



Relationship between the Concentration of Biogenic Amines and Volatile Basic Nitrogen in Fresh Beef, Pork, and Chicken Meat

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ABSTRACT : Changes in the concentrations of biogenic amines (BAs) in fresh beef, pork, and chicken breast and leg were investigated during storage, and the relationship between the content of volatile basic nitrogen (VBN) and BAs was evaluated. As the storage period increased, the levels of putrescine (PUT), cadaverine (CAD) and tyramine (TYM) increased in all the meat samples, except for TYM in beef ($p < 0.001$). The level of BAs in beef, pork and chicken changed but the extent of these changes was different among the kinds of BAs and meats. Measurement of the VBN content was confirmed as a good index for interpreting the specific BAs content in general, such as PUT, CAD, and TYM, as well as evaluating a meat's freshness during storage. However, the kinds of BAs which can be predicted from the VBN content varied in different meats ($p < 0.05$). (**Key Words :** Biogenic Amines, Volatile Basic Nitrogen, Beef, Pork, Chicken)

INTRODUCTION

The organoleptic quality and freshness of meats deteriorate by physical, chemical, and biological changes during storage. Volatile basic nitrogen (VBN) content and microbial counts have been used for the evaluation of meat freshness (Vinci and Antonelli, 2002). The VBN content of meat increases as putrefaction progresses since ammonia is produced during storage as a result of the deamination of amino acids. Accordingly, the total amount of VBN is one of the best indices of the decomposition of fresh meat and poultry (Byun et al., 2003). In Korea, the upper limit of VBN is 20 mg % for fresh meat (Korea Food and Drug Administration, 2002).

The quantity of biogenic amines (BAs) is also to be considered as a marker of the level of microbiological contamination in food (Vinci and Antonelli, 2002; Min et al., 2004b). BAs are organic compounds with a low molecular

weight that are formed through the enzymatic decarboxylation of specific amino acids in various foods during storage (Halasz et al., 1994; Hernandez-Jover et al., 1997; Min et al., 2004a). Besides the microbiological contamination, BAs levels in food are important for assessing health hazards such as certain neurotransmission disorders because of their actions as false neurotransmitters (Silla Santos, 1996). Especially, BAs are produced in foods where high levels of protein are present, for example in meat. The BAs that are often found in foods include cadaverine (CAD), putrescine (PUT), histamine (HIM), tyramine (TYM), serotonin (SER), β -phenylethylamine (PHM), spermine (SPM) and spermidine (SPD). CAD is formed from lysine, PUT from ornithine, HIM from histidine, TYM from tyrosine, SER from TRM, PHM from phenylalanine, SPM from PUT and SPD from SPM (Halasz et al., 1994; Chen et al., 2002).

Using BAs content as an index for the freshness of meat has been tried. Mietz and Karmas (1977) proposed the Chemical Quality Index (CQI) as an index of freshness. CQI is calculated from a sum of concentrations of HIM, PUT, CAD, SPM and SPD. Later, Veciana-Nogues et al. (1997) reported that the TYM content increased significantly during storage and the authors suggested a BA index (BAI) that was the sum of the concentrations of HIM, CAD, TYM and PUT. However, a BAs determination in meat is suitable for detecting initial spoilage (Vinci and

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Received December 18, 2006; Accepted April 2, 2007

Antonelli, 2002) because BAs could be degraded by some microorganisms (Leuschner et al., 1998). Red and white meat differs regarding their nutritional value, production processes, economic aspects, and spoilage (Eerola et al., 1993). However, Vinci and Antonelli (2002) concluded that the BAs levels were indicators of spoilage both in red and white meat. Particularly a determination of the cadaverine concentration could be used to monitor spoilage in both red and white meat and also the tyramine concentration is a useful indicator to control red meat during storage (Vinci and Antonelli, 2002).

The objective of this study was to investigate the changes in the concentration of BAs in fresh beef, pork, and chicken breast and leg during storage and to evaluate the relationship between VBN and the BAs concentration.

