

## Biogenic Amines and Their Role as Index of Freshness in Chicken Meat

G. Y. Ivanov<sup>1\*</sup>, I. V. Ivanova<sup>2</sup>, A. K. Slavchev<sup>3</sup> and K. P. Vassilev<sup>1</sup>

<sup>1</sup>Department of Food Preservation and Refrigeration Technology, University of Food Technologies, 26 Maritsa Blvd., Plovdiv, Bulgaria.

<sup>2</sup>Department of Analytical Chemistry, University of Food Technologies, 26 Maritsa Blvd., Plovdiv, Bulgaria.

<sup>3</sup>Department of Microbiology, University of Food Technologies, 26 Maritsa Blvd., Plovdiv, Bulgaria.

### Authors' contributions

*This work was carried out in collaboration between all authors. Author GYI designed the study, supervised the work and wrote the first draft of the manuscript. Author IVI contributed during sample collection, analysis and acquisition of data. Author AKS contributed during conception and design, analysis and interpretation of results. Author KPV contributed during analysis and managed the literature searches. All authors read and approved the final manuscript.*

### Article Information

DOI: 10.9734/JALSI/2015/18326

#### Editor(s):

- (1) J. Rodolfo Rendón Villalobos, Department of Technological Development, National Polytechnic Institute, México.  
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(2) Anonymous, Indian Veterinary Research Institute, India.

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?iid=997&id=40&aid=9627>

Original Research Article

Received 15<sup>th</sup> April 2015  
Accepted 15<sup>th</sup> May 2015  
Published 6<sup>th</sup> June 2015

### ABSTRACT

**Aims:** To evaluate the biogenic amines formation in chicken breast and thigh meat during chilled storage and their potential use as index of freshness.

**Place and Duration of Study:** Department of Analytical Chemistry, Department of Food Preservation and Refrigeration Technology and Department of Microbiology, University of Food Technology (UFT) Plovdiv, between February 2014 and May 2014.

**Methodology:** The biogenic amines (BA) concentrations in chicken breast and thigh meat samples were determined by HPLC analysis and were monitored during storage at two temperature regimes (5.0±1.0°C and 1.0±1.0°C). The changes in biogenic amines content, microbiological and sensory quality of meat were studied.

\*Corresponding author: Email: [ivanovgalin.uft@gmail.com](mailto:ivanovgalin.uft@gmail.com);

**Results:** It was found that during the storage period, the content of putrescine, cadaverine and tyramine increased in all meat samples. The biogenic amine histamine was not detected. The first signs of chicken meat sensory deterioration were found when BAI values reached 20 mg/kg. Poultry samples with total viable count (TVC) higher than  $10^7$  cfu/g always had  $BAI > 20$  mg/kg.

**Conclusion:** The following limits of biogenic amine index (BAI) (putrescine + cadaverine + histamine + tyramine) for chicken breast and thigh meat quality were proposed:  $BAI < 10$  mg/kg for good quality fresh meat,  $10 \text{ mg/kg} < BAI < 20 \text{ mg/kg}$  for acceptable meat and  $BAI > 20 \text{ mg/kg}$  for unacceptable meat with initial spoilage signs.

*Keywords: Biogenic amines; chicken meat; chilled storage; index of freshness.*

## 1. INTRODUCTION

Chilled meat is perishable food stuff and its quality deteriorates progressively throughout storage. The spoilage during storage of refrigerated chicken meat is due to the microbial activity and the biochemical transformations inside the product. Meat freshness evaluation is becoming increasingly important because of the consumer awareness about food quality and safety [1]. Chicken meat quality could be evaluated by determination of its microbiological and sensorial characteristics. Preliminary information about meat freshness could be obtained by sensory evaluation, but taken alone this analysis is not sufficient for estimation of meat quality and safety. Standard microbiological analyses are time consuming and they could be replaced by the analysis of the chemical changes which are caused by the meat microflora. It also could provide important information about the degree of the meat spoilage caused by the microbial decarboxylases. Under appropriate conditions they can convert some amino acids to their corresponding toxic amine [2]. For identifying incipient spoilage some chemical indices were proposed: meat pH values, total volatile basic nitrogen (TVBN), biogenic amines content etc [3]. Undesired accumulation of biogenic amines in food requires both the availability of precursors (i.e. amino acids) and the presence of microorganisms possessing amino acid decarboxylases [4,5,6,7].

