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Biogenic amines in Jeotkals, Korean salted and fermented fish products

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Abstract

Eleven different types of Korean traditional salted and fermented fish products, Jeotkals, were analyzed for biogenic amine contents by HPLC. Levels of putrescine, cadaverine, histamine, tyramine, spermidine and spermine in eight Jeotkal samples were found to be in the range of 0–70 mg/kg whose concentration changed little for a period of 20 days at 4, 10 and 15 °C. However, the levels of cadaverine, histamine and spermidine were significantly high in the Myeolchi-jeot and increased considerably during storage for longer than 10 days. The contents of spermine increased progressively in Changran-jeot and Myeongran-jeot samples. The amounts of biogenic amines in most Jeotkal samples we observed are within the safe level for human health, although certain samples which had been stored poorly need to be monitored carefully to ensure their safety for human consumption.

Keywords: Biogenic amines; Jeotkals; Korean traditional salted and fermented fish products; HPLC

1. Introduction

Biogenic amines, including tyramine, histamine, putrescine, cadaverine, spermine and spermidine, can be produced and degraded by normal metabolic activities in animals, plants and microorganisms. These amines are mainly produced by microbial decarboxylation amino acids in foods (Brink, Damirik, Joosten, & Huis in't Veld, 1990; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). Typical symptoms of biogenic amine intoxication in human can be nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, bright red rash, oral burning, and hypertension as well as hypotension (Rice, Eitenmiller, & Koehler, 1976). Askar and Treptow (1993) have suggested histamine at a concentration of 500 mg/kg to be hazardous for human health. On the other hand, Brink et al. (1990) reported that 100–800 mg/kg of tyramine and 30 mg/kg of phenylethylamine in foods are toxic, although the threshold levels for other amines are not established yet. Putrescine and cadaverine inhibit intestinal diamine oxidase and histamine-N-methyltransferase which metabolize histamine, resulting in an increase of histamine toxicity (Stratton, Hutkins, & Taylor, 1991). Furthermore, putrescine (Bills, Hildrum, Scanlan, & Libbey, 1973; Warthesen, Scanlan, Bills, & Libbey, 1975), cadaverine (Warthesen et al., 1975), spermidine (Bills et al., 1973; Smith, 1980), spermine and agmatine (Smith, 1980) are reported to be potentially carcinogenic by converting to nitrosamine. Tyramine has also been identified as the major mutagenic precursor in animal (Ochiai, Wakabayashi, Nagao, & Sugimura, 1984).

Most biogenic amines are pharmacologically active, however, oral administration of amines do not generally provoke adverse reactions, because amine oxidases in the intestine rapidly detoxify these compounds (Askar & Treptow, 1986). However, food intoxication may occur (Joosten & Nuñez, 1996) under the situation that the amine-metabolizing activity in human body is over saturated due to an ingestion of high dose of amines

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and/or due to an impairment of metabolic activity in the presence of specific inhibitors (Taylor, 1986).

Jeotkals are the Korean traditional salted and fermented fish products, and are taken popularly not only as side dishes, but also as ingredients in preparing kimchi in Korea. To prepare Jeotkals, salt should be added at the level of 5–20% to raw fish, and then allowed to ferment for a long period of time to develop taste. The fermentation period varies depending on the salt concentration and fermentation temperature; 2 months for most Jeotkals with low salt level (6–18%), and few years for Myeolchi-jeot with high salt (over 20%; Lee, 1993). The favorable taste and flavor may develop gradually during fermentation by several enzymatic reactions and microbial degradation processes. Thus, the fermented fish products contain relatively high amounts of amino acids, the degradation products of fish protein. Since the Jeotkals produced by a small-scale fishery manufacturer by a traditional primitive method and also the low-salt produced Jeotkals are stable only for a limited period, these products could be potentially the source for biogenic amine. At present, systematic data on the occurrence of biogenic amine in Jeotkals are not available. The main objective of this study is to analyze major biogenic amines in Jeotkals and determine their concentrations during the storage.

2. Materials and methods

Eleven different kinds of major Jeotkal samples, according to the type and part of fish used for preparation, were purchased at the local department stores in Seoul, Korea, during March 2000 and transported in ice to the laboratory. The types of samples were as follows: Ojingeo-jeot (sliced squid), Myeolchi-jeot (anchovy), Changran-jeot (pollock entrails), Myeongran-jeot (pollock roe), Saeu-jeot (shrimp), Toha-jeot (toha shrimp), Jogae-jeot (clam), Baendaengi-jeot (big eyed herring), Eorigul-jeot (oyster), Kkolttugi-jeot (small squid) and Agami-jeot (pacific cod gills). The same kind of Jeotkal but prepared by different companies were also obtained for product control, and analyzed for biogenic amine content. All Jeotkal samples were purchased within half a month after distribution to local retail stores, and stored at three different temperatures, 4, 10 and 15 °C to investigate the storage conditions much like common household situations, and then, biogenic amine concentration was analyzed every 5 days for 20 days.

