

## Quantitative Analysis of Biogenic Amines in Raw and Processed Foods of Animal Origin on Korean Domestic Market

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**ABSTRACT :** This study was performed to examine the levels of biogenic amines (BAs) in foods of animal origin such as egg, ham, sausage, milk, cheese and yoghurt distributed on Korean domestic markets, and to compare the results with those of western countries. Egg yolk had more BAs than egg white. BAs detected in ham samples were the highest level in barbecued tender loin ham. Sausage samples had less BAs than ham samples. However, the delicatessen sausages had significantly higher levels of histamine than the other sausage samples ( $p < 0.001$ ). Cadaverine, spermidine and spermine were detected in market milks and their concentrations were very low compared with other samples. In Cheddar cheese, tyramine ( $44.46 \pm 0.83 \mu\text{g/g}$ ) was the major BA. The major BA of stirred yoghurt samples was histamine. These results suggest that BAs of the foods of animal origin distributed on Korean domestic markets were not much different from those of western countries and would not cause any harmful effect to consumers. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 12 : 1764-1768)

**Key Words :** Biogenic Amines, Foods of Animal Origin, Korean Domestic Markets

### INTRODUCTION

The foods of animal origin such as ham, sausage, cheese, yoghurt, etc. have been consumed for the supply of protein. The consumption of those foods has grown gradually as people's income increases. In the past, Koreans consumed mainly fresh meat, but the consumption of processed meat foods has been increasing as people's tastes are being westernized. The consumption of dairy products such as cheese, yoghurt, etc. has increased as well.

Biogenic amines (BAs) are formed by amino acid decarboxylases of microorganisms, and they include  $\beta$ -phenylethylamine (PHM), serotonin (SER), tyramine (TYM), histamine (HIM), tryptamine (TRM), putrescine (PUT), cadaverine (CAD), etc. Fresh meat (Trevino and Steinhart, 1997; Lee and Yoon, 2001; Min et al., 2004), processed meat food (Eerola et al., 1996; Leuschner and Hammes, 1998; Bover-Cid et al., 1999; Bover-Cid et al., 2001; Durlu-Özkaya et al., 2001; Chen et al., 2002), cheese (Vale and Gloria, 1998; Kebary et al., 1999; Valsamaki et al., 2000), fermented vegetables (Kalać et al., 1999), beer (Izquierdo-Pulido et al., 2000), and wine (Fernandes and Ferreira, 2000; Lonvaud-Funel, 2001) are foods which contain BAs. It means that the fermented food and almost all the foods that have free amino acid contain BAs.

An ingestion of BAs can cause headache, hypertension, pyrexia, or heart disease. An ingestion of a large amount of histamine (HIM) can cause the symptoms such as nausea, dyspnea, flush, perspiration, palpitation, hypertension or

hypotension (Franzen and Eytzell, 1969; Taylor, 1986; Bartholomew et al., 1987). The symptoms of hypertension with a bad headache were developed after ingesting foods that contain plenty of tyramine (TYM) (Stratton et al., 1991).

However, there are no data about the contents of BAs in foods of animal origin distributed on Korean domestic markets. Therefore, this study was performed to research how different the contents of BAs in foods of animal origin such as egg, ham, sausage, milk, cheese, yoghurt, etc. on Korean domestic markets are from those of western countries.

### MATERIALS AND METHODS

#### Samples

Egg, ham, sausage, milk, cheese and yoghurt samples were purchased from Korean domestic super-markets, and transported to the laboratory with being kept in an ice box. The 2 weeks old unfertilized eggs and fertilized eggs were separated into egg yolk and white to be used as the egg samples. The samples of ham were the press ham (pork 79%), smoked loin ham (pork 93%), barbeque tender loin ham (pork 95%) and three kinds of sausages. Three types of milk samples were purchased, and Parmesan cheese, Mozzarella cheese, Cheddar cheese and processed cream cheese were used as the cheese samples. Four kinds of stirred yogurt and five drinking yogurts were used as the fermented milk products. All experiments were carried out in three replicates.