MATERIALS AND METHODS

Chemicals

Amine standards (β -phenylethylamine hydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, serotonin creatinine sulfate, tyramine hydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride and 1,7-diaminoheptane), sodium bicarbonate, sodium hydroxide, ammonium acetate, and dansyl chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ammonia and perchloric acid (70%) were purchased from Showa Chemical Co. (Tokyo, Japan) and acetonitrile and acetone (HPLC grade) were purchased from TEDIA (Cincinnati, OH, USA). The chemicals were used without any further purification treatment.

Sample preparation

Totally 18 beef cattle and 18 pigs (6 animals per batch and different stores for each batch) were obtained after 24 h of slaughter (Suwon, Korea). About 1 kg of beef and pork loin (*M. longissimus dorsi*) of the each animal was obtained. Also totally 30 chickens (10 animals per batch) were obtained from a local market for the preparation of breast and thigh samples. All meat samples were immediately placed in an ice box and transferred to the laboratory. Lean flesh was taken from each batch (6 beef loins, 6 pork loins, and 10 chicken breast and thigh in each batch), pooled, ground through a 9 mm plate, and patties (approximately 100 g) were made by using a petri dish. Totally 15 patties per each animal meat sample were made and 3 patties were used for a single storage time. The batch was considered as replication (triplicates) with 3 observation number in each storage time. The patties were packaged in a high density polyethylene (HDPE) film without vacuum and stored in a refrigerator at $4\pm 2^\circ\text{C}$ and considered as Day 0. The analysis was started at the next day (Day 1).

The amount of the volatile basic nitrogen (VBN) and biogenic amines (BAs) of the samples of beef and pork were analyzed at 1, 4, 7, 11 and 15 days, and those of the chicken samples at 1, 3, 5, 7, and 9 days. The experiments were done in triplicate (3 patties of each batch) with wet basis analysis.

Measurement of BAs content

Two grams of the sample was weighed into a 50 ml polypropylene conical tube (Beckton Dickinson & Co., Franklin Lakes, USA) and homogenized (Ultra-Turrax 25, IKA-Labortechnik, Staufen, Germany) in 10 ml of 0.4 M perchloric acid. The homogenized sample was centrifuged for 10 min at 3,000 rpm (Union 5KR, Hanil Co., Incheon, Korea) and the supernatant was filtered through filter paper (Whatman No.1, Whatman International Ltd., Maidstone, England). Ten milliliter of 0.4 M perchloric acid was added to the remnant and mixed thoroughly in a vortex mixer (Vortex- Genie2, Scientific Industries, Inc., Bohemia, USA). This mixture was centrifuged for 10 min at 3,000 rpm and the supernatant was filtered again through the same type of filter paper. Finally, the volume of filtrate collected from both steps was adjusted to 25 ml with 0.4 M perchloric acid. One milliliter of a sample extract was taken into a 15 ml polypropylene conical tube (Beckton Dickinson & Co., Franklin Lakes, USA) and 50 μl of an internal standard (1,000 ppm 1, 7-diaminoheptane) was added. Two hundreds microliters of 2 N sodium hydroxide, 300 μl of saturated sodium bicarbonate and 2 ml of dansyl chloride solution (10 mg dansyl chloride dissolved in 1 ml acetone) were added to a sample extract before incubation for 45 min at 40°C in a water bath. After incubation, 100 μl of liquid ammonia was added to the reaction mixture for the removal of any residual dansyl chloride. After 30 min at an ambient temperature, the volume of the reaction mixture was adjusted to 5 ml with acetonitrile. This reaction mixture was centrifuged for 5 min at $2,500\times g$. The supernatant was filtered with a 0.45 μm syringe filter with a PVDF Membrane (Acrodisc LC13 PVDF minispike, Pall Co., Ann Arbor, USA). Ten microliters of a filtered sample was injected to the HPLC with a diode array detector (Agilent 1100, Agilent Technology Inc., Wilmington, USA) equipped with a Spherisorb ODS2 column (4.6 \times 150 mm i.d., 5 μm , Waters, Milford, USA). Gradient elution program was used with a mixture of 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. Both solvents were vacuum filtered by a membrane filter (47 mm PTFE 0.45 μm , Pall Co., Ann Arbor, USA) and degassed with an ultra-sonicator (5210, Branson Ultrasonic Co., Danbury, USA). The flow rate was 1 ml/min. The gradient began at 50% solvent A and 50% solvent B and ended at 10% solvent A and 90% solvent B at 19 min, respectively. Ten minutes of a waiting time before the next analysis was necessary for an

