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 is 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 (Man-Corr 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 water and performed 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 in 9 ml of tryptic soy broth (TSB) (Oxoid) and incubated at 37°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°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°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 α = 0.05.

**RESULTS**

Part I: survey of chicken fillet from slaughterhouse

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

<table>
<thead>
<tr>
<th>Bacterial count</th>
<th>APC</th>
<th>Coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thigh</td>
<td>Breast</td>
</tr>
<tr>
<td>Minimum</td>
<td>3.30</td>
<td>2.95</td>
</tr>
<tr>
<td>Maximum</td>
<td>5.90</td>
<td>5.00</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>4.41 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.89 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compatibility</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than limit</td>
<td>22</td>
<td>73%</td>
<td>30</td>
<td>100%</td>
<td>19</td>
<td>63%</td>
<td>26</td>
<td>87%</td>
</tr>
<tr>
<td>More than limit</td>
<td>8</td>
<td>27%</td>
<td>0</td>
<td>0%</td>
<td>11</td>
<td>37%</td>
<td>4</td>
<td>13%</td>
</tr>
</tbody>
</table>

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%) 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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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 (2010) (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/cm²), 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/cm² 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.

REFERENCES


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

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Text:

أجريت هذه الدراسة أولاً بغرض تقصي الحالة البكتيرية لفيليه الدواجن المنتج في هذه المجازر وثانياً لمحاولة تحسين تلك الحالة باستخدام الاحماض العضوية من مصادر طبيعية مثل عصير الليمون لخلل أثناء إعدادها في المنزل. لذلك تم جمع عدد 90 عينة من فيليه الدواجن (3 من كل من فيليه الدجاج والأوراق) من مجازر الدواجن حيث تبين أن العينات المحلية وعدة مجموعة كولونيات في عدد فئات لفتيات فيليه الأوراق (4.4 % من عينة فيليه الدجاج) لتعتبر من العينات التي يتناسب فيها عدد الكولونيات بالتفاقيه 3% من العينات بينما تم عزل ميكروب الأوراق 30% % من فيليه الأوراق والصدور على التوالي وكانت 40% % من عينات فيليه الأوراق والصدور طابقية للفئات الفارغة (3% من عينات فيليه الدجاج). حيث أن عدد عينات فيليه الدجاج بدرجة الزواجي 0.2% من عينات فيليه الأوراق والصدور طابقية للفئات الفارغة. بالنسبة لالة لها، فقد أظهرت عدد عينات فيليه الدجاج 0.1% من عينات فيليه الأوراق والصدور طابقية للفئات الفارغة. حيث أن عدد عينات فيليه الدجاج 0.2% من عينات فيليه الأوراق والصدور طابقية للفئات الفارغة. حيث أن عدد عينات فيليه الدجاج 0.1% من عينات فيليه الأوراق والصدور طابقية للفئات الفارغة. حيث أن عدد عينات فيليه الدجاج 0.2% من عينات فيليه الأوراق والصدور طابقية للفئات الفارغة. حيث أن عدد عينات فيليه الدجاج 0.1% من عينات فيليه الأوراق والصدور طابقية للفئات الفارغة. حيث أن عدد عينات فيليه الدجاج 0.2% من عينات فيليه الأوراق والصدور طابقية للفئات الفارغة. حيث أن عدد عينات فيليه الدجاج 0.1% من عينات فيليه الأوراق والصدور طابقية L. وعند اختيار خليط مكسر أو مت 같습니다 (ده 0.2% من عينات فيليه الأوراق) اقتصر عصير الليمون أكثر معنى من فيليه الدجاج في الحالات التي تأتي بديلة على كل من ميكروب السالمونيلا وإي. كولونيا حيث استخدم عد كل منها بقيمته 2 و 3 لو خليطة/جم (على التوالي) بينما أخذت الخل للعجين لعدين بقيمته 1 و 2 لو خليطة/جم (على التوالي)

الكلمات الدائلة: فيليه الدواجن، إي. كولونيا، السالمونيلا، المكور العنقودي، الخل، عصير الليمون