#### Reagent

Amine standards (1,7-diaminoheptane, PHM hydrochloride, PUT dihydrochloride, CAD dihydrochloride,

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**Table 1.** The contents of biogenic amines<sup>1</sup> in unfertilized and fertilized eggs ( $\mu\text{g/g}$ )

	Fertilized		Unfertilized	
	Egg white	Egg yolk	Egg white	Egg yolk
PUT***	0.0±0.00 <sup>b</sup>	6.91±0.02 <sup>a</sup>	0.0±0.0 <sup>b</sup>	6.68±0.24 <sup>a</sup>
CAD	67.60±33.92	64.37±32.58	0.0±0.00	102.62±6.49
TYM**	0.0±0.00 <sup>b</sup>	4.46±1.69 <sup>a</sup>	0.0±0.0 <sup>b</sup>	6.05±0.45 <sup>a</sup>
SPD***	0.0±0.00 <sup>b</sup>	1.96±0.16 <sup>a</sup>	0.0±0.0 <sup>b</sup>	2.17±0.13 <sup>a</sup>
SPM***	0.34±0.06 <sup>b</sup>	33.77±0.75 <sup>a</sup>	0.10±0.10 <sup>b</sup>	32.57±0.51 <sup>a</sup>
TABAs**	67.93±33.86 <sup>bc</sup>	111.47±30.96 <sup>ab</sup>	0.10±0.10 <sup>c</sup>	150.08±7.56 <sup>a</sup>

<sup>a, b, c</sup> Means±SE within the same row with the same superscript were not significantly different. \*\*  $p < 0.01$ . \*\*\*  $p < 0.001$ .

<sup>1</sup> PUT (putrescine), CAD (cadaverine), TYM (tyramine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amines).

**Table 2.** The contents of biogenic amines<sup>1</sup> in hams ( $\mu\text{g/g}$ )

	Press ham	Smoked loin ham	Barbecued tender loin ham
PHM***	7.30±0.09 <sup>c</sup>	30.13±2.16 <sup>b</sup>	77.06±1.53 <sup>a</sup>
PUT***	0.26±0.01 <sup>c</sup>	1.00±0.06 <sup>b</sup>	3.87±0.24 <sup>a</sup>
CAD***	1.00±0.16 <sup>c</sup>	3.41±0.39 <sup>b</sup>	17.78±0.94 <sup>a</sup>
HIM	1.07±0.38	5.12±0.54	10.12±5.71
TYM***	1.59±0.24 <sup>c</sup>	6.77±1.07 <sup>b</sup>	17.53±1.14 <sup>a</sup>
SPD***	3.58±0.26 <sup>c</sup>	9.73±1.28 <sup>b</sup>	27.22±0.75 <sup>a</sup>
SPM***	17.08±0.85 <sup>c</sup>	78.79±10.22 <sup>b</sup>	182.03±6.75 <sup>a</sup>
TABAs***	31.87±0.79 <sup>c</sup>	134.95±14.63 <sup>b</sup>	335.60±13.59 <sup>a</sup>

<sup>a, b, c</sup> Means±SE within the same row with the same superscript were not significantly different. \*\*\*  $p < 0.001$ .

<sup>1</sup> PHM ( $\beta$ -phenylethylamine), PUT (putrescine), CAD (cadaverine), HIM (histamine), TYM (tyramine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amine).

HIM dihydrochloride, SER creatinine sulfate, TYM hydrochloride, SPD trihydrochloride, SPM tetrahydrochloride), sodium bicarbonate, sodium hydroxide, ammonium acetate, dansyl chloride were bought from Sigma Chemical Co. (St. Louis, USA). Ammonia and perchloric acid (70%) were bought from Showa Chemical Co. (Tokyo, Japan). Acetonitrile and acetone (HPLC grade) were purchased from TEDIA (Cincinnati, USA).