Table 1. Mean concentrations of biogenic amines ($\mu\text{g/g}$) and volatile basic nitrogen (VBN, mg %) levels of beef loin during storage at $4\pm 2^\circ\text{C}$ ($n = 3$)

BAs	Storage (days)				
	1	4	7	11	15
PHM	nd ^b	nd ^b	nd ^b	2.1 \pm 0.31 ^a	2.1 \pm 0.13 ^a
PUT	1.0 \pm 0.04 ^c	1.2 \pm 0.04 ^c	3.0 \pm 1.71 ^c	113.9 \pm 6.46 ^b	202.5 \pm 15.27 ^a
CAD	nd ^d	9.4 \pm 1.85 ^c	62.7 \pm 8.65 ^b	70.5 \pm 6.96 ^b	221.4 \pm 26.33 ^a
HIM	1.8 \pm 0.56	4.4 \pm 0.50	2.7 \pm 0.23	5.9 \pm 0.21	7.4 \pm 2.66
SER	17.1 \pm 5.24	13.3 \pm 3.22	9.5 \pm 1.91	9.5 \pm 1.51	10.6 \pm 0.96
TYM	nd ^c	3.1 \pm 0.33 ^{bc}	2.0 \pm 0.19 ^c	6.6 \pm 1.30 ^b	17.4 \pm 2.43 ^a
SPD	4.0 \pm 0.29	4.3 \pm 0.63	2.7 \pm 0.07	5.9 \pm 1.34	6.3 \pm 0.97
SPM	34.0 \pm 0.26	32.0 \pm 2.29	35.0 \pm 1.27	29.0 \pm 2.68	36.6 \pm 4.17
TABA	57.8 \pm 5.90 ^c	67.6 \pm 5.61 ^c	117.3 \pm 10.70 ^c	243.3 \pm 14.87 ^b	504.3 \pm 39.41 ^a
CQI	0.1 \pm 0.02 ^c	0.4 \pm 0.04 ^c	1.8 \pm 0.21 ^c	5.3 \pm 0.51 ^b	10.1 \pm 1.75 ^a
BAI	2.7 \pm 0.53 ^d	18.1 \pm 2.72 ^{cd}	70.4 \pm 10.69 ^c	196.8 \pm 13.70 ^b	448.7 \pm 41.80 ^a
VBN	8.5 \pm 0.25 ^b	8.6 \pm 0.49 ^b	12.3 \pm 0.58 ^b	32.2 \pm 2.95 ^a	36.7 \pm 1.86 ^a

[†] Not detected.

^{a-d} Mean \pm standard deviation within the same row with the same superscript were not significantly different (^b and ^{bc}, ^{bc} and ^c were not significantly different; $p < 0.05$).

Abbreviations: BAs, biogenic amines; PHM, β -phenylethylamine; PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amine; CQI, chemical quality index calculated by the sum of HIM, PUT, CAD, SPM, and SPD; BAI, biogenic amine index calculated by the sum of HIM, CAD, TYM, and PUT.

equilibrium. The column temperature was 40°C . The amounts of the dansyl derivatives of the biogenic amines were quantified by a measurement of the UV-absorption at 254 nm and the fluorescence at 550 nm.

Measurement of volatile basic nitrogen (VBN) content

VBN content was determined by the Conway micro-diffusion technique with slight modification from the method of Miwa and Iida (1973). Meat sample (5 g) was placed into a 50 ml tube and 15 ml of distilled water was added. The sample was homogenized for 2-5 min at $21,000\times g$ and adjusted to 50 ml with distilled water. The homogenate was filtered through a filter paper (Whatman No.1, Whatman International Ltd., Maidstone, England). Grease was plastered on to the cover and the contact surface of a Conway tool. One milliliter of the filtrate was put into the outer space of the Conway tool and 1 ml of 0.01 N H_3BO_3 and 2-3 drops of Conway reagent (0.066% methyl red: 0.066% bromocresol green, 1:1) were added to the inner space. The Conway tool was sealed immediately after adding 1 ml of saturated K_2CO_3 to the outer space. The sealed Conway tool was shaken slowly and incubated at 37°C for 120 min. Then, 0.02 N H_2SO_4 was added to the inner space for a titration. The VBN content was calculated by the following equation:

$$\text{VBN mg \% (mg/100 g sample)} = \frac{(a - b) \times f \times 28.014}{S} \times 100$$

where S is the meat sample weight in grams, b is the volume of added H_2SO_4 in blank in ml, a is the volume of added H_2SO_4 in the sample in ml, and f is the standard

factor of H_2SO_4 (Miwa and Iida, 1973).

