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Occurrence of histamine and histamine-forming bacteria in kimchi products in Taiwan

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Abstract

Twenty kimchi products sold in supermarkets and 17 products sold in retail markets were purchased from southern Taiwan and tested to determine the occurrence of histamine and histamine-forming bacteria. The levels of pH and salt content in all samples ranged from 3.6 to 5.1 and 1.5–16.0%, respectively. The supermarket kimchi products had 1–7.2 log CFU/g of APC and <3–600 MPN/g of total coliform (TC), and the retail market kimchi products had 4–8.03 log CFU/g of APC and <3 to >2400 MPN/g of TC. Only one of the retail market kimchi products contained 20 MPN/g *Escherichia coli*. Although, supermarket kimchi products had an average histamine content of 49.8 mg/100 g, 15 of them had histamine content greater than 5 mg/100 g, the allowable level set by the US Food and Drug Administration (FDA) for scombroid fish and/or product. In contrast, only eight retail market kimchi products had histamine levels greater than 5 mg/100 g. Among the supermarket samples, three contained histamine at 50.2, 273 and 535 mg/100 g, that are more than the 50 mg/100 g hazard action level. Four histamine-producing bacteria capable of producing 13.6–43.1 ppm of histamine in MRS broth supplemented with 0.25% L-histidine were identified as *Lactobacillus para. paracasei* (one strain), *Lb. brevis* (one strain), and *Brevibacillus brevis* (two strains). To our knowledge, this is the first report to demonstrate the occurrence of high contents of histamine and histamine-forming bacteria in kimchi products.

Keywords: Histamine; Hygienic quality; Histamine-forming bacteria; Kimchi

1. Introduction

Biogenic amines are basic nitrogenous compounds occurring in meat, fish, cheese and wine products and are mainly due to amino acid decarboxylation activities of certain microbes (Arnold & Brown, 1978). High levels of histamine in foods can have important vasoactive effects in humans (Lehane & Olley, 2000; Taylor, 1985). Scombroid fish such as tuna, mackerel, bonito and saury, that contain high levels of free histidine in their muscle, are often implicated in scombroid poisoning incidents (Taylor, 1986). However, nonscombroid fish, cheese, and other foods have also been implicated in incidents of histamine poisoning (Taylor, 1985). An incident of histamine poisoning that occurred in Europe was reported to be caused by sauerkraut that had histamine at near the toxic dose level (Mayer & Pause, 1972).

Kimchi, a specially fermented vegetable product, is an important dietary dish of the Korean people. Recently, kimchi has become a popular food item for consumption in Taiwan. Preparation of kimchi involves: Soaking the sliced Chinese cabbage in 10% (w/v) NaCl for 4 h; washing and draining; mixing the cabbage with various ingredients such as red pepper, garlic, ginger and fish sauce or shrimp paste; placing the cabbage mixture

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in an appropriate vessel; and fermenting the mixture in a cellar (or buried container) for several days or weeks. After fermentation, the product is ready for consumption (Cheigh & Park, 1994; Lee, Kim, & Frank, 1995).

Mower and Bhagavan (1989) demonstrated that low levels of tyramine were found in kimchi and commercial samples of Japanese picked vegetables (urume-zuke). Although higher levels of tyramine were found in homemade kimchi and urume-zuke than their commercial counterparts, the tyramine levels were still not high enough to cause illness. Taylor, Leatherwood, and Lieber (1978a) surveyed 50 samples of retail sauerkraut and detected an average histamine content of 5.06 mg/100 g (ranged from 0.91 to 13.0 mg/100 g). These authors also detected histamine at an average level of 4.07 mg/100 g in canned sauerkraut (Taylor, Lieber, & Leatherwood, 1978b). However, Kalac, Spicka, Krizek, Steidlova, and Pelikanova (1999) detected lower histamine content of 0.78 mg/100 g in Czech sauerkraut, although Mayer and Pause (1972) found histamine at 20 mg/100 g and 0.7 mg/100 g in two separate sauerkraut juice samples.

Histamine is formed mainly through the decarboxylation of histidine by exogenous decarboxylases released by many bacterial species known to possess histidine decarboxylase. These bacteria have been isolated not only from fish and other seafood products, but also from other types of foods such as cheese, fermented sausage, and wine (Taylor, 1986). In these fermented foods, several species of histamine-producing lactic acid bacteria belonging to the *Lactobacillus*, *Leuconostoc*, and *Pediococcus* genera have been isolated (Guerrini, Mangani, Granchi, & Vincenzini, 2002; Roig-Sagues, Hernandez-Herrero, Lopez-Sabater, Rodriguez-Jerez, & Mora-Ventura, 1996; Stratton, Hutkins, & Taylor, 1991; Stratton, Hutkins, Summer, & Taylor, 1992).