Among chemical indicators, biogenic amines, particularly putrescine and cadaverine, have been proposed for determining meat quality. According to Balamatsia et al. [8] the biogenic amine determination is important not only because of their toxicity but also for their application as potential freshness indicators. The fresh meat contains very low quantities of these compounds, during the storage period these concentrations increase due to bacterial growth [9]. Galgano et al. [10] and Hernández-Jover et

al. [11] reported that tyramine, putrescine and cadaverine can be produced during storage of the meat. According to Ruiz-Capillas and Jiménez-Colmenero [12] the most prevalent biogenic amines in meat and meat products are tyramine, cadaverine, putrescine and histamine.

The toxic effects of biogenic amines and their potential effects on human health are not fully clarified. According to the scientific opinion on risk based control of biogenic amine formation in fermented foods of EFSA [13] no adverse health effects were observed after exposure to 50 mg histamine (per person per meal) and 600 mg tyramine for healthy individuals. The information in that respect for putrescine and cadaverine is insufficient.

Different studies on the biogenic amines formation in refrigerated chicken meat showed that the tyramine, cadaverine, putrescine and histamine concentrations are increasing in time, while spermine and spermidine concentrations are decreasing during storage [1,8,14,15,16]. Identification and quantification of biogenic amines is less time consuming in comparison with the microbiological analysis and they could be used as a complex indicator for chicken meat freshness evaluation. However, the practical implementation of this parameter requires more studies.

The aim of the present study was to evaluate the biogenic amines formation in chicken breast and thigh meat during chilled storage at two temperature regimes ( $5.0 \pm 1.0^\circ\text{C}$  and  $1.0 \pm 1.0^\circ\text{C}$ ) and their potential use as index of freshness.

## 2. MATERIALS AND METHODS

### 2.1 Meat Samples and Refrigeration Treatment

The refrigerated chicken breast and thigh meat samples were delivered from local processor

(Gradus-1 Ltd.). All samples were obtained twenty four hours *postmortem*. The meat cuts were stored aerobically up to 10 and 15 days at two temperature regimes  $5.0 \pm 1.0^\circ\text{C}$  and  $1.0 \pm 1.0^\circ\text{C}$ , respectively. Analysis were performed the first day when the meat was received, recorded at day 1, then on the 3<sup>rd</sup>, 5<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day of storage at  $5.0 \pm 1.0^\circ\text{C}$  and on the 3<sup>rd</sup>, 5<sup>th</sup>, 8<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day of storage at  $1.0 \pm 1.0^\circ\text{C}$ . At each storage period five samples were analysed.

## 2.2 Microbiological Analysis

Determination of the total viable count and psychrotrophic bacteria counts were performed according to the standard procedures [17,18]. Meat samples (10 g) were dispersed in 90 mL of saline solution (0.85% NaCl) by using of a Stomacher Lab Blender. Decimals dilutions were prepared and plated on Plate Count Agar (PCA). Inoculated plates were incubated in aerobic condition at  $30^\circ\text{C}$  for 3 days for total viable count and at  $7^\circ\text{C}$  for 10 days for psychrotrophic bacteria determination. After incubation period viable colonies were counted.