Statistical analysis

Statistical analysis was performed using SAS 8.01 for Windows (SAS, 2000). One-way ANOVA was performed and Duncan's multiple range test was used to analyze the significant differences among the mean values. Pearson's correlation coefficients were used to determine the relationship between BAs and VBN.

RESULTS AND DISCUSSION

Table 1 shows the changes of BAs contents in beef loin during 15 days of storage. As storage time increased, PHM, PUT, and TYM concentration increased significantly ($p < 0.05$). Especially, PUT and CAD levels increased from 0.97 and none to 202.54 and 221.38 $\mu\text{g/g}$, respectively. This increase of BAs during storage is higher than those reported by Kaniou et al. (2001). Other BAs measured such as HIM, SER, SPD tended to increase their concentration during storage, however a statistical significance was not found. Lee and Yoon (2001) reported a lower value for SPM content when compared to the present results and that of the SPM content increased with an increase of storage in vacuum-packed beef. Yano et al. (1995) observed that TYM was a major BA during storage of beef at 0, 5 and 10°C . Mietz and Karmas (1977) found that the concentration of SPM and SPD increased in a tuna during decomposition process. The values CQI, BAI, and VBN, which are used as indices of freshness, as well as TABA were increased significantly ($p < 0.05$, Table 1).

Table 2. Mean concentrations of biogenic amines ($\mu\text{g/g}$) and volatile basic nitrogen (VBN, mg %) levels of pork loin during storage at $4\pm 2^\circ\text{C}$ ($n = 3$)

BAs	Storage (days)				
	1	4	7	11	15
PHM	nd ^b	nd ^b	nd ^b	6.2±0.52 ^a	7.1±1.08 ^a
PUT	nd ^c	0.1±0.14 ^c	2.5±0.86 ^c	27.7±2.63 ^b	66.5±12.08 ^a
CAD	1.0±0.04 ^b	20.5±1.40 ^b	31.7±15.94 ^b	43.7±3.32 ^b	145.4±24.81 ^a
HIM	0.9±0.28	1.1±0.11	2.3±0.54	1.3±0.73	1.8±0.45
SER	23.1±2.32 ^a	25.4±0.32 ^a	11.5±0.59 ^b	7.6±0.26 ^b	8.7±1.62 ^b
TYM	nd ^d	3.3±0.28 ^{bc}	1.9±0.45 ^c	4.6±0.58 ^b	29.0±0.97 ^a
SPD	2.1±0.08	2.3±0.10	1.6±0.19	6.9±2.15	6.7±2.50
SPM	32.4±2.30 ^b	33.3±0.70 ^b	26.2±2.90 ^c	34.0±1.26 ^b	42.5±0.92 ^a
TABA	59.6±4.06 ^c	86.1±2.05 ^{bc}	77.8±17.81 ^{bc}	132.0±7.01 ^b	307.7±41.34 ^a
CQI	0.1±0.01 ^c	0.6±0.04 ^{bc}	1.2±0.54 ^b	1.7±0.08 ^b	4.2±0.55 ^a
BAI	1.9±0.26 ^c	25.1±1.65 ^{bc}	38.4±16.54 ^{bc}	77.3±5.86 ^b	242.7±36.73 ^a
VBN	10.2±0.89 ^d	10.6±0.41 ^{cd}	16.3±1.40 ^c	26.5±2.69 ^b	39.4±2.54 ^a

¹ Not detected.^{a-d} Mean±standard deviation within the same row with the same superscript were not significantly different (^b and ^{bc}, ^{bc} and ^c were not significantly different; $p < 0.05$).Abbreviations: BAs, biogenic amines; PHM, β -phenylethylamine; PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amine; CQI, chemical quality index calculated by the sum of HIM, PUT, CAD, SPM, and SPD; BAI, biogenic amine index calculated by the sum of HIM, CAD, TYM, and PUT.**Table 3.** Mean concentrations of biogenic amines ($\mu\text{g/g}$) and volatile basic nitrogen (VBN, mg %) levels of chicken breast during storage at $4\pm 2^\circ\text{C}$ ($n = 3$)

