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# Biogenic Amines as Freshness Indicator in Halal and Non-Halal Slaughtered Chicken Meat Using Chromatographic Approach

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**Abstract**-- Biogenic amines are naturally occurring organic bases produced by bacterial decarboxylation of amino acids that have been associated with harmful toxicology effects to humans. The main objective of this work is to investigate the possible role of biogenic amines (BAs) as indicators of spoilage in fresh chicken meat stored at 4°C for 15 days. A reversed-phase high-performance liquid chromatographic method with isocratic elution system is used for the quantification of four biogenic amines (putrescine, histamine, tyramine, cadaverine and spermine as well as spermidine) in chicken meat. Amines were extracted with 5% of trichloroacetic acid (TCA) and derivatised using dansyl chloride. The variation storage time differentiated the chicken meat on the microbiological characteristics. The results obtained shows that histamine, spermidine, tyramine, cadaverine, and putrescine increased slowly while spermine decreased for both chicken breast in halal and non-halal chicken meat. Thus, this BAs could be used as a spoilage index of fresh chicken meat.

**Keywords**- chicken, freshness, biogenic amines, trichloroacetic acid, dansyl chloride

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## I. INTRODUCTION

Biogenic amines (BAs) are organic bases of low molecular weight that possess biological activity. It is usually produced by the decarboxylation of free amino acids, aldehydes and ketones transamination or nitrogen compound hydrolysis [1] [2]. It can be found in various types of foods and beverages, such as wines, beer, meat, fish, and processed food [3]. Moreover, it can be formed or degraded as a result of regular metabolic activity. The two main factors for BAs production are the food and microorganism type [4]–[6].

There are two types of BAs occurring from living organism reactions such as histamine, tyramine, putrescine and cadaverine, where naturally occurring amines are spermine and spermidine [7] [8]. These BAs are also often related to the food spoilage, and the amounts in foods can substantially differ and drastically rise as a result of microbial putrefaction [9]. The excessive intake of BAs in our body can lead to the risk of getting various diseases, such as headache, dizziness, itching, nausea, vomiting, diarrhoea, heart palpitation, and respiratory difficulty [10].

Amino acid decarboxylation is the essential BAs production pathway when microorganisms spoil the fresh meat. The quantity of BAs can be considered as a marker for microbiological contamination level in food [2] [7]. They can, directly and indirectly, cause toxicity when their concentration levels are high. Therefore, it is suitable for detecting incipient spoilage, and their quantities can be related to the freshness of meat [1] [3] [11].

Many analytical methods have been described based on different techniques for BAs determination in foods, such as ion exchange chromatography (IEC), gas chromatography (GC), thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) [12] [13]. Still, HPLC is the most preferred method either using pre-column or post-column derivatisation. The most common derivatised agents are dansyl chloride (DnCl), benzoyl chloride and *o*-phthalaldehyde (OPA), which results in high resolution of separation [6] [8] [14]. For the derivatisation reaction, many researchers suggest that it must be done in the dark due to the sensitivity of the agent to light which can also be affected by temperature [15] [16].

Therefore, this work aimed to evaluate the fresh chicken meat stored at 4°C for 15 days to assess the reaction using BAs as biomarkers using high performance liquid chromatography (HPLC) method.

## II. MATERIALS AND METHODS

### A. Materials

The analytical standards and chemical for sample preparation were obtained from Sigma Aldrich (Selangor, Malaysia). The stock solutions used were putrescine (C<sub>4</sub>H<sub>12</sub>N<sub>2</sub>), cadaverine (C<sub>5</sub>H<sub>14</sub>N<sub>2</sub>), histamine (C<sub>5</sub>H<sub>9</sub>N<sub>3</sub>), tyramine (C<sub>8</sub>H<sub>11</sub>NO), spermidine (C<sub>7</sub>H<sub>19</sub>N<sub>3</sub>), and spermine (C<sub>10</sub>H<sub>26</sub>N<sub>4</sub>). The chemicals used for extraction and derivatisation of the sample are dansyl chloride (C<sub>12</sub>H<sub>12</sub>ClNO<sub>2</sub>S), acetone (CH<sub>3</sub>)<sub>2</sub>CO, sodium bicarbonate (NaHCO<sub>3</sub>), toluene (C<sub>7</sub>H<sub>8</sub>), acetonitrile (C<sub>2</sub>H<sub>3</sub>N), and trichloroacetic acid (C<sub>2</sub>HCl<sub>3</sub>O<sub>2</sub>).

