Microbiological Quality of Organic Chicken Meat

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ABSTRACT

Organic chicken production in the world increases every year according to the consumer demands. Therefore, a higher awareness of the microbial quality of organic poultry meat is important for public health, food safety and product shelf life. In this study was used 240 of frozen organic chicken meat, drumstick (n:80), breast (n:80) and leg quarter (n:80) as the material. Microbiological analyses were conducted to determine the counts of total mesophilic aerobic bacteria (TMAB), total psychotropic aerobic bacteria (TPAB), Pseudomonas spp., coliforms, E. coli, molds, and yeasts in all samples. It was determined that 100%, 100%, 100%, 81.6%, 54.1%, 34.1%, and 83.3% of the analyzed samples (N:240) were contaminated with TMAB, TPAB, Pseudomonas spp., coliforms, E. coli, molds and yeasts, respectively. Moreover, the mean counts of TMAB was 4.99±0.80 log₁₀ cfu/g, the TPAB was 5.29±0.96 log₁₀ cfu/g, the coliforms were 3.53 ± 0.92 log 10 cfu/g, E. coli was 2.45±0.65 log₁₀ cfu/g, Pseudomonas spp. was 4.63±1.10 log₁₀ cfu/g, mold was 2.03±0.42 log₁₀ cfu/g and yeast was 3.68±1.13 log₁₀ cfu/g. These results indicate that organic chicken meat can be contaminated with various microorganisms that affect the shelf life and hygienic quality.

Keywords: Chicken meat, Hygiene, Microbiological quality, Organic

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Organik Tavuk Etlərinin Mikrobiyologik Kalitesi

ÖZ

Organik tavuk eti üretimi tüketici tercihlerine bağlı olarak her geçen gün artmaktadır. Bu artışa bağlı olarak organik tavuk etlerinin mikrobiyel kalitesinin daha yakından bilinmesi hem halk sağlığı hem de gıda güvenliği ile ürunün raf ömrü bakımından önemlidir. Bu arastırmada baget (n:80), göğüs (n:80) ve kalçalı but (n:80) olmak üzere toplam 240 adet donmuş organik tavuk parça eti materyal olarak kullanıldı. Tüm örneklerde toplam mezofilik aerobik bakteri (TMAB), toplam psikrotrof aerobik bakteri (TPAB), Pseudomonas spp., koliform bakteri, E. coli ile küf ve maya sayısının tespiti için mikrobiyologik analizler gerçekleştirilirdi. Analiz edilen tavuk parça etlerinin (N:240) sırasıyla % 100, % 100, % 100, % 81.6, % 54.1, % 34.1 ve % 83.3’unun TMAB, TPAB, Pseudomonas spp., koliform, E. coli, küf ve maya ile kontamine olduğu belirlendi. Ayrıca örneklerdeki (N:240) ortalama TMAB sayısı 4.99±0.80 log₁₀ kōb/g, TPAB sayısı 5.29±0.96 log₁₀ kōb/g, koliform bakteri sayısı 3.53±0.92 log₁₀ kōb/g, E. coli sayısı 2.45±0.65 log₁₀ kōb/g, Pseudomonas spp. sayısı 4.63±1.10 log₁₀ kōb/g, küf sayısı 2.03±0.42 log₁₀ kōb/g ve maya sayısı 3.68±1.13 log₁₀ kōb/g düzeyinde bulundu. Bu sonuçlar organik tavuk etlerinin hem raf ömrü hem de hijyenik kalitesi üzerine etkili mikroorganizmalar ile kontamine olabileceğini göstermektedir.

