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ScienceDirect
Food Chemistry 103 (2007) 1305–1311
Food Chemistry
www.elsevier.com/locate/foodchem

Histamine formation by histamine-forming bacteria in douchi, a Chinese traditional fermented soybean product

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Received 25 May 2006; received in revised form 24 August 2006; accepted 17 October 2006

Abstract

Seven soybean and 19 black bean douchi products sold in the supermarkets in southern Taiwan were purchased and tested to determine the occurrence of histamine and histamine-forming bacteria. The levels of pH, salt content, water content, yeast and mold, and aerobic plate count (APC) in all samples ranged from 4.7 to 5.9, 4.4% to 14.0%, 6.8% to 51.6%, 3.0 to 5.1 log CFU/g, and 5.2 to 9.2 log CFU/g, respectively. None of these samples contained total coliform and *Escherichia coli*. Although black bean douchi products had an average histamine content of 29.0 mg/100 g, 18 of them had histamine contents greater than 5 mg/100 g, the allowable level set by the US Food and Drug Administration (FDA) for scombroid fish and/or products. In contrast, only four soybean douchi products had histamine levels greater than 5 mg/100 g. Among the black bean samples, four contained histamine at 56.3, 62.1, 80.2 and 80.8 mg/100 g, that are above the 50 mg/100 g hazard action level. Eight histamine-forming bacterial strains, capable of producing 11.7–601 ppm of histamine in trypticase soy broth (TSB) supplemented with 1% L-histidine (TSBH), were identified as *Bacillus subtilis* (four strains) *Staphylococcus pasteuri* (one strain) and *Staphylocuccus capitis* (three strains) by 16S rDNA sequencing with PCR amplification. *S. capitis*, which was previously reported to be halotolerant, was a potent histamine-former capable of producing more than 500 ppm of histamine in TSBH in the presence of 0.5–10% NaCl.

Keywords: Histamine; Histamine-forming bacteria; *Staphylocuccus capitis*; Douchi products; 16S rDNA

1. Introduction

Douchi is a traditional fermented soybean product that originated in China a long time ago. It has been used as a food seasoning and for pharmaceutical purposes since before the Han dynasty (206 BC). Even today, douchi is still added to some Chinese traditional medicines (Zhang, Tatsumi, Ding, & Li, 2006). Douchi can be used as an appetizer for consumption with bland foods, such as rice gruel; or can be cooked as a flavouring agent with vegetables, meats and seafoods (Hesseltine & Wang, 1972). The soybean preparation methods for fermentation and brine composition may vary from country to country. However, the essential features are similar. Soybeans or black beans are soaked and steamed until they become soft. The steamed beans are drained, cooled, mixed with parched wheat flour, and then inoculated with a strain of *Aspergillus oryzae*. The inoculated beans are packed with the desired amounts of salt, spices, wine, and water, and then aged for several weeks or months. The finished products are blackish and have a salty taste, and their flavour resembles that of soybean sauce (Hesseltine & Wang, 1972).

Biogenic amines are basic nitrogenous compounds occurring in many foods, especially in fermented foods, such as cheese, kimchi, wine, miso and fermented meat and seafood products, due to amino acid decarboxylation

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0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.10.036

activities of certain microbes during fermentation (Arnold & Brown, 1978; Kung, Tsai, & Wei, 2007; Tsai et al., 2005a, 2005b, 2006). Consumption of foods with high levels of histamine can have important vasoactive effects in humans (Lehane & Olley, 2000; Taylor, 1985). Although no douchi has been incriminated in incidents of histamine poisoning, the other biogenic amines present in douchi products might contribute to enhanced histamine toxicity. Cadaverine and putrescine are known to enhance histamine toxicity by inhibiting histamine-metabolizing enzymes, such as diamine oxidase and histamine methyl transferase (Arnold & Brown, 1978; Bjeldanes, Schutz, & Morris, 1978; Lehane & Olley, 2000), and douchi was reported to contain these two biogenic amines (Yen, 1986).

Histamine is formed mainly through the decarboxylation of histidine by exogenous decarboxylase 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 other types of foods, such as cheese, kimchi, 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 (Kung et al., 2005; Stratton, Hutkins, & Taylor, 1991; Stratton, Hutkins, Summer, & Taylor, 1992; Tsai et al., 2005a). Recently, our research group isolated histamine-formers, *Staphylococcus* spp., *Enterobacter cloacae* and *Candida* spp., from mustard pickle products in Taiwan (Kung et al., 2006).

