**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 β-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 foods (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 (Kalač 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 Eysell, 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 yoghurt and five drinking yogurts were used as the fermented milk products. All experiments were carried out in three replicates.

**Reagent**

Amine standards (1,7-diaminohexane, PHM hydrochloride, PUT dihydrochloride, CAD dihydrochloride,
**Table 1.** The contents of biogenic amines\(^1\) in unfertilized and fertilized eggs (µg/g)

<table>
<thead>
<tr>
<th></th>
<th>Fertilized</th>
<th>Egg white</th>
<th>Egg yolk</th>
<th>Unfertilized</th>
<th>Egg white</th>
<th>Egg yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUT***</td>
<td>0.0±0.00(^a)</td>
<td>6.91±0.02(^b)</td>
<td>0.0±0.00(^a)</td>
<td>6.68±0.24(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>67.60±33.92(^a)</td>
<td>64.37±32.58(^b)</td>
<td>0.0±0.00(^a)</td>
<td>102.6±26.49(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYM**</td>
<td>0.0±0.00(^a)</td>
<td>4.46±1.69(^b)</td>
<td>0.0±0.00(^a)</td>
<td>6.05±0.45(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPD***</td>
<td>0.0±0.00(^a)</td>
<td>1.96±0.16(^b)</td>
<td>0.0±0.00(^a)</td>
<td>2.17±0.13(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPM***</td>
<td>0.34±0.06(^b)</td>
<td>33.77±0.75(^a)</td>
<td>0.10±0.10(^b)</td>
<td>32.57±0.51(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TABAs**</td>
<td>67.93±33.86(^a)</td>
<td>111.47±30.96(^b)</td>
<td>0.10±0.10(^b)</td>
<td>150.08±7.56(^c)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)^\(^b\)^\(^c\) Means±SE within the same row with the same superscript were not significantly different. ** p<0.01, *** p<0.001.

\(^1\) PUT (putrescine), CAD (cadaverine), TYM (tyramine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amines).

**Table 2.** The contents of biogenic amines\(^1\) in hams (µg/g)

<table>
<thead>
<tr>
<th></th>
<th>Press ham</th>
<th></th>
<th>Smoked loin ham</th>
<th></th>
<th>Barbecued tender loin ham</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PHM***</td>
<td>7.30±0.09(^b)</td>
<td></td>
<td>30.13±1.26(^b)</td>
<td></td>
<td>77.06±1.53(^a)</td>
<td></td>
</tr>
<tr>
<td>PUT***</td>
<td>0.26±0.00(^a)</td>
<td></td>
<td>1.00±0.06(^b)</td>
<td></td>
<td>3.87±0.24(^a)</td>
<td></td>
</tr>
<tr>
<td>CAD***</td>
<td>1.00±0.16(^a)</td>
<td></td>
<td>3.41±0.39(^b)</td>
<td></td>
<td>17.78±0.94(^a)</td>
<td></td>
</tr>
<tr>
<td>HIM</td>
<td>1.07±0.38(^a)</td>
<td></td>
<td>5.12±0.54(^b)</td>
<td></td>
<td>10.12±5.71(^a)</td>
<td></td>
</tr>
<tr>
<td>TYM***</td>
<td>1.59±0.24(^b)</td>
<td></td>
<td>6.77±1.07(^b)</td>
<td></td>
<td>17.53±1.14(^a)</td>
<td></td>
</tr>
<tr>
<td>SPD***</td>
<td>3.58±0.26(^a)</td>
<td></td>
<td>9.73±1.28(^b)</td>
<td></td>
<td>27.22±0.75(^a)</td>
<td></td>
</tr>
<tr>
<td>SPM***</td>
<td>17.08±0.85(^a)</td>
<td></td>
<td>78.79±10.22(^b)</td>
<td></td>
<td>182.03±6.75(^a)</td>
<td></td>
</tr>
<tr>
<td>TABAs***</td>
<td>31.87±0.79(^b)</td>
<td></td>
<td>134.95±14.63(^a)</td>
<td></td>
<td>335.60±13.59(^a)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)^\(^b\)^\(^c\) Means±SE within the same row with the same superscript were not significantly different. *** p<0.001.

\(^1\) PHM (β-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 then 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 bicarbonate, 300 µl 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 µl 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 µm syringe filter (Acrodisc® LC13 PVDF minispike, 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 flow rate was 1ml per min. Acetonitrile and acetone (HPLC grade) were purchased from TEDIA (Cincinnati, USA).

**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 µg/g) was detected in egg white of the fertilized eggs while CAD (64.37±32.58 µg/g) and SPM
(33.77±0.75 µg/g) were detected in egg yolk. In the case of unfertilized eggs, almost no BA was detected in egg white, whereas CAD (102.62±6.49 µg/g) and SPM (32.57±0.51 µg/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 6. The contents of biogenic amines1 in stirred yoghurts2 (µg/g)

<table>
<thead>
<tr>
<th>Amines</th>
<th>DY1</th>
<th>DY2</th>
<th>DY3</th>
<th>DY4</th>
<th>DY5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHM</td>
<td>0.08±0.08b</td>
<td>0.32±0.32b</td>
<td>0.39±0.09b</td>
<td>5.18±2.41a</td>
<td></td>
</tr>
<tr>
<td>PUT</td>
<td>0.6±0.07</td>
<td>0.0±0.00</td>
<td>0.36±0.23</td>
<td>0.48±0.10</td>
<td></td>
</tr>
<tr>
<td>HIM</td>
<td>1.81±1.81b</td>
<td>1.91±1.91b</td>
<td>21.20±1.90a</td>
<td>15.80±4.64a</td>
<td></td>
</tr>
<tr>
<td>SPD</td>
<td>1.16±0.07b</td>
<td>0.0±0.00</td>
<td>0.36±0.18b</td>
<td>0.38±0.10b</td>
<td></td>
</tr>
<tr>
<td>SPM</td>
<td>0.26±0.26b</td>
<td>0.28±0.28b</td>
<td>2.33±1.11ab</td>
<td>4.83±1.03a</td>
<td></td>
</tr>
<tr>
<td>TABAs</td>
<td>3.92±1.66b</td>
<td>2.50±2.04b</td>
<td>24.64±1.48a</td>
<td>26.66±7.33a</td>
<td></td>
</tr>
</tbody>
</table>

a,c Mean±SE within the same row with the same superscript were not significantly different. * p<0.05, ** p<0.01, *** p<0.001.
1 PHM (β-phenylethylamine), PUT (putrescine), HIM (histamine), SPD (spermidine), SPM (spermine), TABA (total amount of biogenic amine).
2 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 µg/g) was the major BA, followed by HIM (29.37 µg/g). Voigt et al. (1974) reported that TYM was detected in the range of 0.07-100 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 Maijala 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.

ACKNOWLEDGEMENT

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REFERENCES


