Microbial and chemical spoilage of chicken meat during storage at isothermal and fluctuation temperature under aerobic conditions

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IJVST
DOI:
10.22067/veterinary.v8i1.54563
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Keywords
Pseudomonas spp., microbial spoilage, chicken meat, shelf-life

Abstract
Microbial activity and spoilage of chicken meat from the market in Mashhad, Iran, stored in air at 0, 4, 10 and 15°C was assessed. Microbial spoilage population, Total Volatile-Base Nitrogen (TVB-N), pH and organoleptic changes were determined. Based on the results of sensory analysis, shelf-life of chicken meat stored at 0, 4, 10 and 15°C was 72, 120, 220 and 320 h, respectively. TVB-N that serves as an important indicator increased with storage time and TVB-N values were more than 28 mg/100g at the time of unacceptable sensory analysis. Both of them showed a high correlation coefficient with microbial growth, especially, Pseudomonas spp. However, pH cannot be used as a good indicator of chicken spoilage under aerobic conditions and there was relatively low correlation between this parameter and the sensory analysis. The results from microbiological behavior analysis, organoleptic quality and TVB-N values identified pseudomonas spp. as special spoilage organisms (SSO) of poultry meat stored at 0-15°C and Pseudomonas spp. population level was 7.5 CFU/g at the end of shelf life. The microbial growth under dynamic temperature condition followed the same pattern and the results showed that temperature abuse affects the surviving population of Pseudomonas spp. and it leads to reduction of shelf life.

Abbreviations
TVB-N: Total Volatile-Base Nitrogen
LAB: Lactic Acid Bacteria
TVC: Total Viable Count
PCA: Plate Count Agar
MRS: Man Rogosa Sharpe
CFC: Cetrimide Fucidin Cephaloridine
VRBG: Violet Red Bile Dextrose/Glucose
SSO: Special Spoilage Organisms
CFU: Colony Forming Unit
Introduction

Chicken meat is a nutritious food with a relatively low cost and fat content, consumed all over the world. However, it is highly perishable and its shelf life is relatively short even when refrigerated (Manilla et al., 2011). Biochemical and microbial mechanisms are the leading cause of the rapid loss of quality and freshness of chicken meat (Gram and Huss 1996; Boziaris et al., 2011).

The interactions and changes that exist between various microbial spoilage groups turn meat spoilage into a dynamic process (Gram and Dalgaard 2002; Tsigarida et al., 2003). In fact bacterial spoilage is the important factor that directly reduces the shelf life of meat and affects the health and safety of consumers in the supply chain (Gil et al., 2006). The initial microbial level and storage conditions are major contributing factors which determine the level of metabolites and the Specific Spoilage Organisms (Raab et al., 2008) produced in chicken meat (Drosinos and Nychas 1997; Gram and Dalgaard 2002; Boziaris et al., 2011). The metabolic activities of microorganisms responsible for spoilage constitute the main spoilage mechanism and produce off-odors (Dainty 1996; Gram and Huss 1996).

The main criterion of quality assessment of chicken meat is freshness which corresponds to the presentation of spoilage signs. The expiry point occurs when the acceptable maximum limit of existing bacteria exceeds 7-8 log CFU/g and metabolites resulting from microbial growth produce bad odors, viscosity and chemical modifications in the meat (Smolander et al., 2004; Raab et al., 2008). For quality assessment of meat and deciding about the end of shelf life, there are chemical, microbiological and sensory methods with some accuracy (Olafsdottir et al., 1997). Mostly, all of these methods are put together and are used for assessment of quality changes of meat.