Although a pure *A. oryzae* culture is used for fermentation of douchi, the manufacture process itself is carried out under non-sterile conditions. Consequently, microbial contamination could occur during douchi production. Yen (1986) studied the occurrence of biogenic amines in douchi products in Taiwan. However, no information is available on the hygienic quality and histamine-forming bacteria in this product. This research was therefore undertaken by testing 26 douchi products sold in the supermarkets in Taiwan to better understand their safety, including the contents of total coliform, *Escherichia coli*, and histamine, for the purpose of protecting consumers.

2. Materials and methods

2.1. Materials

Seven soybean and 19 black bean douchi products sold in the supermarkets in southern Taiwan were purchased in August and September of 2005. The douchi products were packaged in plastic bags and kept at room temperature. Once purchased, they were immediately transported to the laboratory for analysis.

2.2. pH value, salt content and water content determination

Samples of douchi product (10 g) were homogenized in sterile blenders with 10 ml of distilled water to make a thick slurry. The pH of this slurry was then measured using a Corning 145 pH meter (Corning Glass Works, Medfield, MA, USA). The salt content in each sample was determined according to the AOAC procedures (1995) by homogenizing 2 g of douchi sample with 18 ml of distilled water. The homogenate was titrated with 0.1 M $AgNO_3$, using 10% w/v K_2CrO_4 solution as an indicator. The water content was determined by the standard gravimetric method by drying 1–3 g of a test sample at 102.0 ± 2.0 °C under atmospheric pressure for 2 h. Consistency of mass is tested by additional drying steps of 1 h until the difference in mass does not exceed 0.5 mg.

2.3. Microbiological analysis and isolation of histamine-forming bacteria

A 25 g portion of the douchi sample was 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 sterile blender was prepared by autoclaving for 15 min at 121 °C. The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 ml aliquots of the diluted solutions were inoculated into 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 douchi samples were expressed as $\log_{10}$ colony-forming units (CFU)/g.

To isolate histamine-forming bacteria, a 0.1 ml aliquot of the sample dilute was spread on histamine-forming bacterium isolation agar (HBI agar) fortified with L-histidine (Niven, Jeffreg, & Corlett, 1981). Following incubation of the differential agar plates for 4 d at 35 °C, colonies with blue or purple colour on the plates were picked and further streaked onto trypticase soy agar (TSA) (Difco) to obtain pure cultures. Their ability to produce biogenic amines was determined by inoculating the isolates in trypticase soy broth (TSB) (Difco) supplemented with 1% L-histidine (TSBH) and incubated without shaking at 35 °C for 24 h. Two millilitres of the culture broth were taken for quantitation of biogenic amines.

Analyses of total coliform and *E. coli* in these douchi samples were conducted using the three-tube most probable number (MPN) methods (FDA, 1992). Lauryl sulphate tryptose broth (LST broth) and brilliant green lactose bile (2%) broth (BGLB broth) were used for presumptive and confirmed tests for total coliform, respectively. *E. coli* was determined by using the LST broth and EC broth. Cultures that showed positive production of gas were then confirmed by eosine methylene blue agar (EMBA) and IMViC test.

Yeasts and molds were determined by using Petrifilm yeast and mold count plates (The 3M Products, 1999) with incubation at 22 °C for 3–5 d. The Petrifilm plates contained antibiotics and an indicator dye stained yeast and mold colonies to provide contrast and facilitate counting. The yeast colonies were small blue-green colonies with defined edges and no foci; while the mold colonies were

large, variably coloured colonies and were with diffuse edges and centre foci.

2.4. Identification of histamine-forming isolates

The presumptive histamine-forming isolates were identified on the basis of morphology, Gram stain, endospore stain, catalase and oxidase reaction. The identity of histamine-forming isolates was further confirmed by amplifying and sequencing approximately 1400 bp of the 16S ribosomal DNA (rDNA) for bacteria (Kuhnert, Capaul, Nicolet, & Frey, 1996; Kuhnert, Heyberger-Meyer, Nicolet, & Frey, 2000). Amplification of histamine-forming bacteria was performed using the universal primers UNI-L (5′-AGAGTTTGATCATGGCTCAG-3′) and UNI-R (5′-GTGTGACGGGCGGTGTGTAC-3′) (Kuhnert et al., 1996, 2000). Bacterial cells were cultured overnight in 2 ml of TSB at 35 °C and then centrifuged at 5000*g* for 10 min. The cell pellet was washed and resuspended in 0.5 ml of TE-buffer (10 mM Tris–HCl, 1 mM EDTA; pH 8.0), and then lysed by 20% sodium dodecyl sulfate (SDS). After the solution was boiled for 20 min and the cellular debris was discarded following centrifugation at 13,000*g* for 3 min, the total DNA in the supernatant was precipitated with 70% ethanol and used as template DNA for PCR.