All standard chemicals were obtained from Sigma chemical Co. and Merck KGaA.

2.1. Preparation of standard amine solutions

Extraction of the samples and HPLC determination of biogenic amines were carried out according to the procedure developed by Ben-Gigirey, de Sousa, Villa, and Barros-Velazquez (1998). The detection limits were approximately 4 mg/kg for putrescine, histamine, tyramine and spermine, and 6 mg/kg for cadaverine and spermidine. Stock solutions of putrescine, cadaverine, spermidine and spermine were prepared at a concentration of 10,000 mg/l distilled water, and diluted to 100 or 1000 mg/l for working solutions.

2.2. Preparation of sample extracts

To extract biogenic amines, 10 ml of 0.4 M perchloric acid was added to 4 g of Jeotkal samples, and the mixture was homogenized and centrifuged at 3000 $\times g$ at 4 °C for 10 min. The supernatant was collected and the residue was extracted again with an equal volume of 0.4 M perchloric acid solution. Both supernatants were combined and the final volume was adjusted to 25 ml with 0.4 M perchloric acid. The extract was filtered through Whatman paper No. 1.

2.3. Derivatization of sample extracts

One milliliter of each sample extracted was mixed with 200 μl of 2 M sodium hydroxide and 300 μl of saturated sodium bicarbonate. Two milliliters of a dansyl chloride solution (10 mg/ml) prepared in acetone were added to the mixture, and then incubated at 40 °C for 45 min. Residual dansyl chloride was removed by the addition of 100 μl of 25% ammonium hydroxide. After 30 min incubation at room temperature, the

Table 1
Biogenic amine contents (mg/kg) of Korean commercial Jeotkals (salted and fermented fish products)[a]

	Amines					
Jeotkals	Put	Cad	His	Tyr	Spd	Spm
Ojingeo-jeot	ND-30	ND	ND	ND	ND	ND
Myeolchi-jeot	92–241	ND-665	155–579	63–244	ND-43	ND-77
Changran-jeot	ND-20	ND	ND	ND	ND	ND-51
Myeongran-jeot	15–136	ND-85	ND	22–171	ND	26–58
Saeu-jeot	ND	ND	ND	ND	ND	33–62
Toha-jeot	ND-30	ND	ND	ND	ND	ND-26
Jogae-jeot	ND	ND	ND	ND	ND	44–61
Baendaengi-jeot	ND-22	ND	ND-11	ND	ND	ND-18
Eorigul-jeot	ND-20	ND-29	ND	ND-31	ND-12	ND-42
Kkolttugi-jeot	ND-32	ND	ND	ND	ND	ND
Agami-jeot	ND-46	ND	ND	ND-14	ND	ND-43

[a] Put, putrescine; Cad, cadaverine; His, histamine; Tyr, tyramine; Spd, spermidine; Spm, spermine. ND, not detected. Ojingeo-jeot (sliced squid), Myeolchi-jeot (anchovy), Changran-jeot (pollock entrails), Myeongran-jeot (pollock roe), Saeu-jeot (shrimp), Toha-jeot (toha shrimp), Jogae-jeot (clam), Baendaengi-jeot (big eyed herring), Eorigul-jeot (oyster), Kkolttugi-jeot (small squid) and Agami-jeot (pacific cod gills). Myeolchi-jeots, Changran-jeots and Myeongran-jeots of three different products and other Jeotkals of two different products were used.

extract was adjusted to 5 ml with acetonitrile. Finally, the mixture was centrifuged at 2500 ×*g* for 5 min and the supernatant was filtered through 0.2 μm-pore-size filters (Millipore Co., Bedford, MA). Biogenic amine standard solutions were diluted to 10 ml with 0.4 M perchloric acid to obtain the range of 2 mg/kg to 100 mg/kg. These solutions were derivatized using the same method in the sample extracts to obtain a calibration curve.