There exists no report on the occurrence of biogenic amines, including histamine, histamine-forming bacteria and related bacteria in kimchi products. Therefore, this research was undertaken by testing 37 kimchi products sold in retail markets and supermarkets in Taiwan so that a better understanding of the safety quality of the products can be accomplished to better protect consumers.

2. Materials and methods

2.1. Materials

Twenty kimchi products sold in supermarkets and 17 kimchi products sold in retail markets were purchased from southern Taiwan from July to September, 2003. The retail market kimchi products were homemade and packaged in aseptic bags, while the supermarket kimchi products were made by food factories and packed in plastic or glass bottles. All kimchi samples were kept at 4 °C and immediately transported to the laboratory for use.

2.2. pH value and salt content

Samples of kimchi product (10 g) were homogenized with 10 ml of distilled water to make thick slurry. The pH of this slurry was then measured using a Corning 145 pH meter (Corning Glass Works, Medfield, MA). The salt content in each sample was determined according to the AOAC procedures (1995).

2.3. Microbiological analysis and isolation of histamine-forming bacteria

A 25-g portion of the kimchi was removed from each sample and homogenized at high speed for 2 min in a sterile blender with 225 ml of sterile potassium phosphate buffer (0.05 M, pH 7.0). The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 ml aliquots of the dilutes were poured with aerobic plate count (APC) agar (Difco, Detroit, MI, USA) containing 0.5% NaCl. Bacterial colonies were counted after the plates were incubated at 35 °C for 48 h. The bacterial numbers in the kimchi samples were expressed as $\log_{10}$ colony forming units (CFU)/g.

To isolate histamine-forming bacteria, a 1-ml aliquot of the sample dilute was taken and mixed with differential plating medium fortified with L-histidine (Joosten & Northolt, 1989). Following incubation of the differential agar plates for 4 days at 35 °C, colonies with blue or purple color on the plates were picked and further streaked on MRS agar (Difco, Detroit, MI, USA) to obtain pure cultures. Histamine-forming bacteria were identified on the basis of Gram stain, catalase and oxidase reaction. Further identification of the pure isolates to the species level was accomplished by a variety of biochemical tests using the API 50CHL and API 50CHB system (BioMeriex, Marcy-l'Etoile, France). To determine their ability to produce biogenic amines, these isolates were incubated without shaking in MRS broth supplemented with 0.25% L-histidine (MRSH) at 35 °C for 24 h. Two milliliters of the culture broth were taken for quantitation of biogenic amines.

Analyses of total coliform and *Escherichia coli* in these kimchi samples were conducted using the methods described by FDA (1992).

2.4. Biogenic amine analysis

Biogenic amines, including tryptamine hydrochloride (Trp), 2-phenylamine hydrochloride (Phe), putrescine dihydrochloride (Put), cadaverine dihydrochloride (Cad), spermidine trihydrochloride (Spd), spermine tetrahydrochloride (Spm), histamine dihydrochloride (Him), tyramine hydrochloride (Tyr), and agmatine sulfate

(Agm), were obtained from Sigma (St. Louis, MO, USA). Trp (61.4 mg), Phe (65.1 mg), Put (91.5 mg), Cad (85.7 mg), Spd (87.7 mg), Spm (86.0 mg), Him (82.8 mg), Tyr (63.2 mg), and Agm (87.7 mg) were dissolved in 50 ml of 0.1 M HCl and used as the standard stock solution (each at 1.0 mg/ml). A series of diluted standard solutions were prepared from the standard stock solutions and used to obtain the standard curve for each biogenic amine.

Each kimchi sample was ground in a Waring Blender for 3 min. The ground samples (5 g) were transferred to 50-ml centrifuge tubes and homogenized with 20 ml of 6% trichloroacetic acid (TCA) for 3 min. The homogenates were centrifuged (10,000*g*, 10 min, 4 °C) and filtered through Whatman No. 2 filter paper (Whatman, Maidstone, England). The filtrates were then placed in volumetric flasks, and TCA was added to bring to a final volume of 50 ml. Samples of standard amine solutions and 2-ml aliquots of the kimchi extracts were derivatized with benzoyl chloride according to the previously described method (Hwang, Chang, Shiau, & Chai, 1997). Two milliliters of each bacterial culture broth were also benzoylated following the same procedures as the kimchi extracts. The benzoyl derivatives were dissolved in 1 ml of methanol, and 20 μL aliquots were used for HPLC analysis.