The evaluation of meat quality by microbial methods and the plate count of specific microorganisms (indicators of spoilage or related changes) is a precise method that needs 2 days or more for incubation and results (Dainty 1996). On the other hand, the strong relationship between spoilage resulting from the growth of bacteria and chemical indices could be an auxiliary and supplementary technique used to make a decision about or predict the end of meat shelf life and assessing its quality (Dainty 1996). The two main parameters related to the microbial growth are total volatile base nitrogen (TVB-N) and changes in pH (Gram and Dalgaard 2002, Boziaris, Kordila et al. 2011). The principal chemical parameter for microbial growth, including Pseudomonas spp. is TVB-N (Fraqueza et al., 2008, Yan et al., 2008, Boziaris et al., 2011, Zhang et al., 2012). The correlation between the microbial growth and TVB-N has been properly established in the case of dark turkey meat in aerobic conditions (Fraqueza et al., 2008, Zhang et al., 2012). There is a body of work that recognize pH determination as an essential indicator of lactic acid bacteria (LAB) growth. Consequently, pH and TVB-N are two important indicators of microbial spoilage in meat and poultry (Hernandez-Herrero, et al., 1999; Fraqueza et al., 2008).

Furthermore, an important item in the distribution and consumption of fresh poultry is the effective monitoring of temperature conditions which affects safety and quality of chicken meat. (Bovill et al., 2001; James et al., 2008; Zhang et al., 2012).

If chicken meat that is a perishable product is stored at improper and fluctuating temperature conditions, rapid microbiological growth happens and the product spoils (Almonacid-Merino and Torres 1993; Raab et al., 2008).

The objectives of the present study were to evaluate shelf life of air-packaged chicken meat and to establish a relationship between the microbial quality and the biochemical parameter TVB-N. The other objectives were to determine special spoilage organisms SSOs, to analyze the effect of constant storage temperature (0, 4, 10 and 15°C) on the growth of different spoilage microorganisms (lactic acid bacteria, Enterobacteriaceae, Pseudomonas spp. and aerobic mesophilic bacteria) in air-packed chicken meat, to evaluate sensory characteristics of fresh chicken meat, and to investigate the effect of temperature fluctuations on the spoilage of air-packaged chicken meat stored under chilled conditions.

Material and methods

Sample preparation and storage conditions

Fresh chicken meat breast fillets were purchased from a local poultry processing plant that was 2 km away from the laboratory (Baharan Company, Mashhad, Iran) and transported to the laboratory in cold boxes within 15 minutes. In the laboratory the breast fillets were sliced to 100g pieces under aseptic conditions. As a sample, each four pieces were wrapped in a low density polyethylene film and were packed in polypropylene bags. Forty four samples were prepared and the samples were randomly divided into 4 groups and stored under controlled isothermal conditions (0, 4, 10 and 15°C) in high-precision (± 0.2°C) low temperature incubators (Model FCC 225E, VELD, Germany) after packaging.

Temperature conditions were continuously controlled by data loggers. After appropriate time intervals, microbiological, sensory and chemicals measurements were made from individual packaged poultry samples.

For the dynamic storage experiments, a non-isothermal low-temperature storage profile was used in order to simulate a continuously changing storage environment and potential abuse periods in the chilled chain. The storage protocol included a periodically alternating 24 h cycle of 8 h at 4°C, 8 h at 10°C and 8 h at 15°C.

Microbiological analysis

At the designated time intervals, 10 g of meat from...
each sample group was aseptically weighed and added to a quarter strength Ringer's solutions (90 ml) after which it was homogenized in a Seward Stomacher* 400 (Seward, London) for 60 s at room temperature. Decimal serial dilutions were prepared and 1 ml samples of appropriate dilutions were spread on the surface of the appropriate media in petri dishes for the enumeration of:

(i) Total viable count (TVC): on Plate Count Agar (PCA) (Merck, Darmstadt, Germany), incubated at 30°C for 48 h (Smolander et al., 2004).
(ii) Lactic acid bacteria (LAB): on Man Rogosa Sharpe (MRS) Agar (Merck, Darmstadt, Germany) overlaid with the same medium, incubated at 30°C for 96 h.
(iii) Pseudomonas spp.: on Cetrimide-Fucidin-Cephaloridine (CFC) Agar (CM559, Oxoid) supplied with the selective supplement (SR 103E, Basingstoke, UK), incubated at 25°C for 48 h.
(iv) Enterobacteriaceae: on Violet Red Bile Dextrose/Glucose (VRBG) Agar (Merck, Darmstadt, Germany) overlaid with the same medium, incubated at 37°C for 24 h.