PCR amplification was performed in 20 μl reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 20 pmol of each primer, a 0.2 mM concentration for each of the four deoxynucleotide triphosphates, 0.5 U of *Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA), and template DNA (10 ng). Amplifications were carried out for 35 cycles (94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s) in a GeneAmp PCR 2400 Thermal Cycler (Applied Biosystems) with an initial denaturation at 94 °C for 4 min and a final extension at 72 °C for 7 min (Kuhnert et al., 1996, 2000). Amplicons were detected by electrophoresis on a 1.5% agarose gel, staining with ethidium bromide. Amplicons were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA), and eluted in Tris–HCl (10 mM, pH 8.5) prior to sequencing. The amplified DNA was directly sequenced with the ABI TaqDye Deoxy Terminator Cycle sequencing kit and ABI Model 377 automated DNA sequencer (Applied Biosystems). The sequences were analyzed with the BLAST (NCBI) for identification of histamine-forming bacteria.

2.5. Biogenic amine analysis

Each douchi 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 biogenic amine solutions and 2 ml aliquots of the douchi extracts were derivatized with benzoyl chloride according to the previously described method (Hwang, Chang, Shiau, & Chai, 1997). Two millilitres of each bacterial culture broth were also benzoylated using the same procedures as for douchi extracts. The benzoyl derivatives were dissolved in 1 ml of methanol, and 20 μl aliquots were used for HPLC injection.

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 chromatographic separation. The gradient elution programme 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.

2.6. Effect of NaCl content on histamine-forming bacteria

The effect of NaCl content on histamine production by histamine-forming bacteria was determined in 50 ml of TSBH medium in flasks containing 0.5%, 5%, 10%, 17% or 24% of NaCl. One hundred microlitres of the 18 h-old bacterial cultures in 5 ml of TSBH medium at 35 °C were inoculated into fresh TSBH to obtain an initial concentration of about 6.0 log CFU/ml. Bacterial growth and histamine production in test TSBH were determined after incubation at 35 °C for 1, 2, 3 and 4 days. For those samples containing 17% and 24% NaCl, analyses were additionally performed at 7 and 10 days of incubation.

2.7. Statistical analysis

Pearson correlation was carried out to determine relationships between pH, salt content, water content, yeast and mold, APC and histamine contents in the 26 douchi samples. All statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 9.0 for windows (SPSS Inc., Chicago, Il. USA). A value of $P < 0.05$ was used to indicate significant deviation.

3. Results and discussion

Values of the pH, salt content, water content, aerobic plate count (APC), yeast and mold, total coliform, and *E. coli* in the douchi products are presented in Table 1. The levels of pH, salt content, water content, yeast and mold, and APC in all samples ranged from 4.7 to 5.9, 4.4% to 14.0%, 6.8% to 51.6%, 3.0 to 5.1 log CFU/g and

Table 1
Values of the pH, salt content, water content, yeast and mold, aerobic plate count (APC), total coliform (TC), and *E. coli* in tested douchi products

Samples	No. of samples	pH	Salt content (%)	Water content (%)	Yeast and mold (log CFU/g)	APC (log CFU/g)	TC (MPN/g)	*E. coli* (MPN/g)
Soybean douchi	7	5.0–5.1 (5.0 ± 0.1)[a] A	5.1–10.1 (7.9 ± 1.7) A	16.9–37.1 (22.5 ± 6.9) A	3.1–3.9 (3.7 ± 0.4) A	5.2–7.4 (6.6 ± 0.7) B	<3	<3
Black bean douchi	19	4.7–5.9 (5.1 ± 0.2) A	4.4–14.0 (9.6 ± 2.1) A	6.8–51.6 (31.5 ± 11.9) A	3.0–5.1 (3.9 ± 0.5) A	5.3–9.2 (7.5 ± 1.0) A	<3	<3

[a] Mean ± SD. Values in the same column with different letters are statistically different ($P < 0.05$).