## 2.3 Analysis of Biogenic Amines by HPLC

Sample treatments were the same for each kind of meat (chicken breast and thigh meat). The meat cuts were minced. 20 ml of 0.4 M  $\text{HClO}_4$  were added to an amount of 5 g minced meat. The samples were homogenised and then centrifuged for 5 min at 2500 rpm. The supernatant was pooled and diluted to 50 ml with 0.4 M  $\text{HClO}_4$ . The centrifuged acid extract was derivatised according to the following procedure: 200  $\mu\text{l}$  of 2N NaOH were added to 1 ml portions of the diluted supernatant, then buffered by adding 300  $\mu\text{l}$  of saturated  $\text{NaHCO}_3$  solution and then 2 ml of dansylchloride solution (10 mg/ml in acetone) were added. The dansylation reaction proceeds at room temperature [19]. 100  $\mu\text{l}$  of  $\text{NH}_4\text{OH}$  were added after 15 min to stop the reaction and to remove residual dansylchloride. The final volume was adjusted to 5 ml by adding acetonitrile. The obtained dansylated solution was filtered and injected into the Liquid chromatograph (Agilent Technologies, Model 1260 Infinity) equipped with UV detector and Spherisorb ODS2 (C18) (4.6 x 150 mm, 5  $\mu\text{m}$ , Waters, Milford, USA) column. The mobile phase was a mixture of acetonitrile and water (80:20). Chromatographic conditions: injected volume 20  $\mu\text{l}$ , flow rate 0.5 ml/min; detection wavelength ( $\lambda$ ) = 254 nm were used. After each run the column

was conditioned for 10 min. Each HPLC run took about 18 min and afterwards the column must be conditioned again for 10 min.

The presence and abundance of biogenic amines (putrescine, cadaverine, histamine, tyramine – Sigma-Aldrich) were determinate by comparing sample peak retention time to standards. Additionally 1.4-diaminoheptane was used as internal standard. One milliliter of the standard solutions was derivatised as previously described for the acid extracts.

## 2.4 Sensory Evaluation

Sensory quality of all studied samples was evaluated by five member expert panel. The color, appearance, texture and aroma acceptability of chicken breast and thigh samples were evaluated. A five point's hedonistic scale was used: 5 points – good quality (without any off – odors or off – flavours); 4 points – acceptable quality (slight changes in meat color and texture); 3 points - medium quality (significant changes in meat color and texture); 2 points – low quality (slight off-odors and/or off-flavors); 1 – very low quality (spoiling stage). Total sensory evaluation score (the average of sensory quality attributes) for each sample was calculated and submitted at this article. The samples with total sensory evaluation score lower than 3 were evaluated as unacceptable for consumption.

## 2.5 Statistical Analysis

Statistical analyses were carried out on the averages of the triplicate results. Data were analyzed by the analysis of variance (one-way ANOVA) method with a significant level of  $P = .05$  [20]. The Duncan's multiple comparison test (SPSS) with a significant difference set at  $P = .05$  was used to compare sample means. Significant differences between means less than 0.05 were considered statistically significant [21]. All statistical procedures were computed using the Microsoft Excel 5.0 software.

## 3. RESULTS AND DISCUSSION

### 3.1 Microbiological Analysis

Total viable count (TVC) represents the total bacterial load in the studied meat sample. The TVC tests could reflect the general hygiene condition of the poultry. The psychrotrophic bacteria count (PBC) represents that part of microbial population which is able to growth at low temperatures. The results for TVC and PBC

of studied poultry samples are shown on Fig. 1. The initial microbial loads of chicken breast and thigh samples were  $3.0 \times 10^4$  cfu/g and  $2.4 \times 10^5$  cfu/g, respectively (Fig. 1). Balamatsia et al. [1] established similar initial contamination of modified atmosphere-packaged chicken fillets. Higher microbial load of thigh samples is probably due to the presence of skin which is more contaminated during the processing. The psychrotrophic bacteria count of breast and thigh meat at the beginning of cold storage was  $3.2 \times 10^4$  cfu/g and  $1.6 \times 10^5$  cfu/g, respectively. The initial values of TVC and PBC were in the same range. Moreover, PBC of the studied samples had a similar variation trend as TVC (Fig. 1). According to Baston et al. [3] during the cold storage of meat the majority of the microorganisms incline to become mostly psychrotrophic-type due to the adaptation at the new environment conditions. This could explain the similarity in the variation trend of TVC and PBC found in the present study. Different limits for total microbial load were proposed to classify meat quality. Values lower than  $10^6$  cfu/g would indicate good quality meat [22]. TVC values between  $10^6$  cfu/g and  $10^7$  cfu/g - acceptable quality and counts higher than  $10^7$  cfu/g - unacceptable [23]. In the present study, an arbitrary value for total microbial load of  $10^7$  cfu/g was taken for the upper acceptability limit of fresh chicken meat.