Anahtar Kelimeler: Hijyen, Mikrobiyologik kalite, Tavuk eti, Organik
INTRODUCTION

Chicken meat is an essential source of animal origin food, which contains amino acids that are necessary for high human nutrition, and it is cheaper than red meat (Marangoni et al. 2015). However, with the changing consumer perception in the last 20 years, poultry production has become more environmentally friendly where animal welfare is more important (Harvey et al. 2016, Dervilly-Pinel et al. 2017). The term “organic” as defined in 2002 by the US Department of Agriculture (USDA), National Organic Program of the Agriculture Marketing Service, applies to specific methods of production of crops and livestock aimed to protect natural resources and conserve biodiversity. In this production system, crops are verified as organic when certain agricultural practices are not performed or specific compounds are not used, including sewage sludge, synthetic fertilizers, genetically modified organisms, and prohibited pesticides. Organic chicken production system aims to raise the wellbeing conditions of animals by raising them in an environment similar to the natural life of the chickens and minimize the chemical risks that may arise from the meat obtained from these chickens (Fanatico et al. 2007, İpek and Sözcü 2015). Statistical data show that the total organic chicken meat production in the European Union was 41 million in 2015 and reached 43 million in 2016 (Anonymous 2018a, Anonymous 2018b) while the organic broiler production in the United States was 9 million in 2008 and reached 22 million in 2016. Organic broiler meat sales in the United States were 451 million USD in 2014 and reached 750 million in 2016 (Anonymous 2018d, Anonymous 2017b, Anonymous 2017a). According to the data of the Ministry of Agriculture and Forest in Turkey, the number of organic broiler farms was 5 in 2012 and reached 23 in 2015. These data indicate that the organic chicken production increases every year to reach a broad consumer mass in the world. Moreover, it requires a better knowledge of the microbiological risks of chicken meat regarding public health and food safety. However, according to the national and international food codices, the organic poultry meat is evaluated based on the determined microbiological parameters and the limits for chicken meat produced by conventional methods. Therefore, the microbial risks related to organic chicken meat are not evaluated on a broader scale regarding food safety and public health; thus, the consumer awareness of microbial hazards remains at a lower level (Harvey et al. 2016). Previous limited studies have shown that like conventional chicken meat, organic chicken meat can also be contaminated with microorganisms in stages from production to consumption. In a study on the microbiological profile of organic chicken meat have reported that the total mesophilic aerobic bacteria, coliform and E. coli counts to be 2.8 log cfu/ml, 1.5 log cfu/ml and 1.3 log cfu/ml, respectively and determined the Salmonella spp. and Campylobacter spp. prevalence to be 20% and 28%, respectively (Scheinberg et al. 2013). Kijlstra and Eijck (2006) have reported that 27% of the organic broiler meat was contaminated with Campylobacter jejuni and 73% with Campylobacter coli, whereas Van Loo et al. (2012) reported 49% of the organic chicken carcasses were contaminated with L. monocytogenes. Previous studies on this subject showed that the scientific studies usually focus on the microbiological quality of conventional chicken meat or the presence of pathogenic microorganisms in organic chicken meat (Lestari et al. 2009, Kim et al. 2017). The present study aims to determine the level of contamination regarding microorganisms used as hygiene indicator (coliform and E. coli) and deteriorative indicator (total mesophilic aerobic bacteria, total psychotropic aerobic bacteria, Pseudomonas spp., mold, and yeast) in organic chicken meat parts offered to consumers.

MATERIAL and METHOD

Sample collection

Within the scope of the regulations in Turkey, the products of the brands of the companies that produce organic chicken meat can be obtained from the super markets and online shopping stores. Thus, 240 organic frozen chicken meat parts (80 each of drumsticks, breasts, and leg quarters) sold in the super markets in Diyarbakir city and online shopping stores that operating at the national level were used as the study material. Frozen chicken meat parts were defrosted at refrigerator. Microbiological analyses were conducted immediately after the defrosting process. The production dates and expiration dates of the chicken meat samples collected were within limits determined by the relevant legislation.

Sample preparation

For the microbiological analysis of each chicken sample, 10 g of each chicken sample was taken under aseptic conditions using a sterile pensette and scalpel and placed in a sterile sampling bag (Bag Filter, France). Then, 90 mL 0.1% sterile peptone water (LAB M, UK) was added to each sampling bag containing 10 g sample and homogenized for 2 minutes in a stomacher (Easy Mix-G560E, France). To determine the number of microorganisms in each sample, the decimal dilutions were prepared in the tubes with 9 mL of 0.1% sterile peptone water.