All the plates were examined visually for the typical colony types and morphological characteristics that were associated with each growth medium. Moreover, some selected colonies of each medium were checked routinely by Gram staining and microscopic examination.

Measurement of pH

The pH values were measured using a pH meter (WTW, USA). The glass electrode was immersed in the homogenate of chicken meat after the end of microbiological analysis at three different points in each package.

Sensory evaluation

A sensory panel who was composed of five panelists carried out the sensory evaluations of the chicken meat samples. All members were blinded to the time and temperature history of the sample being tested. The breast fillet samples were cooked in aluminum foil at 180°C for 20 min. The panel members evaluated the odor of the raw meat and the taste and odor of the cooked meat. The results were recorded in designated forms with descriptive terms, based on the organoleptic characteristics of quality deterioration (taste and odor). Odor was evaluated using a simple three-point scoring system and each attribute (taste and odor) was scored on a continuous 0 to 3 hedonic scale (with 0 being the highest quality score and 2 being the limit of acceptance) (Koutsoumanis et al., 2006).

Measurement of total volatile basic nitrogen (TVB-N)

TVB-N content was measured using the Conway micro-diffusion technique (Conway and Byrne, 1933). Meat samples of 10 g were homogenized in 50 mL distilled water for 10 s and then extracted for 30 min. The homogenate was filtered through a Whatman filter paper grade 4 (Whatman International Ltd., UK). Sulfuric acid (5 %w/v, CID: 1118, PubChem) was added to the filtrate until its pH reached 6.0. One mL of the filtrate and 1.0 mL of saturated K2CO3 (CID: 11430, PubChem) were placed into a Conway's unit. One mL of 0.01 N H2SO4 was added to the inner well of the Conway's unit. The Conway's unit was slowly shaken by hand and incubated at 25°C for 60 min. Then, the substance in the inner well was titrated with 0.01 N NaOH (CID: 14798, PubChem) in the presence of 10 μL Brunswick reagent. TVB-N expressed as milligrams nitrogen per 100 g (mg-N/100 g) was calculated by using the Eq. 1.

$$TVB-N=0.14\left(\frac{v1-v2}{w}\times100\times c\right)$$

(Eq. 1) where w is the sample weight (g); v1 is the volume of added 0.01 N NaOH to blank (mL); v2 is the volume of added 0.01 N NaOH to the sample (mL) and c is the dilution factor.

Data analysis

All calculations were performed using SPSS 16.0 (SPSS Inc., Chicago, IL) and all the Figures were plotted using Microsoft- Excel 2007. All of the experiments were carried out in triplicate.

Results and discussion

Organoleptic assessment

Trained amateur panelists were employed to conduct the organoleptic assessment in the present study. The similarity between the assessments and the typical response given by consumers regarding the shelf life and acceptability of such products is the added advantage of this decision. The organoleptic changes in chicken meat kept in the constant temperature was showed in Fig.1 (0, 4, 10 and 15°C). In three replicated experiments, the organoleptic results were exactly the same. Off-odor development after 65 hours at the temperature of 15°C was the cause of product rejection while it took 72 hours for bad taste to disqualify the product. Due to slower occurrence of bad taste and off-odor at 10°C, the product was rejected after 120 hours. 220 hours were needed for bad taste and off-odor to occur at 4°C while at 0°C presence of bad taste and off-odor was observed after 300 hours. Some consumers describe taste (cooked) and bad odor (raw) as the most important characteristics of determining shelf-life in some types of meat such as chicken. There are studies such as Raab et al. (2008) and Smolander et al. (2004) that reported chicken meat stored at the same constant temperature has a shelf-life of 5 to 7 days while, the level of microbial activity, TVB-N and pH were still in the acceptable range.