BAs	Storage (days)				
	1	3	5	7	9
PHM	nd ^b	nd ^b	1.6±0.46 ^b	4.3±1.62 ^a	4.7±0.35 ^a
PUT	3.3±0.66 ^c	2.7±0.22 ^c	4.5±1.00 ^c	49.5±10.61 ^b	207.0±23.88 ^a
CAD	1.5±0.35 ^b	1.0±0.55 ^b	0.1±0.14 ^b	3.8±1.31 ^b	91.1±22.02 ^a
HIM	nd ^c	6.2±2.44 ^b	7.7±1.17 ^b	16.7±1.17 ^a	9.4±2.33 ^b
SER	6.4±3.33 ^b	7.8±0.38 ^b	11.0±0.39 ^{ab}	11.6±2.74 ^{ab}	15.8±0.49 ^a
TYM	1.3±0.21 ^b	5.5±1.75 ^b	5.7±0.49 ^b	3.8±1.95 ^b	130.5±27.78 ^a
SPD	7.9±0.90 ^a	6.3±0.30 ^{ab}	5.7±0.38 ^b	7.6±0.42 ^a	7.5±0.13 ^a
SPM	38.8±4.01 ^d	60.2±1.00 ^{bc}	63.7±1.66 ^b	53.2±4.00 ^c	77.4±0.48 ^a
TABA	59.1±8.13 ^b	89.6±5.18 ^b	100.1±1.26 ^b	150.6±14.52 ^b	543.5±74.84 ^a
CQI	0.1±0.02 ^c	0.1±0.04 ^c	0.2±0.00 ^c	1.1±0.10 ^b	3.6±0.57 ^a
BAI	6.1±0.99 ^b	15.4±4.87 ^b	18.0±0.27 ^b	73.9±12.92 ^b	438.0±75.46 ^a
VBN	10.2±0.47 ^c	12.7±0.95 ^{bc}	13.8±0.75 ^{bc}	19.4±0.41 ^b	32.7±5.83 ^a

¹ Not detected.^{a-c} Mean±standard deviation within the same row with the same superscript were not significantly different (^a and ^{ab}, ^{ab} and ^b were not significantly different; $P < 0.05$).Abbreviations: BAs, biogenic amines; PHM, β -phenylethylamine; PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amine; CQI, chemical quality index calculated by the sum of HIM, PUT, CAD, SPM, and SPD; BAI, biogenic amine index calculated by the sum of HIM, CAD, TYM, and PUT.

The changes of the BAs concentration in pork loin had a similar trend to those in the beef loin in this study, except for a decrease in the SER content after 7 days of storage. HIM content did not show any difference during storage. However, the PUT, CAD, and TYM concentration increased with an increase of the storage for 15 days. PHM was only detected in the pork loin stored for 11 and 15 days. CQI, BAI, and VBN levels also increased during storage (Table 2). Nakamura et al. (1979) reported that the PUT content in pork samples was found to increase markedly during storage at 4°C which agrees well with our result. CAD and

PUT levels were similar to those reported by Hernandez-Jover et al. (1996). However, Szerdahelyi et al. (1993) did not find CAD in fresh pork.

BAs content during storage of chicken breast and leg are shown in Table 3 and 4, respectively. In the chicken breast, it was found that the concentrations of PUT, CAD and TYM increased with the storage time ($p < 0.05$). The concentration of SER increased slightly during chicken storage, which differed from the results for the beef or pork samples. The concentration of SPM increased during storage ($p < 0.05$), although Bardócz (1995) suggested that

Table 4. Mean concentrations of biogenic amines ($\mu\text{g/g}$) and volatile basic nitrogen (VBN, mg %) levels of chicken leg during storage at $4\pm 2^\circ\text{C}$ ($n = 3$)