2.4. Chromatographic separations

A HPLC unit (Waters 2690) equipped with a Waters 996 photodiode array detector and Millennium 2010 software was employed. A Nova-Pak C18, 4 μm, 150 by 3.9 mm column (Waters) was used, with ammonium acetate (0.1 M; solvent A) and acetonitrile (solvent B) as the mobile phases at the flow rate of 1 ml/min. The program was set for a linear gradient starting from 50% of solvent B to reach 90% of the solvent at 19 min. The sample volume injected was 20 μl and the sample was monitored at 254 nm.

3. Results and discussion

Table 1 shows different biogenic amine contents in 11 kinds of Jeotkals. Most samples, including Changran-jeot, Ojingeo-jeot, Saeu-jeot, Toha-jeot, Jogae-jeot, Baendaengi-jeot, Eorigul-jeot, Kkolttugi-jeot and Agami-jeot had low levels of amines (below 70 mg/kg). On the other hand, Myeolchi-jeot sample had higher level of biogenic amine than the other samples tested, specifically, cadaverine concentration (665 mg/kg) was highest. The amounts of histamine, tyramine and putrescine were also rather high, reaching to 155–579 mg/kg, 63–244 mg/kg and 92–241 mg/kg, respectively. Spermidine and spermine contents were determined to be 43 and 77 mg/kg, respectively. In the case of Myeongran-jeot, relatively high levels of biogenic amines were detected; 22–171 mg/kg for tyramine and 15–136 mg/kg for putrescine. In contrast, spermine and cadaverine contained less than 100 mg/kg.

Changes in the concentrations of various biogenic amines in the Myeolchi-jeot sample during storage for 20 days at 4, 10 and 15 °C are shown in Fig. 1; concentration of cadaverine increased from 480 mg/kg to 1083–1205 mg/kg, whereas that of histamine from 521 mg/kg to 751–824 mg/kg during the first 10 days of storage, and then decreased thereafter. Spermidine contents increased from 43 mg/kg to 309–351 mg/kg after the storage for 5 days, but decreased thereafter. Putrescine and spermine contents did not change much during the storage (150 and 40 mg/kg, respectively), except tyramine with a slight increase (320 mg/kg after 5 days).

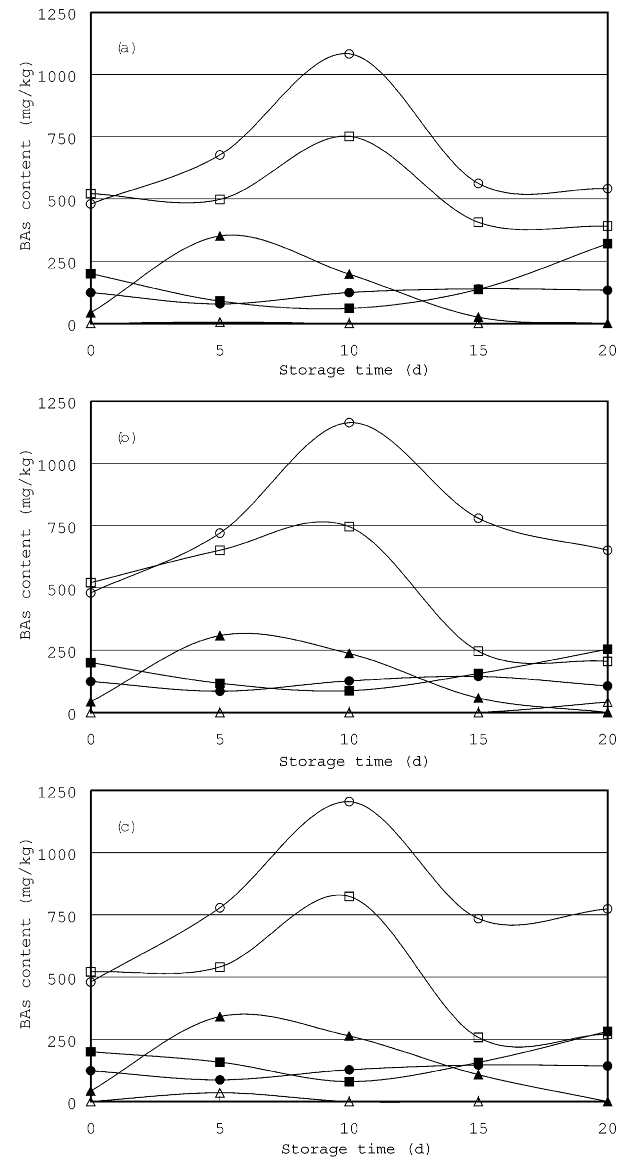

Fig. 1. Changes in biogenic amine concentrations during Myeolchi-jeot (salted and fermented anchovy) storage at 4 °C (a), 10 °C (b) and 15 °C (c). ●: Putrescine, ○: Cadaverine, ■: Tyramine, □: Histamine, ▲: Spermidine, △: Spermine.