Total mesophilic aerobic bacteria (TMAB): Enumeration of TMAB was performed by using Plate Count Agar (PCA) (LAB M, UK). Duplicate pour plates were made from each dilution. Plates were incubated at 37 °C for 24-48 h. All colonies from the appropriate dilution were counted as mesophilic aerobic bacteria (Harrigan 1998).
Total psychrotrophic aerobic bacteria (TPAB): Enumeration of TPAB was performed by using Plate Count Agar (PCA) (LAB M, UK). Duplicate pour plates were made from each dilution. Plates were incubated at 5 °C for 7 days. All colonies from the appropriate dilution were counted as psychrotrophic aerobic bacteria (Harrigan 1998).

Coliform bacteria: Enumeration of coliforms was performed by using Violet Red Bile Lactose Agar (VRBLA) (Merck, Germany). Duplicate pour plates were made from each dilution. The plates were incubated at 37 °C for 24-48 h. Pink-red colonies from the appropriate dilution were counted as coliform bacteria (Harrigan 1998).

E. coli: For the enumeration of E. coli was used Tryptone Bile X Glucuronide (TBX) Agar (Merck, Germany). Duplicate pour plates were made from each dilution. Plates were incubated at 44 °C for 24 h. Typical blue-green colonies from the appropriate dilution were counted as E. coli (ISO 2001).

Pseudomonas spp.: To determine Pseudomonas spp., the prepared dilutions were inoculated onto Pseudomonas Agar (Oxoid, UK) plates containing Pseudomonas CFC Selective Supplement (Oxoid, UK) and glycerol. Duplicate spread plates were made from each dilution. These plates were incubated for 48 hours at 30 °C, and then end of the incubation all colonies from the appropriate dilution were enumerated as Pseudomonas spp. (Harrigan 1998).

Mold and yeast: To determine mold and yeast counts, 10% tartaric acid added Potato Dextrose Agar (PDA) (LAB M, UK) was used. Duplicate pour plates were made from each dilution, and then the plates were incubated for five days at 22 °C (Andrew 1992).

Following the incubation, the colonies with soft mucoid consistency, oval or rounded edges were evaluated as yeast, whereas those with a "puffy cotton" mycelium appearance were evaluated as molds.

Data analysis

In this study, the minimum detectable limit for Pseudomonas spp. was designated to be 10^6 and 10^9 for the remaining microorganisms. The colonies growing in petri dishes were enumerated and recorded as cfu/g. Then, these data were converted to log_{10} cfu/g unit and evaluated using standard deviation, minimum-maximum values and averages in the SPSS (16.0) software program.

RESULTS

It was determined that 100%, 100%, 100%, 81.6%, 54.1%, 34.1%, and 83.3% of the analyzed samples (n:240) were contaminated with TMAB, TPAB, Pseudomonas spp., coliform, E. coli, mold and yeasts, respectively (Table 1). In chicken meat parts, the mean TMAB count was 4.99±0.80 log_{10} cfu/g; the mean TPAB count was 5.29±0.96 log_{10} cfu/g, the mean coliform count was 3.53±0.92 log_{10} cfu/g, the mean E. coli count was 2.45±0.65 log_{10} cfu/g, the mean Pseudomonas spp. count was 4.63±1.10 log_{10} cfu/g, the mean mold count was 2.03±0.42 log_{10} cfu/g, and the mean yeast count was 3.68±1.13 log_{10} cfu/g (Table 1).

Table 1. Distribution and number of microorganisms detected in organic chicken meat parts (log_{10} cfu/g)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>The average microorganism numbers in the analyzed samples (N: 240)</th>
<th>Distribution of microorganisms according to chicken meat parts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>TMAB</td>
<td>3.04</td>
<td>6.90</td>
</tr>
<tr>
<td>TPAB</td>
<td>3.25</td>
<td>7.94</td>
</tr>
<tr>
<td>Coliform</td>
<td>1.30</td>
<td>5.60</td>
</tr>
<tr>
<td>E. coli</td>
<td>1.27</td>
<td>3.99</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>2.00</td>
<td>6.32</td>
</tr>
<tr>
<td>Mold</td>
<td>1.27</td>
<td>3.04</td>
</tr>
<tr>
<td>Yeast</td>
<td>1.30</td>
<td>5.44</td>
</tr>
</tbody>
</table>