### Analysis of biogenic amines

BAs were analyzed by the method of Eerola et al. (1993) with a modification. Internal standard was put into 2 g of the samples. After homogenizing the sample in 10 ml of 0.4 M perchloric acid, the homogenized sample was centrifuged for 10 min at the speed of 3,000×g and the supernatant was filtered through a filter paper (Whatman no. 1, Whatman International Ltd., Maidstone, England). The total volume of filtrate was adjusted to 25 ml by using 0.4 M perchloric acid after centrifuging again at the previous condition the residue that had been thoroughly mixed with 10 ml of 0.4 M perchloric acid and then re-filtering the supernatant. Two hundreds microliters of 2 N sodium hydroxide, 300  $\mu\text{l}$  saturated sodium bicarbonate and 2 ml of dansyl chloride were added to the sample extract, and incubated the mixture at 40°C for 45 min. After the incubation, 100  $\mu\text{l}$  ammonia was added and after 30 min, the volume was adjusted to 5 ml with acetonitrile. This reaction mixture was centrifuged for 5 min at the speed of 2,500×g and then the supernatant was filtered by 0.45  $\mu\text{m}$

syringe filter (Acrodisc<sup>®</sup> LC13 PVDF minispikes, Pall Co., Ann Arbor, USA). Ten microliters of the filtrate were injected into HPLC with diode array detector (Agilent 1100, Agilent Technology Inc., Wilmington, USA). The column was Spherisorb OSD2 column (4.6×150 mm i.d., 5  $\mu\text{m}$ ) (Waters, Milford, USA). Gradient elution program was used with the mixture of 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. Two menstrua were applied to vacuum filtration by using membrane filter (47 mm PTFE 0.45  $\mu\text{m}$ , Pall Co., Ann Arbor, USA), and degassed by using ultrasonicator (5210, Branson Ultrasonic Co., Danbury, USA). The flow rate was 1 ml per min. Acetonitrile was set at 50% at the beginning, and finished at 90% after 19 min for the gradient. Ten minutes of waiting time before each analysis was given to maintain the equilibrium. The temperature of column was 40°C, and UV-absorption was detected at 254 nm.

### Statistical analysis

Statistical analysis was performed with SAS system for windows V8 (SAS, 2000). The significant differences among samples were analyzed by using One-way ANOVA and Duncan's multiple range tests.

## RESULTS AND DISCUSSION

As shown in Table 1, a large amount of CAD (67.60±33.92  $\mu\text{g/g}$ ) was detected in egg white of the fertilized eggs while CAD (64.37±32.58  $\mu\text{g/g}$ ) and SPM

**Table 4.** The contents of biogenic amines<sup>1</sup> in milks<sup>2</sup> ( $\mu\text{g/ml}$ )

	MILK-1	MILK-2	MILK-3
CAD***	0.0 $\pm$ 0.00 <sup>c</sup>	14.33 $\pm$ 0.14 <sup>b</sup>	18.52 $\pm$ 0.63 <sup>a</sup>
SPD	0.04 $\pm$ 0.02	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01
SPM	0.15 $\pm$ 0.02	0.36 $\pm$ 0.24	0.30 $\pm$ 0.20
TABAs***	0.19 $\pm$ 0.04 <sup>c</sup>	14.74 $\pm$ 0.37 <sup>b</sup>	18.88 $\pm$ 0.42 <sup>a</sup>

<sup>a,b,c</sup> Means $\pm$ SE within the same row with the same superscript were not significantly different. \*\*\*  $p < 0.001$ .

<sup>1</sup> CAD (cadaverine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amine).

<sup>2</sup> Produced by three different companies.

(33.77 $\pm$ 0.75  $\mu\text{g/g}$ ) were detected in egg yolk. In the case of unfertilized eggs, almost no BA was detected in egg white, whereas CAD (102.62 $\pm$ 6.49  $\mu\text{g/g}$ ) and SPM (32.57 $\pm$ 0.51  $\mu\text{g/g}$ ) were detected in high concentration in egg yolk. As a result, egg yolk seems to contain more BAs than egg white does. The presence of BA in eggs reflects its freshness as BA is formed by microbial decarboxylation of amino acids. SPM is not BA but one of polyamines that are present naturally in food (Bardocz, 1995).