BAs	Storage (days)				
	1	3	5	7	9
PHM	0.4±0.44 ^d	0.1±0.10 ^d	10.3±0.48 ^a	5.9±0.67 ^b	2.5±0.46 ^c
PUT	0.3±0.28 ^b	1.3±0.19 ^b	7.8±0.29 ^b	13.7±10.29 ^b	163.6±16.39 ^a
CAD	1.6±0.82 ^b	3.4±2.27 ^b	3.6±0.11 ^b	6.0±0.97 ^b	40.3±5.54 ^a
HIM	nd ^d	4.4±0.58 ^c	5.4±0.85 ^c	11.9±0.94 ^a	8.3±1.20 ^b
SER	8.9±1.09	14.2±8.01	14.2±0.95	26.5±3.49	8.0±0.39
TYM	3.7±0.65 ^b	4.4±2.47 ^b	6.4±1.67 ^b	7.2±2.30 ^b	46.7±9.00 ^a
SPD	6.8±0.63	15.3±3.02	9.1±1.49	8.2±2.33	9.6±0.22
SPM	46.6±4.88	92.4±19.84	70.5±0.65	84.7±14.33	68.9±3.98
TABA	68.5±4.00 ^c	135.6±26.46 ^{bc}	127.1±5.11 ^{bc}	164.2±29.64 ^b	348.1±32.30 ^a
CQI	0.04±0.02 ^b	0.1±0.06 ^b	0.2±0.01 ^b	0.3±0.05 ^b	2.7±0.31 ^a
BAI	20.3±7.73 ^b	13.6±3.65 ^b	23.1±2.84 ^b	38.9±10.42 ^b	259.1±31.94 ^a
VBN	7.9±0.61 ^d	8.5±0.34 ^d	11.5±0.58 ^c	23.4±1.22 ^b	31.2±0.89 ^a

^d Not detected.

^{a-d} Mean±standard deviation within the same row with the same superscript were not significantly different (^b and ^{bc}, ^{bc} and ^c were not significantly different; $p < 0.05$).

Abbreviations: BAs, biogenic amines; PHM, β -phenylethylamine; PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amine; CQI, chemical quality index calculated by the sum of HIM, PUT, CAD, SPM, and SPD; BAI, biogenic amine index calculated by the sum of HIM, CAD, TYM, and PUT.

Table 5. Pearson's correlation coefficients between volatile basic nitrogen (VBN) value, chemical quality index (CQI), biogenic amine index (BAI) versus biogenic amines concentration

	Species	PHM	PUT	CAD	HIM	SER	TYM	SPD	SPM
VBN	Beef	0.99	0.97	0.84	0.89	-0.61	0.87	0.86	-0.02
	Pork	0.94	0.98	0.94	0.40	-0.81	0.89	0.88	0.75
	Chicken breast	0.87	0.98	0.94	0.49	0.94	0.93	0.30	0.77
	Chicken leg	0.12	0.84	0.85	0.77	0.13	0.83	-0.21	0.14
CQI	Beef	0.90	0.99	0.96	0.89	-0.56	0.97	0.80	0.26
	Pork	0.84	0.97	0.99	0.48	-0.72	0.96	0.76	0.76
	Chicken breast	0.82	0.999	0.97	0.38	0.89	0.96	0.39	0.71
	Chicken leg	-0.11	0.999	0.999	0.37	-0.41	0.999	-0.05	-0.07
BAI	Beef	0.73	0.999	0.99	0.26	0.86	0.99	0.33	0.74
	Pork	0.83	0.98	0.999	0.39	-0.64	0.98	0.76	0.83
	Chicken breast	0.73	0.999	0.99	0.26	0.86	0.99	0.33	0.74
	Chicken leg	-0.13	0.999	0.999	0.36	-0.41	0.999	-0.08	-0.11

Abbreviations: BAs, biogenic amines; PHM, β -phenylethylamine; PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amine; CQI, chemical quality index calculated by the sum of HIM, PUT, CAD, SPM, and SPD; BAI, biogenic amine index calculated by the sum of HIM, CAD, TYM, and PUT.