The contents of biogenic amines in the test samples were determined with a Hitachi liquid chromatograph (Hitachi, Tokyo, Japan) consisting of a Model L-7100 pump, a Rheodyne Model 7125 syringe loading sample injector, a Model L-4000 UV–Vis detector (set at 254 nm), and a Model D-2500 Chromato-integrator. A Lichrospher 100 RP-18 reversed-phase column (5 μm, 125 × 4.6 mm, E. Merck, Damstadt, Germany) was used for separation. The gradient elution program began with 50:50 (v/v) methanol:water at a flow rate of 0.8 ml/min for the first 0.5 min, followed by a linear increase to 85:15 methanol:water (0.8 ml/min) during the next 6.5 min. The methanol:water mix was held constant at 85:15 (0.8 ml/min) for 5 min, and then decreased to 50:50 (0.8 ml/min) during the next 2 min.

A set of biogenic amine standards and their mixtures were analyzed together with test samples. During analysis, a standard solution was also injected intermittently between test samples to check chromatographic consistency. Each sample was injected twice. The peak heights of the biogenic amine standard solutions were used to prepare standard curves, and then for determination of the amine concentrations in the test samples.

3. Results and discussion

Values of the pH, salt content, APC, total coliform, and *E. coli* in the kimchi products are presented in Table 1. The levels of pH and salt content in all samples ranged from 3.6 to 5.1%, and 1.5 to 16.0%, respectively. These results are in agreement with those previously reported by Mheen and Kwon (1984) and Lee (1996) for kimchi in Korea. The supermarket kimchi products had 1–7.2 log CFU/g of APC and <3–600 MPN/g of total coliform (TC), and the retail market kimchi products had 4–8.03 log CFU/g of APC and < 3 to >2400 MPN/g of TC. Only one of the retail market kimchi products contained 20 MPN/g *E. coli*. The rates of unacceptable supermarket and retail market kimchi products were 30.0% (6/20) and 70% (12/17), respectively, based on the regulatory level for APC in Taiwan; and 25.0% (5/20) and 52.9% (9/17), respectively, using the Taiwanese regulatory standard for total coliform (Table 2). Based

Table 1
Values of the pH, salt content, APC, TC and *E. coli* in tested kimchi products

Source of samples	No. of sample	pH	Salt content (%)	APC (log CFU/g)	TC (MPN/g)	*E. coli* (MPN/g)
Supermarket kimchi	20	3.6–5.1 (4.2 ± 0.4)[a]	1.5–16.0 (5.5 ± 3.8)	1–7.2 (5.9 ± 4.7)	<3–600 (66.0 ± 71.7)[b]	<3
Retail market kimchi	17	3.8–5.1 (4.3 ± 0.3)	2.0–14.5 (6.3 ± 3.4)	4–8.03 (7.0 ± 3.8)	<3–>2400 (689 ± 203)[c]	<3–20

[a] Mean ± SD.
[b] Calculated based on the assumption that the number <3 MPN/g was zero.
[c] Calculated based on the assumption that the number >2400 was 2400.

Table 2
The percentage (%) of unacceptable kimchi products as judged by Taiwanese regulatory levels of APC, TC and *E. coli*[a]

Source of samples	Percentage (%) of unacceptability		
	APC	TC	*E. coli*
Supermarket kimchi	30.0 (6/20)[b]	25.0 (5/20)	0 (0/20)
Retail market kimchi	70.6 (12/17)	52.9 (9/17)	5.9 (1/17)

[a] The regulatory levels of APC, TC and *E. coli* for general food and frozen vegatable products in Taiwan are <1 × 10^5 CFU/g, 10 MPN/g, and <3 MPN/g (Negative), respectively.
[b] Number of unacceptable samples/number of total samples tested.