For chicken thigh and breast samples stored at  $5.0 \pm 1.0^\circ\text{C}$  such values were exceeded on day 5 and day 8, respectively (Fig. 1A). The TVC and PBC of chicken samples stored at  $1.0 \pm 1.0^\circ\text{C}$  remained below the values of  $10^7$  cfu/g up to the 10<sup>th</sup> day of the storage (Fig. 1B). These results demonstrate the significant impact of storage temperature on the microbial growth in chicken meat. Delayed microbial growth in chicken samples stored at  $1.0 \pm 1.0^\circ\text{C}$  is a main reason for the extended shelf-life and sensory acceptability of these samples in comparison with the samples stored at  $5.0 \pm 1.0^\circ\text{C}$ .

### 3.2 Sensory Evaluation

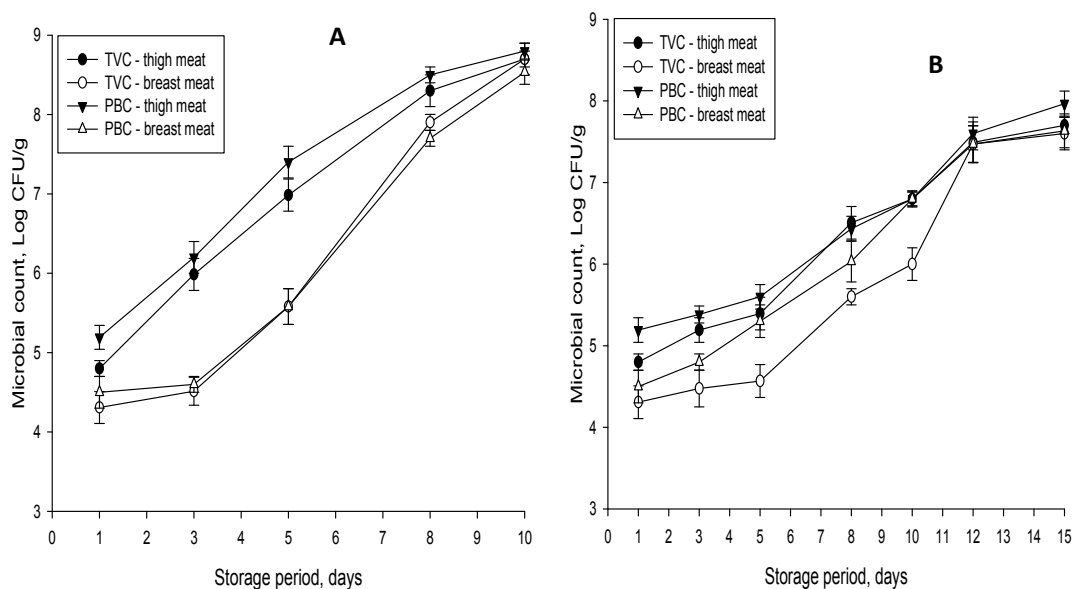
Sensory evaluation is one of the most popular means for assessing meat freshness. At the present study, greatest sensory score received by chicken breast and thigh samples at the first stages of cold storage (Fig. 2).

With the increasing of storage time some alterations in meat texture and flavour were observed. The appearance of off flavours and off odours as well as some great textural changes at

the last stages of the storage indicated for meat deterioration processes started. Usually, such changes are recognized as first signs of deterioration and makes meat sensory quality unacceptable (less than 3 points). It was found (Fig. 2A), that the chicken thigh and breast samples stored at  $5.0 \pm 1.0^\circ\text{C}$  preserved acceptable sensory quality for 5 and 8 days, respectively. These results are in agreement with the findings of other authors [8,24,25] who established that the shelf-life of chicken breast meat stored at  $4.0^\circ\text{C}$  is between 7 and 9 days. The longer shelf-life of chicken breast meat in comparison with thigh meat could be due to the lower microbial load of this product (Fig. 1). The results (Fig. 2B) showed that the chicken thigh and breast samples stored at  $1.0 \pm 1.0^\circ\text{C}$  preserved their acceptable sensory quality up to the 10<sup>th</sup> day of storage. The decreasing of storage temperature with  $4.0^\circ\text{C}$  (from  $5.0 \pm 1.0^\circ\text{C}$  to  $1.0 \pm 1.0^\circ\text{C}$ ) delayed significantly deterioration processes in chicken meat which results in approximately double increase of its shelf-life.