the SPM concentration often decreased during a food spoilage since SPM can be used as a nitrogen source by some microorganisms. In this regards, the CQI value as an index of a meat freshness might be disputable. Vinci and Antonelli (2002) reported that CAD was the BA produced in the greatest quantity in chicken, and the total amount of BAs in red and white meat was similar after 30 days; the total was about double in the white meat compared to the red meat after 5 and 15 days. Also the authors suggested that the difference of the changes in the concentration for different meats is probably due to the presence of shorter muscular fibers and shorter protein chains in chicken compared to those of beef, which results in facilitating an attack by proteolytic enzymes and increasing the quantities of the amino acid precursors for the biosynthesis of BAs

(Vinci and Antonelli, 2002). Silva and Glória (2002) reported, however, that PUT and CAD were not detected in chicken meat until 10 days of storage. These contradictory results could be attributed to the differences in the microflora present in the meat samples (Hernandez-Jover et al., 1996).

As can be seen in Table 4, the concentration of PUT (0.28-163.56 $\mu\text{g/g}$) in the chicken legs changed more than that of CAD (1.63-40.28 $\mu\text{g/g}$) which was similar to the data of chicken breast. In contrast to these findings, Silva and Glória (2002) detected only low levels of PUT and CAD in chicken breast. TYM content, which was 3.70 $\mu\text{g/g}$ at the initial stage of storage, increased to 43.73 $\mu\text{g/g}$ at 9 days of storage ($p < 0.05$).

In beef, a high correlation was found between VBN and

BAs production tested except for SER and SPM (Table 5). Therefore, the formation of BAs in beef, especially for PHM and PUT, could be predicted over time by using a regression equation from the VBN content. In pork loin, the Pearson's correlation coefficients of PHM, PUT, CAD, TYM with VBN were higher than 0.89. In the chicken breast, all the BAs were positively correlated with VBN (Table 5). The correlation coefficient between the VBN and PUT, CAD, and SER in chicken breast were higher than the other BAs. In the chicken leg meat, the PHM, SER, SPD and SPM levels were not significantly correlated with VBN. However, the VBN value may predict the concentration of PUT, CAD, HIM and TYM during storage. The Pearson's correlation coefficient (r^2) of each pair of CQI and BAI versus PUT, CAD, and TYM were higher than 0.96 in all meat species (Table 5). However, the values of CQI during storage were relatively low in all meat species (Table 1-4).

Our results suggest that the PUT, CAD and TYM concentration are highly correlated with the VBN concentration in beef, pork, and chicken during storage. The differences among the BAs used in the VBN-based regression models during storage may be due in part to differences in the dominant microbial flora within the meat and poultry samples. Ground beef inoculated with *Proteus morgani* contained histamine at a concentration of 595 pg/g while the level of histamine of meat without *P. morgani* was 8.26 pg/g (Teodorovic et al., 1994).

The difference of the concentration in total biogenic amines (TABAs) among different meat species was evaluated after 7 days of storage by analysis of variance. The results showed a significant difference ($p < 0.01$) among species. The result demonstrated that the concentration of TABA of pork was the lowest and that of the chicken breast and thigh was the highest. There was no difference was found between chicken breast and thigh meat.

In conclusion, the level of BAs in beef, pork and chicken changed but the extent of these changes was different among the different BAs and meats. The measurement of the VBN content was confirmed as a good index for inferring the specific BAs content as well as evaluating a meats' freshness during storage. The kinds of BAs which can be predicted from the VBN content varied in different meats. These variations were possibly due to the differences in the dominant microbial flora among the three kinds of meat tested.

ACKNOWLEDGEMENT

This work was supported by a Korea Research Foundation Grant (KRF-2001-042-G00008) and partially supported by grant No. R11-2002-100-00000-0 from ERC program of the Korea Science & Engineering Foundation.