Chemical changes

Production of TVB-N during the storage of chicken meat at 0, 4, 10 and 15°C is shown in Fig. 2. The initial
Figure 1
Sensory evaluation of chicken meat stored at constant temperature: A (0°C), B (4°C), C (10°C) and D (15°C). Columns: black (smell), lined (taste), gray (total sensory score). Line represents the score limit (0: highest quality, 1: acceptable quality, 2: the limit of acceptance, and 3: unacceptable quality)

Figure 2
Changes of TVB-N (mg/100gr) during the storage of chicken meat at 15°C (●), 10 (×), 4°C (△) and 0°C (○). Red line represents the acceptance limit
The pH values at sometimes during storage may be attributed to the lowering of the pH values (Hernandez-Herrero et al., 1999; Fraqueza et al., 2008). Finally, over time the growth of pseudomonas overtake LAB and it causes increased production of TVB-N compounds and results in increased pH.

**Microbial changes under isothermal conditions**

Fig. 4 provides changes of TVC, Pseudomonas spp., Enterobacteriacea, and LAB counts at the constant storage temperatures of 0, 4, 10, and 15°C for packaged chicken meat.

The primary TVC level in the samples was 5.07 log CFU/g right after preparation which is in agreement with the range reported by other works, on the most frequent contamination levels of 2 to 5 log CFU/g for mesophilic in refrigerated chicken (Goksoy et al., 2004; Zhang et al., 2012). Increase of storage time caused an increase in the TVC counts and the increased rate was accelerated with the rise of storage temperature, reaching 7-8 log CFU/g at the end of shelf life, approximately 80, 124, 214 and 350 h for 0, 4, 10 and 15°C, respectively (Fig. 5).

The same pattern held true for Pseudomonas spp. counts as the initial Pseudomonas spp. level counts for 0, 4, 10 and 15°C as shown in Fig. 5. Other studies report the same levels of Pseudomonas spp. for chicken which is 2.7-3.8 (Bruckner et al., 2012; Mead et al., 1993) and 3.6 log CFU/g (Abu-Ruwaida et al., 1994). There is an increase from 3.01 log CFU/g to 8.9, 7.9, 7.5 and 7.6 log CFU/g at 0, 4, 10 and 15°C in Pseudomonas spp. counts during storage. On the other hand, there was an increase in Enterobacteriaceae counts from 2.8 log CFU/g to 4.3, 4.8, 5.6 and 6.0 log CFU/g at isothermal storage temperatures of 0, 4, 10 and 15°C as shown in Fig. 7. The initial LAB count increased from 4.40 log CFU/g to 4.5, 5.6, 5.9 and 6.9 log CFU/g at 0, 4, 10 and 15°C, respectively (Fig. 8). There was no marked difference between the groups in terms of the numbers of Pseudomonas spp. organisms at the end of shelf life. Under the temperatures of 0, 4, 10 and 15°C, there was a fairly rapid increase over the time span of 63, 94, 214 and 300 hours which is in agreement with the lag and exponential growth phase (Fig. 6).

Therefore, it can be concluded that under all the temperature conditions, the count of Pseudomonas spp. at the end of shelf life was higher than the other spoilage bacteria (LAB and Enterobacteriaceae). The microbial profile described in the present study has also been reported in some studies on aerobically stored chilled meat (Goksoy et al. 2004; Davis and Conner 2007; Zhang et al., 2012). It has been reported that Pseudomonas spp. is a dominant...
Spoilage of chicken meat during storage at different temperatures under aerobic conditions

Figure 4
Microbial counts of different microorganisms growing in poultry samples stored at 0°C (a), 4°C (b), 10°C (c), 15°C (d). Columns: Total aerobic viable (TVC) (black), Pseudomonas spp. (horizontal lined), Enterobacteriaceae (gray), Lactic acid bacteria (LAB) (hachure).

Figure 5
Growth of total aerobic viable (TVC) on poultry samples stored at different constant temperatures: 0°C (●), 4°C (●), 10°C (●), 15°C (●).

Figure 6
Growth of Pseudomonas spp. on poultry samples stored at different constant temperatures: 0°C (●), 4°C (●), 10°C (●), 15°C (●).
bacteria in different types of meat stored under aerobic conditions at temperatures ranging from 0 to 25°C (Ercolini et al. 2006; Koutsoumanis et al. 2006; Nychas et al. 2008; Bruckner et al., 2012; Zhang et al., 2012).