5.2 to 9.2 log CFU/g, respectively. The average APC level of the black bean douchi (7.5 log CFU/g) was significantly ($P < 0.05$) higher than that of the soybean douchi (6.6 log CFU/g). None of these samples contained total coliform and *E. coli* (Table 1). The higher salt contents (>4.4%) in these douchi samples apparently had some inhibitory effect on the growth of the coliform. In general, no correlation existed among the pH values, salt contents, water content, yeast and mold, APC, and histamine contents in the 26 tested samples.

Table 2 summarizes the contents of biogenic amines in the tested douchi products. The average content of various biogenic amines in tested samples was more than 3.1 mg/100 g. In general, higher levels of putrescine, tryptamine, 2-phenylethylamine, histamine, and tyramine were detected in black bean douchi than in the soybean douchi. Yen (1986) reported similar findings for the levels of biogenic amines in Taiwanese douchi products and higher contents of biogenic amines were detected in black bean douchi products. The difference in the contents of biogenic amines between the black bean and soybean douchi products could be attributed to the variation of the substrate materials, the microbiological composition, and the conditions and duration of fermentation (Chin & Koehler, 1983; Nout, Ruiker, & Bouwmeester, 1993; Yen, 1986).

Table 3 shows the distribution of histamine contents in the tested douchi products, with 22 (84.6%) of them containing greater than 5 mg/100 g, the allowable level set by the US Food and Drug Administration (FDA) for scombroid fish and/or products (USFDA, 2001, chap. 7). Although black bean douchi products had an average histamine content of 29.0 mg/100 g, 18 of them had histamine contents, above the 5 mg/100 g FDA allowable level. In contrast, only four of the tested soybean douchi products had histamine levels greater than 5 mg/100 g. Among the black bean douchi samples, four contained histamine at 56.3, 62.1, 80.2 and 80.8 mg/100 g, levels greater than the 50 mg/100 g hazard action level (Taylor, 1989). However, histamine is not the only compound responsible for scombrotoxicosis, since ingestion of pure histamine does not automatically cause toxic symptoms (Bjeldanes et al., 1978). The toxic effects of histamine are increased in the presence of other amines, such as putrescine and cadaverine, which inhibit histamine-metabolizing enzymes in the small intestine (Arnold & Brown, 1978; Bjeldanes et al., 1978; Lehane & Olley, 2000).

The tested douchi samples produced 20 purple colonies on the differential HBI agar plates. Only eight of them (40%) produced histamine in TSBH medium. The remaining 12 isolates were false-positive histamine-formers. Lopez-Sabater, Rodriguez-Jerez, Hernandez-Herrero, Roig-Sagues, and Mora-Ventura (1996) also found that 63.1% of the presumptive histamine-producers that they isolated from Niven's medium were actually false-positives when grown in a histidine-supplemented culture

Table 2
Contents of biogenic amines in tested douchi products

Samples	Number of samples	Content of biogenic amine (mg/100 g)								
		Put[a]	Cad	Try	Phe	Spd	Spm	His	Tyr	Agm
Soybean douchi	7	ND[b]-36.0 (6.6 ± 13.0)[c]	ND-12.8 (3.1 ± 4.7)	ND-15.1 (4.1 ± 7.0)	ND-19.1 (3.8 ± 5.5)	ND-25.4 (7.6 ± 9.9)	ND-21.9 (6.3 ± 9.5)	ND-23.4 (11.4 ± 10.9)	ND-23.7 (6.2 ± 6.7)	ND-30.1 (4.3 ± 7.9)
Black bean douchi	19	ND-59.6 (18.6 ± 16.2)	ND-19.1 (3.8 ± 5.5)	ND-44.0 (13.8 ± 15.6)	ND-23.9 (7.2 ± 7.5)	ND-71.9 (8.7 ± 17.2)	ND-24.2 (5.8 ± 8.0)	ND-80.8 (29.0 ± 25.1)	ND-52.9 (13.0 ± 9.6)	ND-29.2 (4.1 ± 8.6)

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

Table 3
Distribution of the histamine contents in the 26 tested douchi products

Histamine content (mg/100 g)	Number of soybean douchi	Number of black bean douchi
<4.9	3 (42.9%)	1 (5.2%)
5.0–24.9	4 (57.1%)	11 (57.9%)
25.0–49.9	0	3 (15.8%)
50.0–100	0	4 (21.1%)
Total	7	19

broth. Thus, our results confirm the previous observations that Niven's medium may yield false-positive isolates of histamine-producers, because other alkaline products of bacterial origin can also cause colour changes of the colonies on the agar plates (Ababouch, Afilal, Rhafiri, & Busta, 1991; Tsai, Kung, Lee, Lin, & Hwang, 2004; Tsai et al., 2005b).