### 3.3 Biogenic Amines Analysis

The biogenic amines formation in meat is associated with amino acid decarboxylase activity of microorganisms during storage and can be used as a quality indicator for poultry products. The changes in biogenic amines content of studied chicken breast and thigh samples during the storage at  $5.0 \pm 1.0^\circ\text{C}$  are shown on Fig. 3. At the beginning of the storage period concentrations of putrescine, cadaverine and tyramine were in the range from 1.2 mg/kg to 3.4 mg/kg. The biogenic amine histamine was not detected in studied poultry samples during the whole storage period. During the first 5 days of storage at  $5.0 \pm 1.0^\circ\text{C}$  biogenic amines content of poultry samples did not changed significantly ( $P = .05$ ). After this period, gradual increase of biogenic amines concentrations was observed. The tyramine content of chicken breast samples increased significantly ( $P = .05$ ) and at the 10<sup>th</sup> day of storage reached up to 25.5 mg/kg. At the same storage period the putrescine and cadaverine concentrations increased from 4.5 mg/kg to 17.0 mg/kg and from 7.2 mg/kg to 15.8 mg/kg, respectively. Similar trends in biogenic amines accumulation were found during storage of chicken thigh samples. Significant increase in biogenic amines concentration of these samples was observed after 8<sup>th</sup> day of storage. At the 10<sup>th</sup> day the content of putrescine, cadaverine, and tyramine in chicken thigh meat was 15.2 mg/kg, 12.8 mg/kg and 18.7 mg/kg, respectively.



**Fig. 1. Changes in the total viable count (TVC) and psychrotrophic microorganisms count (PBC) of studied chicken breast and thigh meat samples during storage at 5.0±1.0°C (A) and 1.0±1.0°C (B)**

The changes in biogenic amines content of poultry samples stored at 1.0±1.0°C are shown on Fig. 4. At the beginning of the storage, biogenic amines concentrations varied from 1.3 mg/kg to 2.8 mg/kg. During the first 10 days of storage period the biogenic amines content of poultry did not changed significantly ( $P = .05$ ). After this period the concentration of putrescine, cadaverine and tyramine increased and on the 15<sup>th</sup> day reached 21.3 mg/kg, 18.4 mg/kg and 26.7 mg/kg, respectively. At the same stage of storage the putrescine, cadaverine and tyramine content of chicken thigh samples was 23.2 mg/kg, 15.2 mg/kg and 27.5 mg/kg, respectively. The results (Fig. 3 and Fig. 4) showed delayed formation of biogenic amines in samples stored at lower temperatures. Probably, this is due to the greater inhibitory effect of lower temperatures on the microbial growth and lower decarboxylase activity of the bacteria responsible for the production of biogenic amines in the muscle tissue. Our results are in agreement with the study of Hutařová et al. [26] who found dependence of biogenic amines formation with temperature and length of storage of eviscerated pheasants. The generation of biogenic amines is a complex biochemical process, but mainly it is due to the activity of the meat microflora. Therefore, the biogenic amines formation in

poultry (Figs. 3 and 4) is related to variations of the microbial population (Fig. 1). Amino acid decarboxylases are enzymes present in many microorganisms such as species of the genera *Bacillus*, *Clostridium*, *Pseudomonas*, *Photobacterium*, as well as in genera of the family *Enterobacteriaceae*, such as *Citrobacter*, *Klebsiella*, *Escherichia*, *Proteus*, *Salmonella* and *Shigella*, and *Micrococcaceae* such as *Staphylococcus*, *Micrococcus* and *Kocuria*. [6, 10]. According to Dainty [9] the biogenic amines putrescin, cadaverine, histamine and tyramine are found in very low levels in fresh meat, and their formation is associated with bacterial spoilage.