Sensory analysis is a most important criteria for selection of meat products by consumers as a view of quality, hygiene and freshness (Mancini and Hunt 2005). As shown in Table 1 in this study, a high correlation coefficients was obtained between Pseudomonas spp. count and sensory analysis (taste and odor).

The correlation coefficients between Pseudomonas spp. and the spoilage criteria were greater than those between TVC, LAB, Enterobacteriaceae and the spoilage criteria (Table 1). Therefore, it can be concluded that Pseudomonas spp. is the SSO in chicken meat stored at low temperatures. These data show the maximum number of bacteria at the end of shelf life based on the sensory analysis and TVB-N was 107-108 CFU/g. This finding is

<table>
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<tr>
<th>Table 1</th>
<th>Correlation coefficients of the quality characteristics of chicken meat stored at 4°C.</th>
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<tr>
<td></td>
<td>Pseudomonas spp.</td>
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<tr>
<td>Pseudomonas spp.</td>
<td>0.972</td>
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<tr>
<td>Total viable count (TVC)</td>
<td>0.9744</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>0.628</td>
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<tr>
<td>Lactic acid bacteria (LAB)</td>
<td></td>
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<tr>
<td>TVB-N</td>
<td>0.787</td>
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<td>pH</td>
<td>0.739</td>
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consistent with the conclusions of the other studies where they determined that meat is not suitable for consumption when the number of Pseudomonas spp. reaches 107 - 108 CFU/g (Gil et al., 2006; Davis and Conner 2007; Raab et al., 2008; Zhang, Han et al., 2012). These results concerning shelf life at different storage temperatures are in close agreement with a few other works (Gospavic et al., 2008; Raab et al., 2008; Zhang et al., 2012).

The gaseous composition that constitutes the atmosphere around foods has a great impact on the growth and life of SSO (Nychas et al., 2008; Dougeraki et al., 2012). In this experiment, an oxygen permeable film covered the aerobic atmosphere of the storage. This environment is particularly suitable for psychrotrophic aerobes, allowing them to grow quickly and dominate the microbial composition of the spoilage (Dougeraki et al., 2012; Adams & Moss 1995).

As a product of bacterial spoilage, TVB-N is commonly adopted as an index for meat quality and shelf life assessment (Connell, 1990). The high correlation coefficient (R=0.95) between poultry TVB-N content and microbial count that was obtained in this study shows the significant role of microbial count in TVB-N content of samples. The TVB-N is an indicator of microbial growth (Bozias et al., 2011; Fraqueza et al., 2008) and its variations explain the significance of the number of microorganisms, particularly Enterobacteriaceae and Pseudomonas spp. As a result, TVB-N formation is related to bacterial growth in this study. A slow increase of TVB-N is observed with the decrease of temperature since low temperature diminishes the bacterial growth rate (Fig. 2).

**Microbial changes under non isothermal conditions**

As shown in Fig. 9, the TVC count of samples stored at fluctuating temperatures increased initially from 5.47 log CFU/g to 8.98 log CFU/g after 140 h in the end of shelf life. The bacterial lag phase is about 30h at dynamic storage conditions. In constant temperature conditions, the TVC count reached 7.2 log CFU/g. This upper limit microbiological acceptability might be a consequence of the temperature abuse effect on bacterial growth rate (Mielnik et al., 1999; Tuncer and Sirel, 2008; Zhang et al., 2012; Bovill et al., 2001) and it also suggests that dynamic conditions for meat storage can affect the growth of bacteria.

About the behavior of the growth of Pseudomonas spp., the findings show that there was no significant difference between samples stored at constant temperature and those stored at fluctuating condition (P>0.05). The results also showed that temperature abuse effects on surviving populations of Pseudomonas in meat products when temperature variations were above 5°C (Fig.9). These results are consistent with other works (Davis and Conner 2007; Zhang et al., 2012).