Table 2 shows the analysis of BAs detected in ham samples that are distributed on Korean domestic markets. PHM, PUT, CAD, HIM, TYM, SPM and SPD were detected. All BAs detected in barbecued tenderloin ham were in highest levels. Hernandez-Jover et al. (1997) reported that various cooked ham and other meat products had a wide range of biogenic amines contents from none to 78.1 mg/kg. The reason for that would be the use of low

hygienic raw materials or the length of storage time for raw meat before processing. The barbecued tenderloin ham had higher level of SPM than the other samples. It may reflect that tenderloin has higher amount of SPM because SPD and SPM are naturally occurring polyamines in fresh pork and their formation is not due to food spoilage or fermentation processes (Hernandez-Jover et al., 1997). In the case of sausage samples, in general the BAs detected were relatively low in level (Table 3). CAD detected in frankfurters was the highest. Delicatessen sausage samples had significantly higher level of HIM than other sausage samples ( $p < 0.001$ ). The level of biogenic amines would reflect the freshness of meat. The levels of SPM of all the sausage samples were relatively higher than those of other amines. A similar result was reported by Hernandez-Jover et al. (1997), where 27.3-40.6 mg/kg of SPM was detected in fresh pork. SPM content of fresh pork is higher than sausage due to the dilution of meat with fat and other ingredients used in the manufacturing process (Hernandez-Jover et al., 1997).

The results of analyzed BAs from market milk samples are shown in Table 4. Only CAD, SPD and SPM were detected and their levels were very low. The contents of CAD in two milk samples were relatively higher ( $p < 0.001$ ). A similar result from goat milk was reported by Novella-Rodriguez et al. (2002), where only CAD, SPD and SPM were detected in low concentration.

Table 5 describes the contents of BAs in cheese samples.

**Table 3.** The contents of biogenic amines<sup>1</sup> in sausages ( $\mu\text{g/g}$ )

	Vienna	Frankfurter	Delicatessen1	Delicatessen2
PHM	4.33 $\pm$ 0.97	5.39 $\pm$ 0.76	5.85 $\pm$ 0.47	4.24 $\pm$ 0.89
PUT*	0.28 $\pm$ 0.05 <sup>b</sup>	0.57 $\pm$ 0.05 <sup>a</sup>	0.69 $\pm$ 0.14 <sup>a</sup>	0.63 $\pm$ 0.06 <sup>a</sup>
CAD**	1.35 $\pm$ 0.15 <sup>b</sup>	15.53 $\pm$ 2.26 <sup>a</sup>	3.02 $\pm$ 3.02 <sup>b</sup>	1.09 $\pm$ 0.04 <sup>b</sup>
HIM***	4.34 $\pm$ 0.21 <sup>b</sup>	5.05 $\pm$ 0.61 <sup>b</sup>	12.75 $\pm$ 0.61 <sup>a</sup>	11.29 $\pm$ 1.37 <sup>a</sup>
TYM	2.60 $\pm$ 0.23	4.73 $\pm$ 1.91	1.73 $\pm$ 0.33	6.75 $\pm$ 2.79
SPD	2.99 $\pm$ 0.37	5.32 $\pm$ 2.16	2.78 $\pm$ 0.08	7.78 $\pm$ 2.57
SPM*	13.15 $\pm$ 1.89 <sup>b</sup>	21.21 $\pm$ 3.46 <sup>ab</sup>	22.93 $\pm$ 0.67 <sup>a</sup>	28.40 $\pm$ 3.25 <sup>a</sup>
TABAs	29.05 $\pm$ 3.74 <sup>b</sup>	57.81 $\pm$ 5.55 <sup>a</sup>	49.75 $\pm$ 2.34 <sup>a</sup>	60.18 $\pm$ 6.44 <sup>a</sup>

<sup>a,b</sup> Means $\pm$ SE within the same row with the same superscript were not significantly different. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

<sup>1</sup> PHM ( $\beta$ -phenylethylamine), PUT (putrescine), CAD (cadaverine), HIM (histamine), TYM (tyramine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amine).