In this respect the so-called "biogenic amine index" (BAI) could be used for evaluation of the poultry products freshness. Wortberg and Woller [27] and Hernández-Jover et al. [11] defined BAI as a sum of the putrescine + cadaverine + histamine + tyramine concentrations in meat. In the present study, the first signs of chicken meat sensory deterioration were found when BAI values reached 20 mg/kg. Moreover, the samples with total viable count (TVC) higher than  $10^7$  cfu/g always had BAI > 20 mg/kg. Based on the results from sensory and microbiological analysis of studied poultry samples and the

acceptability limits of  $10^7$  cfu/g for TVC and 3 point for sensory evaluation score, the following limits of BAI for chicken breast and thigh meat quality were proposed: BAI < 10 mg/kg for good quality fresh meat, 10 mg/kg < BAI < 20 mg/kg for acceptable meat and BAI > 20 mg/kg for

unacceptable meat with initial spoilage signs. For the practical implementation of these BAI limits further investigations on the effect of breed and feeding conditions, as well as microbial contamination on the biogenic amines content of cold stored poultry are needed.

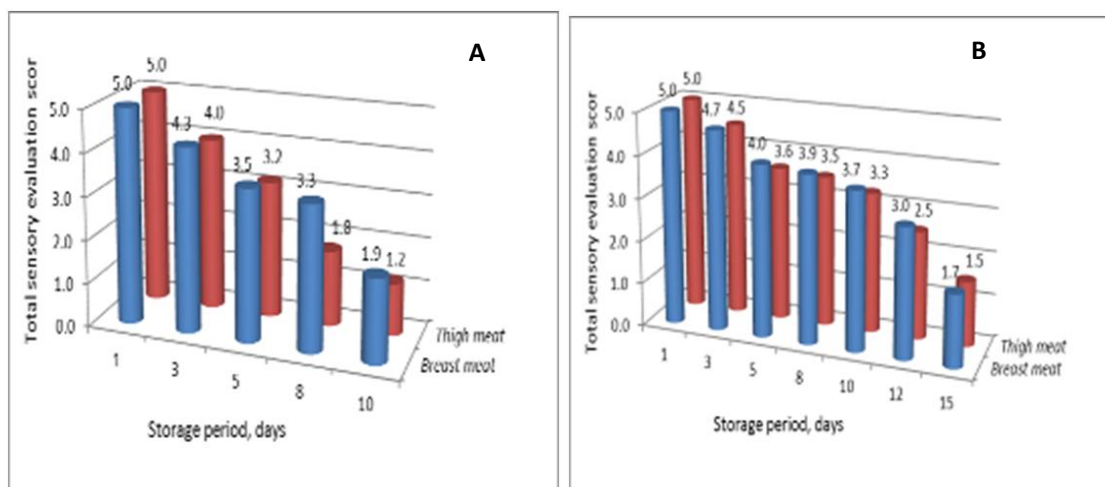


Fig. 2. Changes in total sensory evaluation score of studied chicken breast and thigh meat samples during storage at 5.0±1.0°C (A) and 1.0±1.0°C (B)

