and poultry carcasses by causing intracellular acidification. Citric acid showed to have the highest inhibitory effect because of its ability to diffuse through the cell membrane.

On the contrary of this Seoknam *et al*. (2003) and El-Khawas and Hassan (2015) reported that acetic acid was more effective than citric acid. This difference may be due to the different medium used. Foster and Hall (1990) mentioned that difference between the effect of acetic and citric acids may be referred to that, lethal effects of these weak acids depend on concentration, pH of the environment and the dissociation constant of each acid beside adapted or resistant strains due to sub-lethal conditions.

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الوقوف على الحالة الصحية لفيليه الدواجن المنتج في مجازر الدواجن وتحسينها باستخدام الاحماض العضوية من مصادر طبيعية

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أجريت هذه الدراسة أولا بغرض تقصى الحالة البكتيرية لفيليه الداوجن المنتج فى هذه المجازر وثانيا لمحاولة تحسين تلك الحالة باستخدام الأحماض العضوية من مصادر طبيعية مثل عصير الليمون والخل أثناء إعدادها فى المنزل. لذلك تم جمع عدد ٦٠ عينة من فيليه الدواجن (٣٠ من كل من فيليه الصدور والأوراك) من مجازر الدواجن حيث تبين أن العد الهوائى الكلى وعد مجموعة الكولفورم لعينات فيليه الأوراك (٤.٤ و ١.٨٣ لو خلية/جم) أعلى معنويا عنها فى عينات فيليه الصدور (٣.٨٩ و ١.٤٢ لو خلية/جم) على التوالى. كما تم عزل عترة واحدة من كل من الميكروب المكور العنقودى والسالمونيلا تيفيميوريم بنسبة ٣.٣% من العينات بينما تم عزل ميكروب إى-كولاى من ٣٠% و ١٠% من فيليه الاوراك والصدور على التوالى وكانت ٣٦.٧% و ٨٠% من عينات فيليه الأوراك والصدور مطابقة للمواصفات القياسية المصرية على التوالى. بالنسبة للجزء الثانى، فقد أحدث غمر عينات الفيليه فى الخل أو عصير الليمون (٢% حمض خليك أو سيتريك) لمدة ٢٥ دقيقة اختزالا فى العد الهوائى الكلى وعد الميكروب المكور العنقودى بقيمة لوغاريتم واحد من غير فروق معنوية بين اى من الحمضين، بينما كان تأثير عصير الليمون أكبر معنويا من الخل فى التأثير على كل من ميكروب السالمونيلا وإى-كولاى حيث احدث تخفيضا لعد كل منهما بقيمة ٢ و ٣ لو خلية/جم (على التوالى) بينما احدث الخل اختزالا لعد كل منهما بقيمة ١ و ٢ لو خلية/جم (على التوالى).

الكلمات الدالة: فيليه الدواجن، إى-كولاى، السالمونيلا، المكور العنقودى، الخل، عصير الليمون