### B. Sampling

The halal chicken was obtained from the morning market at Semarak and local supermarket, while the non-halal chicken was obtained from the morning market at Mantin. The non-halal chicken was found to be dead at the time the chicken was obtained without undergoing the slaughtering process. The acquired part of the chicken for this research was the chicken breast, which was stored for 15 days in the freezer with 4°C prior analysis [17]–[19].

### C. Sample Preparation

5 g of grounded chicken meat sample was weighed in 50 mL centrifuge tube and 7 mL of 50 g/L trichloroacetic acid (TCA) was added. Then, the tube was homogenised for 2 minutes by using vortex and centrifuged at 5000g at 4°C for 25 minutes in a refrigerated centrifuge. The supernatant was filtered through Whatman No.1 filter paper. The procedure above was repeated twice with the addition of 7 mL and 6 mL of TCA, respectively. Finally, the extractant was stored in 1 mL microtubes and kept frozen at 4°C.

### D. Derivatisation

Solution derivatisation was prepared by adding 1000 mg/L of dansyl chloride into 100 mL of acetone in a volumetric flask. 1 mL of the extractant or standard was transferred into the centrifuge tube. Then 0.5 mL of Na<sub>2</sub>CO<sub>3</sub> and 1 mL of 10 mg/mL of dansyl chloride were added in the same centrifuge tube. The mixture was then shaken and incubated at 40°C for 45 minutes in the water bath. After that, 250 µL of ammonia was added to remove the excess of unreacted dansyl chloride. The solution was then extracted three times with 1 mL of diethyl ether and evaporated to dryness under nitrogen gas. The derivatised solution was then diluted with 0.5 mL acetonitrile. It was then filtered using a 0.45 µm nylon membrane into HPLC vials [14] [20].

### E. Chromatographic Analysis

The sample was analysed using high performance liquid chromatography (HPLC) (Agilent 1260 Infinity, USIM, Negeri Sembilan) to determine the presence of biogenic

amine in the samples. Biogenic amine separations were performed under isocratic conditions on a Teknokroma Tracer Extrasil ODS2 column (15×0.46 cm id., 5 µm) equipped with a Supelco Ascentis C18 (2×0.40 cm id., 5 µm) guard column. The mobile phase was a gradient elution program with a binary mixture of A: Acetonitrile (ACN) and B: deionised water, as shown in Table I. In an ultrasonic bath, the mixture was degassed. The flow rate and the volume of the sample injection were set at 1.2 mL/min and 15 µL, respectively. The eluent was monitored by diode array detector at a wavelength of 198 nm. To flush the HPLC system, a 10-minute pure acetonitrile injection was used between each sample. The BAs were identified by retention time and quantified by peak area. The determination of standards retention time was repeated ten times while the identification of BAs in the halal and non-halal chicken breast was performed at 1, 3, 5, 10, and 15 days of storage.

TABLE I

GRADIENT ELUTION PROGRAM USED FOR HPLC ANALYSIS

Gradient elution program		
Time (min)	A%	B%
1	65	35
10	80	20
12	90	10
16	100	0
23	100	0
25	65	35
30	65	35

## III. RESULTS AND DISCUSSION

### A. Method reliability studies for biogenic amines detection.

The established method analysis for BAs determination was tested by assessing the selectiveness and precision of the peak obtained. Selectivity is the capability to locate the analyte at a specified retention time, while precision refers to the degree of proximity cluster of data with a repeated measurement under the same condition. In this research, the peaks of BAs were separated and precised very well using the proposed method and instrument. The method was repeated four times using different wavelengths, which are 198, 245, 254, and 340 nm, where the chromatogram of biogenic amine obtained are shown in Figure 1. The data showed that the desired peak was separated at the same retention time at 6.374, 7.055, 7.463, 11.201, 12.346, and 15.505 minutes for putrescine, cadaverine, histamine, tyramine, spermine, and spermidine.