As shown in Fig. 2 for Changran-jeot sample, the content of spermine was 51 mg/kg initially and increased to 260 mg/kg after the storage at 15 °C for 20 days. Fig. 3 shows the biogenic amine contents in the Myeongran-jeot sample, which is a similar order of magnitude with that of the Changran-jeot sample. Spermine contents increased from 73 to 216 mg/kg at 15 °C after 5 days. Concentrations of putrescine, cadaverine and tyramine in the samples of Changran-jeot were 150 mg/kg and those in Myeongran-jeot were 70 mg/kg.

The initial level of biogenic amines in all samples, except Myeolchi-jeot, Changran-jeot and Myeongran-jeot, showed a minimal changes during the storage longer than 20 days (data not shown).

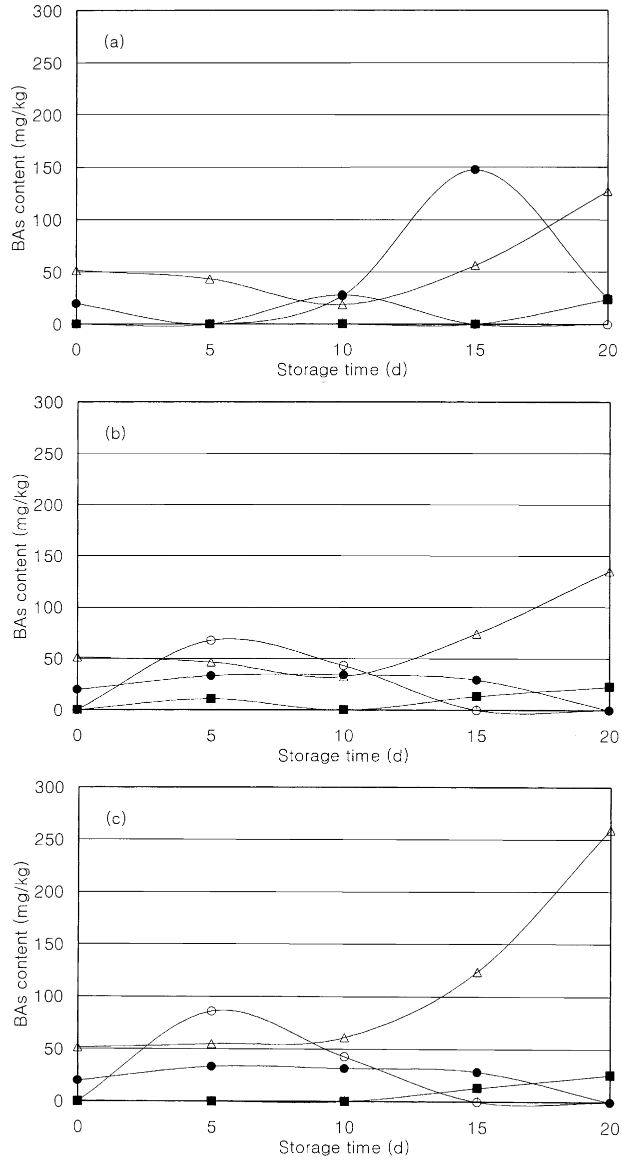

Fig. 2. Changes in biogenic amine concentrations during Changran-jeot (salted and fermented pollock entrails) storage at 4 °C (a), 10 °C (b) and 15 °C (c).●: Putrescine, ○: Cadaverine, ■: Tyramine, □: Spermine.