Table 4 lists the identity of these eight histamine-forming bacteria, as determined by 16S rDNA sequencing, followed by comparison to the reference strains, using NCBI database analysis. The PCR amplicons of strains D4-1, D6-8, D9-2 and D17-1 had a 100% homology with *Bacillus subtilis*. The PCR amplicons from strain D10-1 had a 99% homology with *Staphylococcus pasteuri*, while those from strains D15-1, D15-2 and D16-1 aligned with *Staphylocuccus capitis* at 99% (Table 4). These eight histamine-forming isolates, namely *B. subtilis* (four strains), *S. pasteuri* (one strain) and *S. capitis* (three strains), by 16S rDNA sequencing, produced substantial amounts of histamine (11.7–601 ppm) in TSBH medium (Table 4). Some of them also produced different amounts of cadaverine, 2-phenylethylamine, spermine and tyramine through the actions of their respective decarboxylase enzymes on various amino acids that also existed in the culture medium (Table 4).

The three *S. capitis* strains isolated from the test products were potent histamine-formers, producing 237.5–601 ppm of histamine in TSBH, whereas the *S. pasteuri* strain was a weak histamine-former and it produced 20.0 ppm of histamine (Table 4). *Staphylococcus* spp. were the most frequently reported histamine-formers in fermented salted fish, accounting for nearly 50% of histamine-forming microorganisms, and they showed powerful histamine-forming activity (Yatsunami & Echigo, 1991, 1992). *Staphylococcus epidermidis* and *S. capitis*, isolated from salted Spanish anchovies, produced more than 1000 and 400 ppm of histamine, respectively (Hernandez-Herrero, Roig-Sagues, Rodriguez-Jerez, & Mora-Ventura, 1999). The *S. capitis*, that was recently isolated from mustard pickle products in Taiwan, was a potent histamine-former, capable of producing more than 1000 ppm of histamine in TSBH medium (Kung et al., 2006).

The four *B. subtilis* strains that accounted for 50% of the total histamine-forming isolates from the tested samples in this study were weak histamine-formers. The *Bacillus* spp. isolated from salted anchovies produced low

Table 4
Identification of histamine-forming bacteria isolated from douchi products by 16S rDNA, based on the output results from NCBI database analysis, and their production of histamine and other biogenic amines (ppm) in culture broth

Strain	Organism identified	Percentage identity (%)	Genbank accession number	His[a]	Cad	2-Phe	Spm	Tyr
D4-1	*B. subtilis*	100	AY894690.1	16.3	1.0	9.0	7.8	ND[b]
D6-8	*B. subtilis*	100	AY894690.1	32.4	1.0	ND	ND	1.0
D9-2	*B. subtilis*	100	AY894690.1	11.7	ND	ND	ND	ND
D17-1	*B. subtilis*	100	AY894690.1	14.4	ND	ND	10.0	ND
D10-1	*S. pasteuri*	99	AJ717376.1	20.0	1.2	ND	ND	ND
D15-1	*S. capitis*	99	L37599.1	238	1.8	ND	6.5	ND
D15-2	*S. capitis*	99	L37599.1	601	ND	16.1	ND	3.3
D16-1	*S. capitis*	99	L37599.1	287	1.4	ND	1.6	ND

[a] His, histamine; Cad, cadaverine; 2-Phe, 2-phenylethylamine; Spm, spermine; Tyr, tyramine.
[b] ND, not detected (amine level less than 1 ppm).

levels of histamine at 10.5 and 12.4 ppm, respectively (Hernandez-Herrero et al., 1999; Rodriguez-Jerez, Mora-Ventura, Lopez-Sabater, & Hernandez-Herrero, 1994). The *Bacillus* spp. isolates that Kim et al. (2004) most frequently detected in canned anchovies also produced negligible amounts of histamine in the culture borth. The recently isolated *Bacillus coagulans* and *Bacillus megaterium* from fermented fish products in Taiwan were also identified as weak histamine-forming bacteria (Tsai et al., 2006). *Bacillus amyloliquefaciens*, *B. subtilis* and *B. megaterium*, that were isolated from miso products in Taiwan, were found to be weak histamine-formers (Kung et al., 2007).