*SD: Standard deviation
† The number of analyzed samples (n: 80)
DISCUSSION

Organic food sector has become a fast-growing sector in the international food market during the past decade (Willer and Lernoud 2016). The increased consumer sensitivity to healthy eating and eco-friendly products has contributed to this rapid growth. Like conventional foods, possible microbial contaminations in organic foods can cause serious problems regarding public health and food safety. Moreover, these contaminants can cause undesirable conditions including shortening of the shelf-life or deterioration. Therefore, like conventional chicken meat, it is important to know the microbiological quality of organic chicken meat to prevent the risks mentioned above or undesirable consequences. The information on TMAB in meat and meat products is used as an indicator to determine whether the production and preservation are performed under appropriate conditions (Sofos 1994). In the present study, the TMAB in the chicken drumstick, breast, and leg quarter samples were found to be 5.15±0.63 \( \log_{10} \text{cfu/g} \), 5.10±0.95 \( \log_{10} \text{cfu/g} \), and 4.71±0.72 \( \log_{10} \text{cfu/g} \), respectively, and the TMAB did not exceed the maximum acceptable level for chicken meat 7 \( \log_{10} \text{cfu/g} \) determined by the International Commission on Microbiological Specifications for Foods in any sample (Table 1) (ICFMH 1986). Scheinberg et al. (2013) reported the average TMAB in 50 fresh organic chicken carcasses (analyzed by the rinse method) as 2.8±0.7 \( \log_{10} \text{cfu/ml} \), while Hardy et al. (2013) reported the highest and the lowest TMAB in 50 fresh organic chicken carcasses at 3.4 \( \log_{10} \text{cfu/ml} \) and 4.8 \( \log_{10} \text{cfu/ml} \), respectively. The higher results obtained in our study were associated with variables including the number of samples analyzed, sample type, and the analysis methods used in the studies and geographical differences.

Psychrotrophic bacteria, *Pseudomonas* spp., *Shewanella putrefaciens* and the psychrophilic strains of *Enterobacteriaceae* are the dominant bacteria in the microbiological flora in chilled or frozen chicken meats (Galbo et al. 1988, Mead 2004a, Adams and Moss 2007, Ray and Bhunia 2007). Information on the microbiological load regarding these bacteria is regarded as important regarding preservation of product quality (Russell 2000, Alvarez-Astorga et al. 2002). Depending on the type of microorganism, the level of deterioration can vary between 10\(^8\) cfu/g and 10\(^9\) cfu/g, and like other food products, TPAB and *Pseudomonas* spp. counts higher than 10\(^8\) cfu/g in chicken meat indicate deteriorated product (Adams and Moss 2007). In the present study, the average TPAB was 5.29±0.96 \( \log_{10} \text{cfu/g} \), and *Pseudomonas* spp. was 4.63±1.10 \( \log_{10} \text{cfu/g} \). Moreover, the TPAB and *Pseudomonas* spp. counts did not exceed 10\(^8\) cfu/g in any sample; however, in 6.2% of the drumstick and breast samples and 3.7% of the breast samples, the TPAB was found to be 10\(^7\) cfu/g. There are no previous studies that determine the TPAB and *Pseudomonas* spp., counts in the frozen organic chicken meat while the TPAB in conventional chicken meat was between 3.11 \( \log_{10} \text{cfu/g} \) and 5.63 \( \log_{10} \text{cfu/g} \), and *Pseudomonas* spp. counts varied between 1 \( \log_{10} \text{cfu/g} \) and 4.62 \( \log_{10} \text{cfu/g} \) (Efe and Gümüşsoy 2005, Günen and Büyükyörük 2005, Patsias et al. 2008, Atlan and İşleyici 2012, Santosh et al. 2014). In the Turkish Food Codex Microbiological Criteria Regulation (Anonymous 2018c), there is no limit for the TPAB or *Pseudomonas* spp. counts in fresh or frozen chicken meat.