**Table 5.** The contents of biogenic amines<sup>1</sup> in various cheeses ( $\mu\text{g/g}$ )

	Parmesan	Mozzarella	Cheddar	Processed cream
PHM**	0.0 $\pm$ 0.00 <sup>b</sup>	0.0 $\pm$ 0.00 <sup>b</sup>	2.31 $\pm$ 0.66 <sup>a</sup>	0.23 $\pm$ 0.12 <sup>b</sup>
PUT***	3.56 $\pm$ 0.22 <sup>a</sup>	0.85 $\pm$ 0.24 <sup>c</sup>	2.87 $\pm$ 0.20 <sup>b</sup>	0.61 $\pm$ 0.10 <sup>c</sup>
CAD**	1.20 $\pm$ 0.21 <sup>b</sup>	0.15 $\pm$ 0.15 <sup>b</sup>	4.46 $\pm$ 1.08 <sup>a</sup>	0.0 $\pm$ 0.00 <sup>b</sup>
HIM***	1.42 $\pm$ 0.04 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	29.37 $\pm$ 2.68 <sup>a</sup>	0.0 $\pm$ 0.00 <sup>b</sup>
TYM***	3.61 $\pm$ 0.65 <sup>b</sup>	1.79 $\pm$ 0.1.79 <sup>bc</sup>	44.46 $\pm$ 0.83 <sup>a</sup>	0.0 $\pm$ 0.00 <sup>c</sup>
SPD*	30.70 $\pm$ 1.91 <sup>a</sup>	17.46 $\pm$ 8.74 <sup>ab</sup>	26.55 $\pm$ 5.79 <sup>a</sup>	4.40 $\pm$ 3.52 <sup>b</sup>
SPM	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	6.36 $\pm$ 6.36	6.12 $\pm$ 2.74
TABAs***	40.48 $\pm$ 2.09 <sup>b</sup>	20.25 $\pm$ 6.78 <sup>bc</sup>	116.38 $\pm$ 11.13 <sup>a</sup>	11.35 $\pm$ 5.82 <sup>c</sup>

<sup>a,b,c,d</sup> Means $\pm$ SE within the same row with the same superscript were not significantly different. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

<sup>1</sup> PHM ( $\beta$ -phenylethylamine), PUT (putrescine), CAD (cadaverine), HIM (histamine), TYM (tyramine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amine).

**Table 6.** The contents of biogenic amines<sup>1</sup> in stirred yoghurts<sup>2</sup> ( $\mu\text{g/g}$ )

	SY1	SY2	SY3	SY4
PHM*	0.08±0.08 <sup>b</sup>	0.32±0.32 <sup>b</sup>	0.39±0.09 <sup>b</sup>	5.18±2.41 <sup>a</sup>
PUT	0.60±0.07	0.0±0.00	0.36±0.23	0.48±0.10
HIM**	1.81±1.81 <sup>b</sup>	1.91±1.91 <sup>b</sup>	21.20±1.90 <sup>a</sup>	15.80±4.64 <sup>a</sup>
SPD***	1.16±0.07 <sup>a</sup>	0.0±0.00 <sup>c</sup>	0.36±0.18 <sup>b</sup>	0.38±0.10 <sup>b</sup>
SPM**	0.26±0.26 <sup>b</sup>	0.28±0.28 <sup>b</sup>	2.33±1.11 <sup>ab</sup>	4.83±1.03 <sup>a</sup>
TABAs**	3.92±1.66 <sup>b</sup>	2.50±2.04 <sup>b</sup>	24.64±1.48 <sup>a</sup>	26.66±7.33 <sup>a</sup>

<sup>a,b,c,d</sup> Means±SE within the same row with the same superscript were not significantly different. \*  $p<0.05$ , \*\*  $p<0.01$ , \*\*\*  $p<0.001$ .

<sup>1</sup> PHM ( $\beta$ -phenylethylamine), PUT (putrescine), HIM (histamine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amine).

<sup>2</sup> Produced by four different companies.