Enterobacteriaceae number is considered as a hygiene indicator that has a high correlation with the sensory analysis of odor of the poultry (Smolander et al., 2004). During storage at fluctuating conditions, the number of Enterobacteriaceae increased from 3.6 log CFU/g to 7.01 log CFU/g (Fig.9). Comparing the Enterobacteriaceae number between these results and the constant temperature storage (0, 4, 10 and 15°C) results (3.8 to 6.0 log CFU/g) indicates that Enterobacteriaceae were more dependent on changes in temperature during storage time (Smolander et al., 2004; Zhang et al., 2012). Cold-tolerant Enterobacteriaceae also occurs on chilled meat stored aerobically. However, they do not contribute to the microbial associations in terms of numbers. Although rarely, if ever, contributing significantly to the spoilage flora on meat and meat products, Enterobacteriaceae have been considered as indicators of food safety (Nychas et al., 2008).

The results of this study showed that temperature abuses compared to constant temperature during storage of chicken meat lead to more shelf life reduction. The shelf life of chicken meat in fluctuating conditions was about 140 h (Fig.9). In this study, the count of Pseudomonas spp. in air-packaged chicken meat showed an increase from 3.01 log CFU/g to about 7.5 log CFU/g during the constant temperature storage conditions. The correlation between the growth of Pseudomonas spp. and the spoilage chemical indicators (TVB-N, sensory analysis and pH) is statistically significant. Therefore, Pseudomonas spp. is considered as the SSO for air-packaged chicken meat. Although the temperature abuses during storage of chicken meat led to shelf life reduction, a similar pattern was observed for the growth of Pseudomonas spp. during fluctuating storage conditions.

**Acknowledgment**

This research was financially supported by grant no. 28263 from the Research Council of the Ferdowsi University of Mashhad.

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پرزی فساد شیمیایی و میکروبی گوشت مرغ در طی نگهداری در دمای ثابت و متغیر در شرایط هوازی

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دریافت مقاله: 23/12/1394
پذیرش نهایی: 09/09/1395

چکیده

در این بررسی فعالیت میکروبی و سایر شاخص‌های فساد گوشت مرغ خریداری شده از مراکز عرضه شهر مشهد در شرایط دمایی ثابت (0، 4، 10 و 15 درجه سانتی‌گراد)، در شرایط هوازی، مورد ارزیابی قرار گرفت. جمعیت باکتری‌های عامل فساد، ازت بازی کل (TVB-N)، pH و تغییرات حسی تعبیه شد. بر اساس نتایج ارزیابی حسی، زمان ماندگاری گوشت مرغ ذخیره شده در 0، 4، 10 و 15 درجه سیلسیوس به ترتیب 72، 120، 220 و 320 ساعت بود. TVB-N به عنوان یک شاخص مهم فساد در طی زمان نگهداری، افزایش یافته و مقدار آن در زمانی که ارزیابی حسی بیانگر غیر قابل قبول بودن گوشت بود، مقدار بیش از 28 mg/100g را نشان داد. نتایج ارزیابی حسی و تغییرات pH ضریب همبستگی بسیار خوبی با تغییرات TVB-N مقداری بخش از آن راشید میکروبی، به خصوص سودوموناس داشت. اما بررسی تغییرات pH نشان داد که این شاخص نمی‌تواند به عنوان معیار خوبی برای ارزیابی فساد مرغ به‌کارگرفته شود در شرایط هوازی مدت نظر باشد. تغییرات pH حسی نشان داد که نتایج ارزیابی pH راشید میکروبی و ارزیابی حسی نشان داد که سودوموناس ارگانیسم ویژه فساد مرغ در شرایط دمایی 15 لیتری از همین تأثیر گذار در طی نگهداری مرغ بود. رشد میکروبی در شرایط دمایی متغیر نیز از همین نتیجه‌گیری می‌شود. نتایج حساسیت درجه حرارت در طی نگهداری بر

واژگان کلیدی: سودوموناس، فساد میکروبی، گوشت مرغ، عمر ماندگاری