The detection of an indicator in food or the presence of this indicator microorganism above a certain limit in the food indicates that food may be contaminated with pathogenic microorganisms. Coliform and *E. coli* bacteria are the most widely used indicator microorganisms (Rangel 2005, Lima et al. 2017). Coliform group bacteria are considered as indicators of a direct or indirect fecal contamination, while *E. coli* is considered as a direct indicator of fecal contamination. Therefore, the detection of coliform and *E. coli* bacteria in poultry meat is often adopted to determine the hygienic quality of the product. In the present study, of the 240 frozen organic chicken meat parts, coliforms were detected in 81.6% and *E. coli* was detected in 54.1%. The average coliform counts in drumstick, breast and leg quarter samples were 3.65±0.75 \( \log_{10} \text{cfu/g} \), 3.71±1.13 \( \log_{10} \text{cfu/g} \) and 3.08±0.67 \( \log_{10} \text{cfu/g} \), respectively while the average *E. coli* counts were 2.41±0.66, 2.67±0.65 and 2.18±0.52 \( \log_{10} \text{cfu/g} \), respectively (Table 1). In contrast to our results, Scheinberg et al. (2013) determined that the coliform (1.5 \( \log_{10} \text{cfu/ml} \)) and *E. coli* (1.3 \( \log_{10} \text{cfu/ml} \)) count in organic fresh chicken meats obtained by the rinse method were lower. The differences between the results could be related to the inadequacy of the rinse method used for microorganism recovery by these researchers in the detection of microorganisms (Jørgensen et al. 2002, Fletcher 2006, Berrang et al. 2017). In the Turkish Food Codex Microbiological Criteria Regulation (2011) there are no limits for *E. coli* and the coliform counts in chicken meat. Mold and yeasts are a part of the aerobic flora and contaminate the products through environmental sources such as air, water, soil, tools, and equipment. Although these organisms do not cause foodborne poisoning, they play an important role in the degradation of food and the shortening of shelf life (Petruzzii et al. 2017, Synder and Worobo 2018). In the present study, the average mold counts in drumstick, breast and leg quarter samples were found to be 2.08±0.45 \( \log_{10} \text{cfu/g} \), 2.07±0.35 \( \log_{10} \text{cfu/g} \), 1.93±0.42 \( \log_{10} \text{cfu/g} \), respectively, while the average yeast counts were found to be 3.66±1.06 \( \log_{10} \text{cfu/g} \), 4.10±0.98 \( \log_{10} \text{cfu/g} \), 3.36±1.21 \( \log_{10} \text{cfu/g} \), respectively. Moreover, 34.1% of 240 chicken meat parts were contaminated with mold, and 83.3% of them were contaminated...
with yeasts. There are no results reported on the mold and yeast counts in organic chicken meats in the literature while Kingsbury (2006) have reported that only 6% of the chicken samples were contaminated with mold and yeasts.

In organic poultry production, restrictions on the use of antibiotics and antiparasitic drugs, and outdoor breeding of the animals play a role in increasing the microbial risks of organic poultry meats (Thamsborg 2001, Engvall 2001, Mead 2004b). The TMAB, TPAB, E. coli, coliform, Pseudomonas spp., mold and yeast counts in the present study were different (lower or higher results) from the results of the studies carried out with conventional frozen chicken meats depending on the type of microorganism (Eglezos et al. 2008, Patias et al. 2008, Santosh et al. 2014, Fernandes et al. 2016). Thus, it is difficult to ascertain that the TMAB, TPAB, E. coli, coliform, Pseudomonas spp., mold and yeast counts in organic chicken meat analyzed in this study were higher than those reported in conventional chicken meat.

CONCLUSION

This is the first study to determine the counts of some microorganisms affecting the hygienic quality and the shelf-life of frozen organic chicken meat parts sold at the retail level in Turkey. Especially, the presence of coliform (81.6%) and E. coli (54.1%) bacteria in organic meat parts indicates that fecal pathogenic microorganisms can be found in organic chicken meats. The hygienic conditions at all stages (from the farm to the table), compliance with the implementation of international food safety systems, and consumption of the organic chicken meats following an adequate/efficacious heat treatment according to the general hygiene rules are essential to increase the shelf-life and reduce the microbial risks of organic chicken meat.

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