Table 3
The levels of biogenic amines in tested kimchi products

Source	No. of samples	Range of amine level (mg/100 g)								
		Put[a]	Cad	Try	Phe	Spd	Spm	His	Tyr	Agm
Supermarket kimchi	20	ND[b]∼7.3 (2.06 ± 1.33)[c]	ND∼155 (14.3 ± 9.2)	ND∼11.4 (1.0 ± 1.05)	ND	ND∼8.8 (0.65 ± 0.51)	ND∼12.1 (1.96 ± 1.31)	ND∼535 (49.8 ± 32.5)	ND∼4.2 (0.46 ± 0.48)	ND
Retail market kimchi	17	ND∼5.1 (0.67 ± 0.79)	ND∼4.8 (1.59 ± 1.44)	ND	ND	ND∼8.2 (0.48 ± 0.52)	ND	ND∼18.6 (5.59 ± 4.57)	ND∼3.5 (0.40 ± 0.65)	ND

[a] Put, putrescine; Cad, cadaverine; Try, tryptamine; Phe, 2-phenylethylamine; Spd, spermidine; Spm, spermine; His, histamine; Tyr, tyramine; Agm, agmatine.
[b] ND, not detected (amine level less than 0.1 mg/100 g).
[c] Means ± S.D.

on these microbiological indices, the supermarket kimchi had a better hygienic quality than retail market kimchi. The higher rate of unacceptability for retail market kimchi products could be due to the use of poor quality raw vegetables for production or unclean processing techniques during homemade fermentation.

Table 3 summarizes the results of biogenic amine contents in tested kimchi samples. None of the 37 samples contained 2-phenylethylamine and agmatine. In general, higher levels of putrescine, cadaverine, tryptamine, spermine, and histamine were detected in supermarket kimchi samples than the retail market kimchi. Except for histamine and cadaverine, the average content of various biogenic amines in tested samples was less than 3 mg/100 g. The average histamine content in supermarket kimchi was 49.8 mg/100 g, and 5.59 mg/100 g in retail market kimchi. Fig. 1 shows the typical HPLC profiles of the 9 standard biogenic amines, the supermarket kimchi that had the highest level of histamine at 535 mg/100 g and cadaverine at 155 mg/100 g, and the MRSH culture medium of *Brevibacillus brevis* strain 17MT that contained 43.1 ppm of histamine.

Table 4 shows the distribution of histamine contents in tested samples, with 15 (75%) supermarket and eight (47.1%) retail market kimchi products containing greater than 5 mg/100 g of histamine, the allowable limit of the US Food and Drug Administration (FDA) for scombroid fish and/or product. Only three out of the 20 supermarket kimchi products had histamine content exceeding 50 mg/100 g at 50.2, 273 and 535 mg/100 g. These three supermarket kimchi samples with greater than 50 mg/100 g of histamine could be hazardous to the health of consumers based on the data collected from numerous outbreak reports (Taylor, 1989). Bartholomev, Berry, Rodhouse, and Gilhouse (1987), which demonstrated that histamine at greater than 100 mg/100 g in fish would be toxic and unsafe for consumption. Taylor et al. (1978a) surveyed 50 samples of retail sauerkraut and revealed an average histamine content of 5.06 mg/100 g (ranged from 0.91 to 13.0 mg/100 g). However, a higher average histamine level at 4.07 mg/100 g was found in canned sauerkraut (Taylor et al., 1978b). A low histamine average content of 0.78 mg/100 g was reported in Czech sauerkraut by Kalac et al. (1999). Mayer and Pause (1972) tested two samples of sauerkraut juice and reported their histamine contents as 20 and 0.7 mg/100 g. In this study, the levels of histamine in tested kimchi products, especially the supermarket kimchi samples, are considerably higher than those in the above reported sauerkraut (Kalac et al., 1999; Mayer & Pause, 1972; Taylor et al., 1978a; Taylor et al., 1978b). The higher histamine content of the supermarket kimchi samples observed in this work may be related to the addition of fermented fish products, such as fish sauce or shrimp paste, during kimchi fermentation according to traditional Korean manufacturing procedures (Cha et al., 2003a; Cha et al., 2003b; Cheigh & Park, 1994; Lee, 1996), whereas the retail market kimchi samples, which were homemade in Taiwan, contained less fermented fish products. The fermented fish products and fish paste were frequently found to contain high contents of histamine (Fardiaz & Markakis, 1979). For example, Sanceda, Kurata, and Arakawa (1996) reported histamine levels of 430 mg/L in *nampla* and 1380 mg/L in Korean anchovy sauce.

Although high content of histamine was detected in some of the kimchi products tested in this study, only very few cases of food-borne histamine intoxication was reported due to consumption of kimchi products. Symptoms of histamine poisoning are not particularly definitive. Therefore, histamine intoxication is frequently confused diagnostically with an allergic reaction. Also, histamine poisoning is not a reportable illness even in those countries that keep surveillance records (Taylor, 1986).