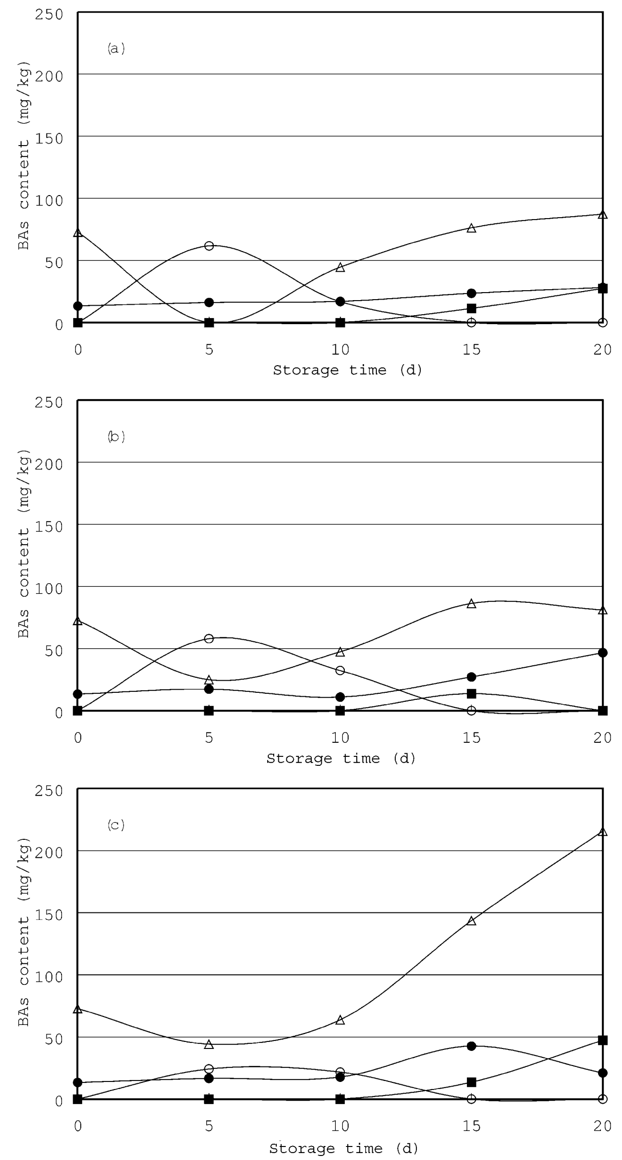

Fig. 3. Changes in biogenic amine concentrations during Myeongran-jeot (salted and fermented pollock roe) storage at 4 °C (a), 10 °C (b) and 15 °C (c). ●: Putrescine, ○: Cadaverine, ■: Tyramine, □: Spermine.

In the case of the Myeolchi-jeot sample, the decrease in cadaverine, histamine and spermidine levels were observed between 5 and 10 days after storage. A similar decrease in the histamine level during the ripening of salted anchovies was reported by Hernández-Herrero, Roig-Sagués, Rodríguez-Jerez, and Mora-Ventura (1999). These authors suggested that a part of the amine content might have been diffused into the brine with other nitrogen fractions during the process of ripening.

Dapkevicius, Nout, Rombouts, Houben, and Wymenga (2000) reported that *Lactobacillus sake* strains degrade histamine in fermented fish pastes, while Leuschner and Hammes (1998) reported that *Micrococcus varians* strains degrade tyramine in fermented sausage. Therefore, in the case of the Myeolchi-jeot sample, the decrease in biogenic amines might be due to not only diffusion into the brine but also by microbial degradation.

A hazard level of histamine for human health has been suggested to be 500 mg/kg (Askar & Treptow, 1993). Putrescine and cadaverine can increase histamine toxicity (Stratton et al., 1991). Moreover, putrescine, cadaverine, spermidine and spermine have also been implicated to be the potential precursors for the formation of nitrosamines (Bills et al., 1973; Smith, 1980; Warthesen et al., 1975). While the histamine content varied between 155 and 579 mg/kg in the commercial Myeolchi-jeot sample, cadaverine and putrescine levels were detected up to 665 and 241 mg/kg, respectively. Myeolchi-jeot, therefore, might not be completely safe for human consumption under certain storage conditions at high temperature.

Furthermore, histamine content in the Myeolchi-jeot sample during the storage increased to the level of nearly 1000 mg/kg which is considered to be critical to induce histamine poisoning (Askar & Treptow, 1993). Significant increments of cadaverine and spermidine in Myeolchi-jeot might be also potentially hazardous for health, because inactivation of histamine metabolizing enzymes by biogenic diamines may yield nitrosamines (Bills et al., 1973; Smith, 1980; Stratton et al., 1991; Warthesen et al., 1975). Overall biogenic amine contents in Changran-jeot and Myeongran-jeot appeared to increase during storage, especially spermine showed even higher increase up to 260 and 216 mg/kg, respectively. It is therefore, important to investigate the possibility that biogenic polyamines in Myeolchi-jeot, Changran-jeot and Myeongran-jeot could be converted into nitroso compounds.

In summary, most commercial Jeotkal samples except for Myeolchi-jeot, Changran-jeot and Myeongran-jeot contained only trace amounts (far below 200 mg/kg) of biogenic amines, and the concentrations did not change significantly during various storage conditions, indicating that these samples are safe for human consumption. However, further studies are required to control the concentrations of biogenic amines in Jeotkals under proper storage condition, since the contents of biogenic amines tend to increase as the storage temperature increases.

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