REFERENCES

- Bardócz, S. 1995. Polyamines in food and their consequences for food quality and human health. *Trends in Food Sci. Technol.* 6:341-346.
- Byun, J. S., J. S. Min, I. S. Kim, J. W. Kim, M. S. Chung and M. Lee. 2003. Comparison of indicators of microbial quality of meat during aerobic cold storage. *J. Food Prot.* 66:1733-1737.
- Chen, M.-T., Y.-S. Lin, H.-T. Tsai and H.-L. Kuo. 2002. Efficiency of hurdle technology applied to raw cured meat (Si-raw) processing. *Asian-Aust. J. Anim. Sci.* 15(11):1646-1652.
- Eerola, S., R. Hinkkanen, E. Lindfors and T. Hirvi. 1993. Liquid chromatographic determination of biogenic amines in dry sausages. *J. AOAC Int.* 76: 575-577.
- Halasz, A., A. Barath, L. Simon-Sarkadi and W. Holzapfel. 1994. Biogenic amines and their production by microorganisms in food. *Trends in Food Sci. Technol.* 5:42-49.
- Hernández-Jover, T., M. Izquierdo-Pulido, M. T. Veciana-Nogues and M. C. Vidal-Carou. 1996. Biogenic amine sources in cooked cured shoulder pork. *J. Agric. Food Chem.* 44:3097-3101.
- Hernandez-Jover, T., M. Izquierdo-Pulido, M. T. Veciana-Nogues, A. Marine-Font and M. C. Vidal-Carou. 1997. Biogenic amine and polyamine contents in meat and meat products. *J. Agric. Food Chem.* 45:2098-2102.
- Kaniou, I., G. Samouris, T. Mouratidou, A. Eleftheriadou and N. Zantopoulos. 2001. Determination of biogenic amines in fresh unpacked and vacuum-packed beef during storage at 4°C. *Food Chem.* 74:515-519.
- Korea Food and Drug Administration. 2002. Food Code, Seoul, Korea.
- Lee, K. T. and C. S. Yoon. 2001. Quality changes and shelf life of imported vacuum-packaged beef chuck during storage at 0°C. *Meat Sci.* 59:71-77.
- Leuschner, R. G., M. Heidel and W. P. Hammers. 1998. Histamine and tyramine degradation by food fermenting microorganisms. *Int. J. Food Microbiol.* 39:1-12.
- Mietz, J. L. and E. Karmas. 1977. Chemical quality index of canned tuna as determined by high-pressure liquid chromatography. *J. Food Sci.* 42:155-158.
- Min, J. S., S. O. Lee, A. Jang, M. Lee and Y. Kim. 2004a. Production of biogenic amines by microflora inoculated in meats. *Asian-Aust. J. Anim. Sci.* 17:1472-1478.
- Min, J. S., S. O. Lee, A. Jang, M. Lee and Y. Kim. 2004b. Quantitative analysis of biogenic amines in raw and processed foods of animal origin on Korean domestic market. *Asian-Aust. J. Anim. Sci.* 17:1764-1768.
- Miwa, K. and H. Iida. 1973. Studies on ethylalcohol determination in "Shiokara" by the microfiltration method. *Bulletin of the Japan. Soc. Fish.* 39:1189-1194.
- Nakamura, M., Y. Wada, H. Saway and T. Kawabata. 1979. Polyamine content in fresh and processed pork. *J. Food Sci.* 44:515-517.
- SAS. 2000. The SAS system for windows (Release 8.01). SAS Institute, Inc., Cary, NC, USA.
- Silla Santos, M. H. 1996. Biogenic amines The importance in foods. *Int. J. Food Microbiol.* 29:213-219.
- Silva, C. M. G. and M. B. A. Glória. 2002. Bioactive amines in

- chicken breast and thigh after slaughter and during storage at $4\pm 1^{\circ}\text{C}$ and in chicken-based meat products. *Food Chem.* 78:241-248.
- Szerdahelyi, E. N., P. Freudenreich and K. Fischer. 1993. Untersuchungen ber den Gehalt biogener Amine in Schweinefleisch (Biogenic amines in pork). *Fleischwirtschaft.* 73:789-790.
- Teodorovic, V., S. Buncic and D. Smiljanic. 1994. A study of factors influencing histamine production in meat. *Fleischwirtsch.* 74:170-172.
- Veciana-Nogues, M. T., A. Marine-Font and M. C. Vidal-Carou. 1997. Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts, ATP-related compounds, volatile amines, and organoleptic changes. *J. Agric. Food Chem.* 45:2036-2041.
- Vinci, G. and M. L. Antonelli. 2002. Biogenic amines: quality index of freshness in red and white meat. *Food Control* 13:519-524.
- Yano, Y., N. Kataho, M. Watanabe, T. Nakamura and Y. Asano. 1995. Changes in the concentration of biogenic amines and application of tyramine sensor during storage of beef. *Food Chem.* 54:155-159.