High levels of putrescine and cadaverine were also found in kimchi products in this study (Table 3). Putrescine and cadaverine have been shown to potentiate histamine toxicity when present in spoiled fish by inhibiting the intestinal histamine metabolizing enzyme (Antoine et al., 2002; Bjeldanes, Schutz, & Morris, 1978; Taylor & Sumner, 1987). Since the diamines putrescine and cadaverine can react with nitrite to form heterocyclic carcinogenic nitrosamines such as nitrosopyrrolidine and nitrosopiperidine, respectively, (Seel et al., 1994; Vandekerckhove, 1977), their presence in kimchi products needs to be monitored.

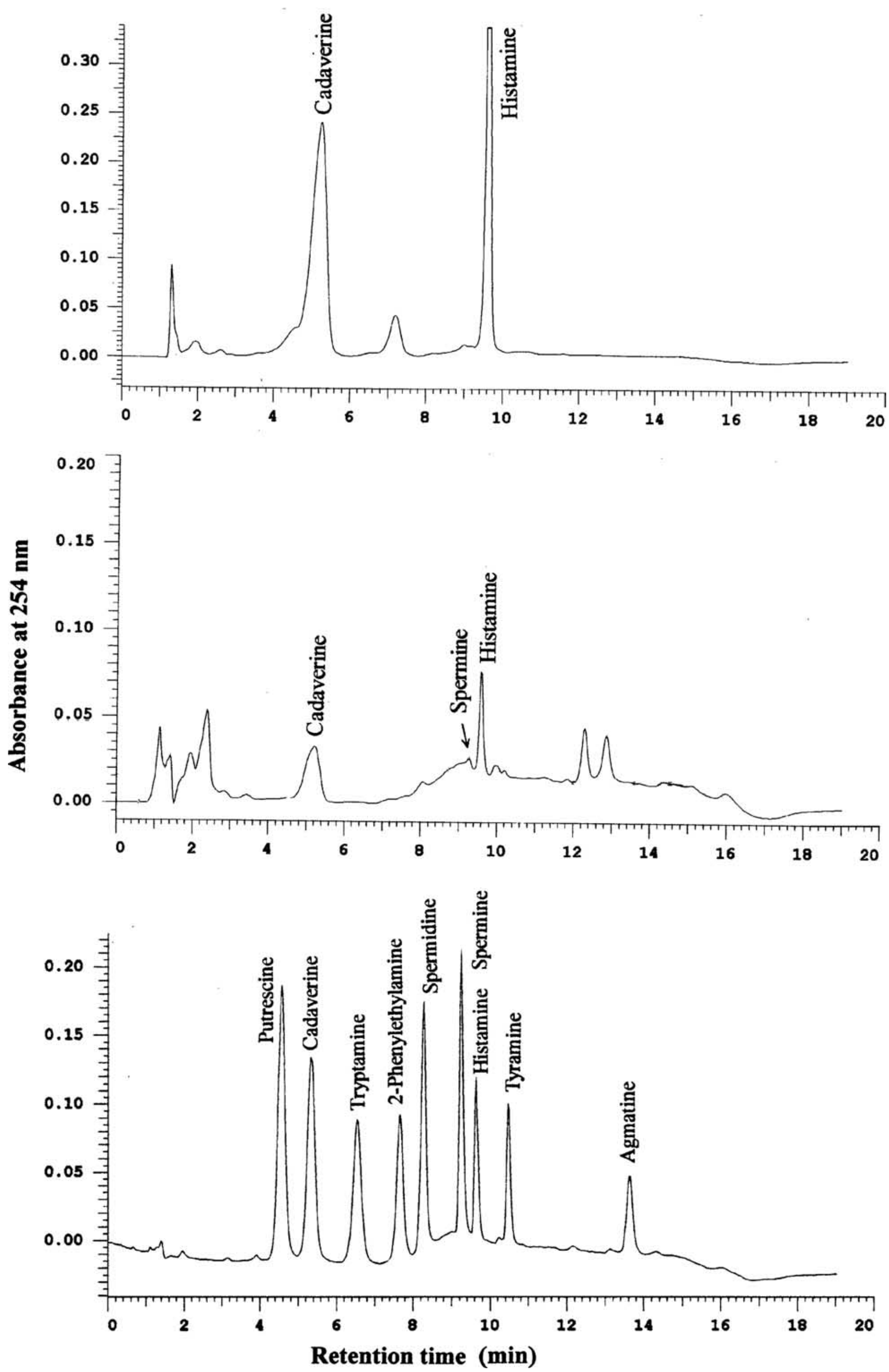

Fig. 1. Typical HPLC profiles of a standard biogenic amine mixture solution (lower), the extract of the kimchi sample that had the highest histamine content of 535 mg/100 g (upper), and the extract of the MRSH culture broth of *Bb. brevis* strain 17HT (middle).