**Table 7.** The contents of biogenic amines<sup>1</sup> in drinking yoghurts<sup>2</sup> ( $\mu\text{g/ml}$ )

	DY1	DY2	DY3	DY4	DY5
CAD**	0.0±0.00 <sup>c</sup>	10.98±1.48 <sup>ab</sup>	16.70±2.20 <sup>a</sup>	7.26±4.89 <sup>bc</sup>	0.0±0.00 <sup>c</sup>
TYM***	0.0±0.00 <sup>b</sup>	0.25±0.01 <sup>a</sup>	0.20±0.07 <sup>a</sup>	0.0±0.00 <sup>b</sup>	0.02±0.02 <sup>b</sup>
SPD	0.06±0.00	0.11±0.01	0.13±0.02	0.21±0.09	0.07±0.02
SPM	0.09±0.01	0.13±0.01	0.23±0.13	1.63±1.36	0.05±0.01
TABAs**	0.15±0.01 <sup>b</sup>	11.48±1.50 <sup>a</sup>	17.25±2.17 <sup>a</sup>	9.09±6.13 <sup>ab</sup>	0.14±0.01 <sup>b</sup>

<sup>a,b,c</sup> Means±SE within the same row with the same superscript were not significantly different. \*  $p<0.05$ , \*\*  $p<0.01$ , \*\*\*  $p<0.001$ .

<sup>1</sup> CAD (cadaverine), TYM (tyramine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amine).

<sup>2</sup> Produced by five different companies.

HIM, TYM, CAD and PUT were detected in Parmesan cheese, but the concentration was lower than that was reported by Halász et al. (1994). In Cheddar cheese, TYM (44.46±0.83  $\mu\text{g/g}$ ) was the major BA, followed by HIM (29.37  $\mu\text{g/g}$ ). Voigt et al. (1974) reported that TYM was detected in the range of 0-0.7 mg/100 g of cheese. Relatively lower levels of BAs were detected in Mozzarella and processed cream cheese which are unripened cheeses. Biogenic amines were lower in unripened than in ripened cheeses, and the main amines in ripened cheeses were TYM, CAD and PUT (Novella-Rodriguez et al., 2003).

Table 6 shows the contents of BAs in stirred yoghurt samples. PHM, PUT, HIM, SPD and SPM were detected. The contents of BAs in SY1 and SY2 were very low, but somewhat higher levels of BAs were detected in SY3 and SY4. The major BA was HIM, and it was detected at the highest level in SY3. BAs detected in drinking yoghurts are shown in Table 7. The concentrations of BAs detected in drinking yoghurt samples were low. However, the contents of CAD in several yogurts (DY2, DY3 and DY4) were relatively higher. Market milk samples and yoghurt samples contain lower level of BAs than the processed meat products.

In summary, TABAs in yolk of an egg was 2.01-2.70 mg, and 0.00-2.45 mg in egg white. The average weight of an egg is 60 g, and it contains about 60% of albumen, and 30% of yolk. Therefore, when an egg is consumed, about 2.01-5.14 mg of BAs is ingested. On the other hand, about 3.19-33.56 mg of BAs would be ingested in consuming 100 g of sausages while drinking 200 ml of milk would result in an intake of about 0.04-3.78 mg of BAs. A sliced cheese that is distributed on a market is about 20 g, and one sliced

cheese contains about 2.23-2.33 mg of BAs. Therefore, even the highest level of TYM detected in Cheddar cheese of this study would not create any danger because Majjala and Eerola (1993) reported that 100 mg of TYM caused a megrim. About 0.25-2.67 mg/100 g of BAs was contained in the stirred yoghurt and 150 ml of a drinking yoghurt contained about 0.02-2.95 mg of BAs. It was reported that 10-100 mg/100 g food of TYM, 100-800 mg/kg of TYM and 30 mg/kg of PHM are the levels of toxic manifestations (Taylor, 1986; Brink et al., 1990; Stratton et al., 1991).

The results of this study suggest that levels and kinds of BAs contained in foods of animal origin distributed on Korean domestic markets were similar to those reported in western countries and would not cause any harmful effect to consumers.

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