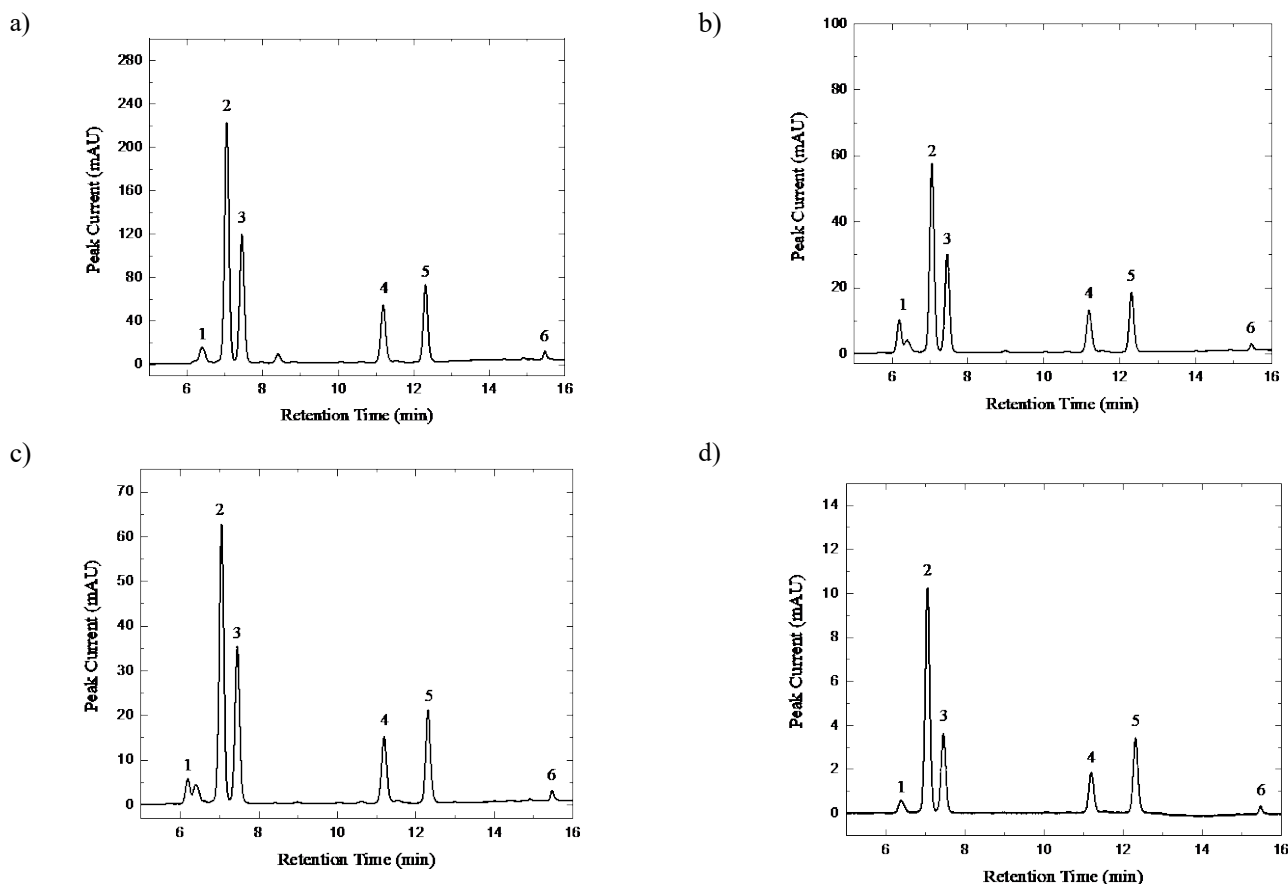


Fig. 1: Biogenic amines chromatogram in different UV detection a) 198, b) 245, c) 254, and d) 340. 1=PUT, 2=CAD, 3=HIS, 4=TYR, 5=SPD and 6=SPM

Repeatability assessment was done by repeating the dansylation process for six different standards of BAs within ten times. The relative standard deviation and the average value of the retention time for the BAs were calculated and tabulated in Table II. The relative standard deviation of the BAs obtained were between 0.265% and 1.004%, which indicate that the data is clustered around the mean. The HPLC analysis of standard to determine the retention time is shown in Figure 2.

TABLE II

REPEATABILITY DETERMINATION FOR THE RETENTION TIME OF BIOGENIC AMINES

Biogenic Amine	Retention Time (min)
	Mean + RSD
Putrescine	6.374 ± 0.602%
Cadaverine	7.055 ± 1.004%
Histamine	7.463 ± 0.764%
Tyramine	11.201 ± 0.522%
Spermidine	12.346 ± 0.476%
Spermine	15.505 ± 0.265%

### B. Determination of Biogenic Amines Content in Chicken Meat

According to Table III, IV and V, the BA content in chicken meat was affected by the time of storage. The value of spermine showed the highest amount in both types of different slaughtered meat for day 1 and decreased significantly until day 15. It indicates that the meats were still fresh because spermine is one of the indicators of food freshness. The decrease of spermine content happened because the nitrogen from its molecular structure was used as the source for microorganisms and also might be due to the enzymatic reaction of polyaminooxidase [7].