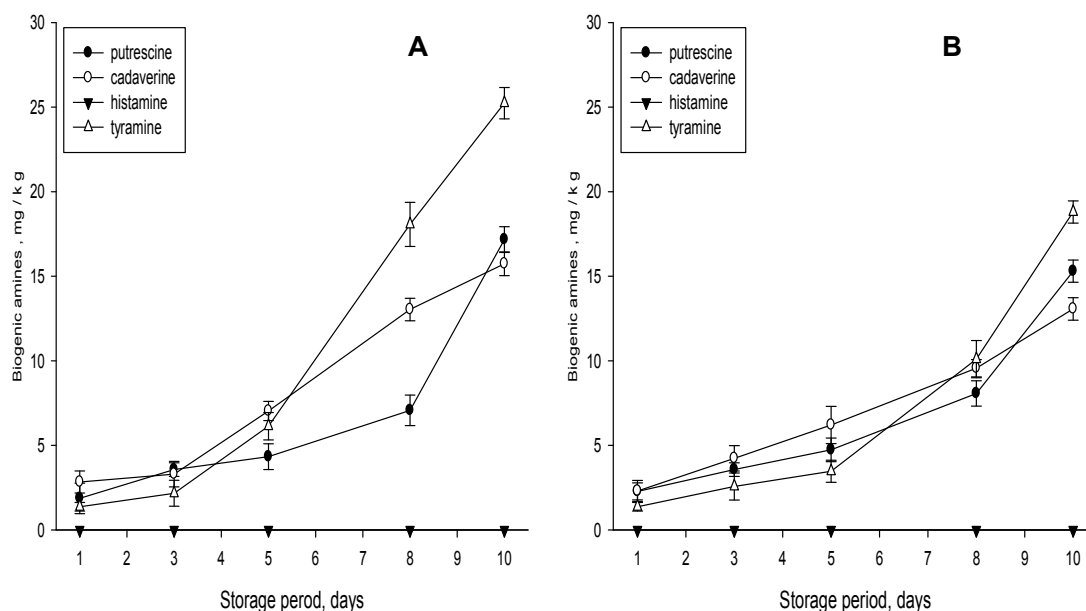
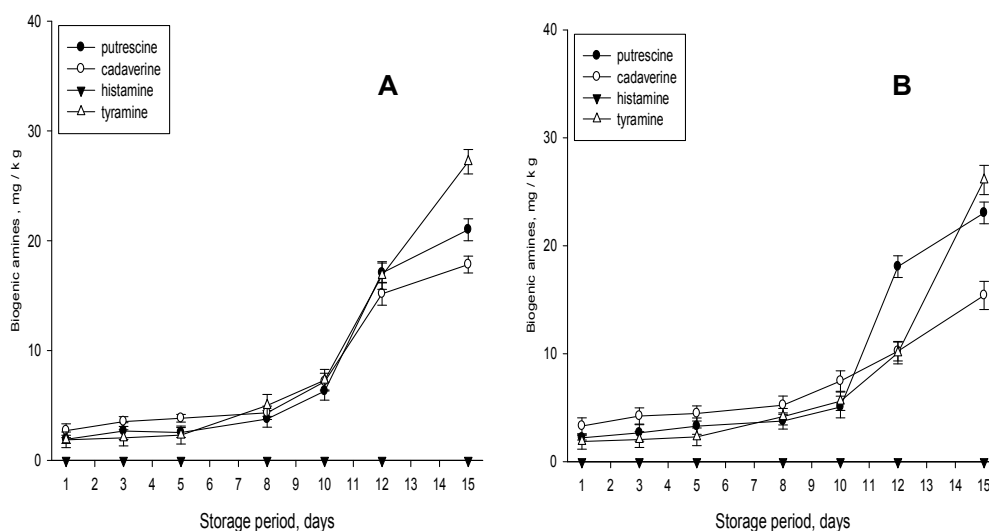


Fig. 3. Changes in the concentration of biogenic amines in studied chicken breast (A) and thigh (B) meat samples during storage at 5.0±1.0°C



**Fig. 4. Changes in the concentration of biogenic amines in studied chicken breast (A) and thigh (B) meat samples during storage at  $1.0\pm 1.0^{\circ}\text{C}$**

#### 4. CONCLUSION

The shelf-life of the poultry samples stored at  $5.0\pm 1.0^{\circ}\text{C}$  and  $1.0\pm 1.0^{\circ}\text{C}$  was evaluated on the basis of the microbiological and sensory parameters. Based on the obtained results the BAI (putrescine + cadaverine + histamine + tyramine) values higher than 20 mg/kg of muscle are proposed as the upper limits for initiation of spoilage in fresh chicken meat stored aerobically. The results obtained demonstrate the applicability of biogenic amine index (BAI) as indicator for poultry quality and freshness evaluation.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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