The tested kimchi products produced 25 purple colonies on the differential plating medium. Following incubation in MRSH broth, four out of the 25 isolates (16.0%) showed the ability to produce histamine, as determined by HPLC analysis of the culture broth. These four histamine-forming bacterial isolates were identified as *Lactobacillus brevis* (1 isolate), *Lactobacillus para. paracasei* (1 isolate), and *Bb. brevis* (2 isolates) which produced 13.6 – 43.1 ppm of histamine in the culture broth (Table 5). *Lb. para. paracasei* strain 17A produced 4.5 ppm of spermine, but low levels of cadaverine, while *Lb. brevis* strain 17B produced 5.6 ppm

Table 4
Distribution of histamine content in tested kimchi products

Content of histamine (mg/100 g)	Supermarket kimchi		Retail market kimchi	
	No. of samples	% of samples	No. of samples	% of samples
<4.9	5	25.0	9	52.9
5.0–49.9	12	60.0	8	47.1
50.0–99.9	1	5.0	0	0
>100	2	10.0	0	0
Total	20		17	

Table 5
Histamine and other biogenic amines (ppm) produced in culture broth by histamine-forming bacteria isolated from supermarket kimchi products

Strain	Histamine former	His[a]	Put	Cad	Phe	Spm
17A	*Lb. para. paracasei*	15.1	ND[b]	0.3	ND	4.5
17B	*Lb. brevis*	13.6	0.2	0.8	4.3	5.6
17MH	*Bb. brevis*	16.3	0.1	ND	3.8	6.8
17MT	*Bb. brevis*	43.1	ND	11.2	ND	8.8

[a] His, histamine; Put, putrescine; Cad, cadaverine; Phe, 2-phenylethylamine; Spm, spermine.
[b] ND, not detected (amine level less than 0.1 ppm).

of spermine and 4.3 ppm of 2-phenylethylamine, but low levels of cadaverine and putrescine (Table 5). *Bb. brevis* strain 17MH produced 6.8 ppm of spermine and 3.8 ppm of 2-phenylethylamine, but low levels of putrescine, while *Bb. brevis* strain 17MT produced 11.2 ppm of cadaverine and 8.8 ppm of spermine in the culture broth (Table 5).

Previous reports by Joosten and Northolt (1989), Maijala (1993), and Bover-Cid and Holzapfel (1999) indicated that *Lb. brevis* isolated from meat, meat products, and cheese was the most intensive biogenic amine former, particularly for tyramine formation. Kung et al. (2004) isolated *Lb. brevis* from natural cheese as the histamine-former in Taiwan. The *Lb. brevis* culture isolated in this study also produced 13.6 ppm of histamine in MRSH medium. Since *Lb. brevis* could be used as a starter culture for kimchi fermentation (Cheigh & Park, 1994), the kimchi industry should pay attention to its histamine-producing ability. On the contrary, *Lb. para. paracasei* has been reported rarely as a histamine-former although it produced 15.1 ppm of histamine in MRSH medium in this study. Bover-Cid and Holzapfel (1999) indicated that *Lb. para. paracasei* was an intensive tyramine-producer. These two histamine-producing lactic acid bacteria could exist naturally in the ingredients of kimchi, such as cabbage, red pepper, garlic and ginger (Cheigh & Park, 1994). Although *Bb. brevis* was never identified as a histamine-former, it accounted for 50% (2/4) of the total histamine-forming isolates in this study. Specifically, the *Bb. brevis* strain 17MT produced 43.1 ppm of histamine in MRSH medium. Therefore, it is important to study the optimal conditions of *Bb. brevis* growth and histamine-production during kimchi fermentation.

4. Conclusion

Based on the microbiological indices, the supermarket kimchi products had a better hygienic quality than the retail market kimchi products. However, the supermarket kimchi products had a higher average histamine content of 49.8 mg/100 g than the average 5.59 mg/100 g content of the retail market kimchi products. *Lb. para. paracasei* (one strain), *Lb. brevis* (one strain), and *Bb. brevis* (two strains) were the four weak histamine formers isolated from these samples. To our knowledge, this is the first report to demonstrate the presence of high contents of histamine and other biogenic amines and histamine-forming bacteria in kimchi products.

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