Putrescine, Cadaverine, Histamine, Spermidine, and Tyramine value increased until day 3, which might be due to microbial activity by amino acid decarboxylation, cells biochemical, and enzymatic activity. Spermine and spermidine are two amines involved in cellular metabolism, essential for organism growth, development, and proliferation of cells. That is why the current peak of spermine decreased, but spermidine increased in time. Table III shows the peak current of BAs detected in halal slaughtered (HS) chicken meat from morning market Semarang.

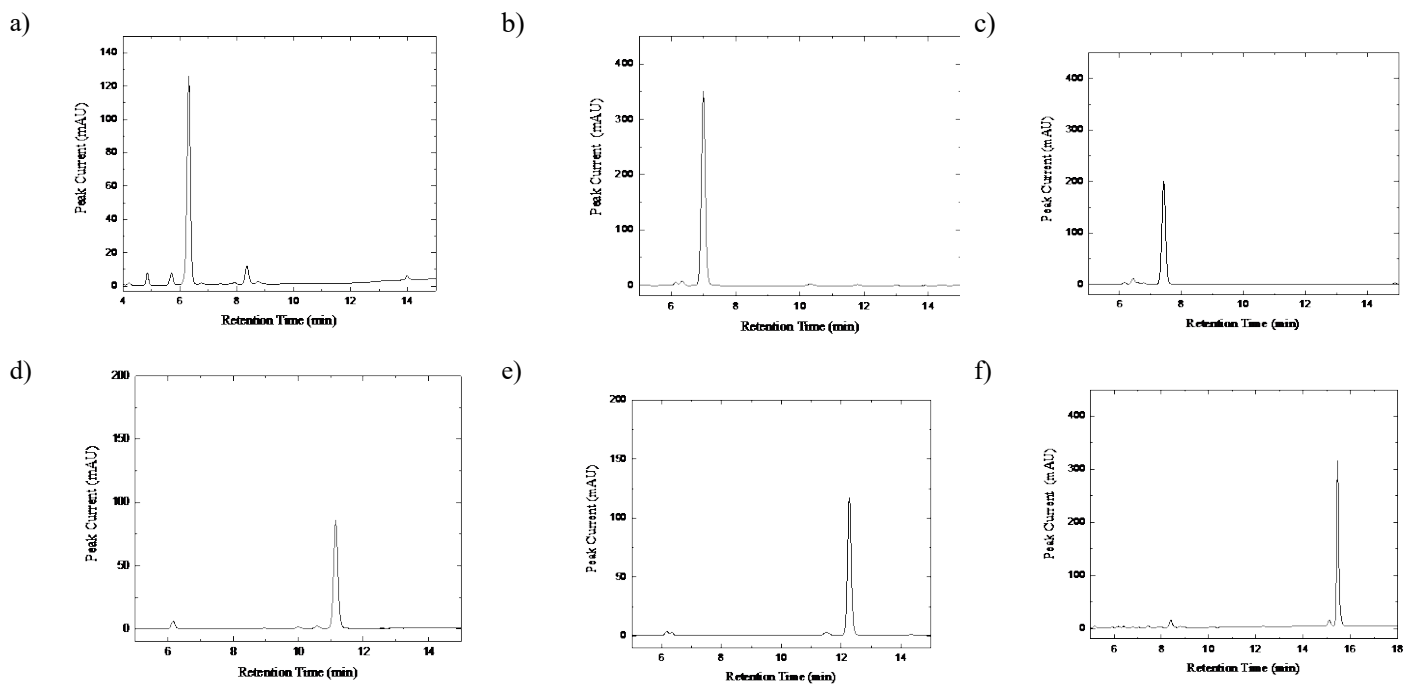


Fig. 2: Biogenic amines standard retention time; a) PUT, b) CAD, c) HIS, d) TYR, e) SPD and f) SPM

TABLE III

PEAK CURRENT OF BIOGENIC AMINES IN CHICKEN MEAT FROM MORNING MARKET SEMARAK

Biogenic Amines Peak Current (mAU)						
Days	PUT	HIS	SPM	SPD	TYR	CAD
1	36.571	15.171	79.514	19.245	n.d	21.084
3	51.911	59.970	59.083	33.985	15.027	40.530
7	161.718	n.d	46.876	46.876	73.966	n.d
15	148.465	20.410	23.490	38.086	n.d	n.d

n.d: not detected

From halal slaughtered (HS) chicken meat from a local supermarket, spermine shows the highest initial value of peak current, which was 27.213 mAU, while the lowest value was cadaverine with 11.044 mAU. Spermidine was not found on the first day of the sample analysis. This might be due to the spermine had not yet synthesised to spermidine. Overall, BAs show increases in trend along the 15 days of storage except for spermine. Table IV shows the summary for the peak found in the chicken meat. Galgano et al. [2] have reported that BA content increased in red and white meat after 5 days of storage. Thus, this could explain the attack of proteins by proteolytic enzymes with consequent major availability of amino acid precursors for the BAs production [2] [21].

In the meat sample analysed, the initial assessed amount of Putrescine was about 36.571 mAU and only after 15 days of

storage, the Putrescine level showed a significant increase. Changes in Putrescine levels are generally associated with microbial spoilage, storage temperature, and storage time of meat, as confirmed in literature [22] [23].

TABLE IV

PEAK CURRENT OF BIOGENIC AMINES IN CHICKEN MEAT FROM LOCAL SUPERMARKET

Day	Biogenic Amines Peak Current (mAU)					
	PUT	CAD	HIS	TYR	SPD	SPM
1	15.842	11.044	22.220	14.231	n.d	27.213
3	6.730	15.596	n.d	n.d	n.d	14.693
7	n.d	n.d	n.d	11.181	8.721	21.058
10	113.956	n.d	44.006	28.291	n.d	64.381
15	121.257	79.809	34.798	22.855	17.177	83.200

n.d: not detected

TABLE V  
PEAK CURRENT OF BIOGENIC AMINES IN CHICKEN MEAT  
FROM MORNING MARKET MANTIN

Biogenic Amines Peak Current (mAU)			
Days	PUT	HIS	SPM
1	20.49	9.38	34.15
3	70.28	17.102	29.16
7	157.9	n.d	7.52
10	199.47	n.d	n.d
15	135.66	n.d	n.d

For non-halal slaughtered (NHS) chicken meat from morning market Mantin, the BAs detected were only putrescine, histamine, and spermine. This might be due to the error in the derivatisation method of the chicken extraction, or the BAs do not exist at all in non-halal chicken. The error that might happen during derivatisation was the biogenic amine might not react entirely with dansyl chloride because some other factors affect the dansylation reaction of Bas, which are temperature, pH, and the amount of dansyl chloride solution [15].

Putrescine and histamine increased in peak current by time, while Spermine decreased by time. The least peak current of biogenic amine found in the first day is histamine with only 9.38 mAU. The peak current of BAs in the non-halal chicken breast from the morning market at Mantin is tabulated in Table V.

#### C. Comparison of Halal and Non-Halal Chicken Slaughtered

The initial peak of spermine was highest in HS chicken meat from morning market Semarang with 79.514mAU compared to HS chicken meat from the local supermarket and NHS chicken meat from morning market Mantin, which is 34.15mAU. As shown in Table II, III, and IV, Putrescine showed the value increase over the storage time for both types of slaughtering method. Cadaverine, Spermidine, and Tyramine were not found in NHS chicken meat from Mantin. This might be due to incomplete reaction during the derivatisation process, where either the method are not sensitive enough to detect the smaller amounts of this BAs, or inexistence in the chicken meat [3].

Based on the data obtained for spermidine and spermine, HS chicken from morning market at Semarang indicates a higher quality of chicken meat compared to HS chicken meat from local supermarkets and NHS from the morning market Mantin. The current peak of these two BAs was higher in HS chicken meat from the morning market in Semarang than in HS chicken meat in the local supermarket. However, the content of spermine in NHS chicken meat from morning

market Semarang is higher than the local supermarket. This might happened due to the chicken from the local supermarket was slaughtered earlier than the other two.

#### IV. CONCLUSION

The results of this research have highlighted that the shelf life of the meat stored at 4°C for 15 days showed no significant differences in both types of different slaughtered meat. This indicates that the freshness of the meat decreases upon the time of storage while increasing the storage time increases the values of BAs except for spermine. This report suggests that BAs should be included in the quality level as measures of freshness and that further research should be carried out to examine the meat handling and storage phase.

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