The effects of NaCl content, at 0.5%, 5%, 10%, 17% or 24% in TSBH medium, on the growth and histamine production of *S. capitis* strain D15-2 are shown in Fig. 1. At 0.5% and 5% NaCl contents, histamine production was accelerated and exceeded over 500 ppm in four days, along with bacterial growth. Higher levels of histamine were detected in medium containing 5% NaCl than 0.5% NaCl at the same incubation time. When the medium NaCl content was increased to 10%, bacterial growth was inhibited for four days and then enhanced, to eventually reach about 7.5 log CFU/ml in 10 days. Low levels of histamine (below 200 ppm) were produced by this culture during the first seven days of incubation. The bacteria then produced over 1000 ppm of histamine within 10 days of growth. However, when the NaCl content in TSBH medium was increased to 17% or 24%, the bacterial growth was inhibited, and histamine formation increased only slightly, reaching about 50 ppm in 10 days. Hernandez-Herrero et al. (1999) reported that NaCl contents in the range of 0.5–10% had a stimulatory effect on histamine formation for *S. capitis* and *S. epidermidis*, whereas NaCl levels in excess of 20% inhibited their growth and histamine formation. Therefore, our results on histamine formation by *S. capitis* strain D15-2 were similar to those of Hernandez-Herrero et al. (1999), except that histamine formation in the medium in the presence of 20% NaCl was not completely inhibited. Furthermore, our results are in agreement with the previous report that *S. capitis* isolated from mustard pickle products had the capability of producing >1000 ppm of histamine at elevated NaCl content (10%) in TSBH medium (Kung et al., 2006).

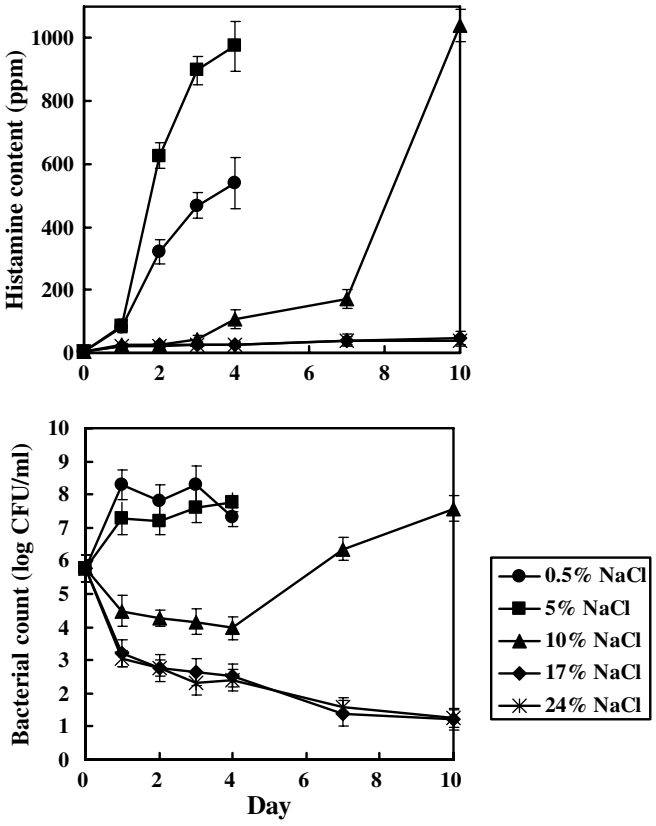

Fig. 1. The growth and histamine production of *S. capitis* strain D15-2 at 35 °C in TSBH medium containing 0.5%, 5%, 10%, 17% or 24% of NaCl.

4. Conclusion

This study to determine the safety of 26 douchi products sold in Taiwan showed that soybean douchi products had lower levels of APC than had black bean douchi products. The histamine contents in most of the douchi product exceeded the 5 mg/100 g USFDA guideline value, and 15.4% (4/26) of the tested samples contained >50 mg/100 g of histamine. Consumption of these douchi products with higher histamine content might lead to scombroid poisoning in consumers. While the isolates of *B. subtilis* and *S. pasteuri* were identified as weak histamine-formers, the *S. capitis* isolates were proven to be prolific histamine-formers

with a consistent ability to produce >500 ppm histamine at elevated NaCl content (10%) in TSBH medium.

Acknowledgements

The study was supported by the National Science Council, ROC (Contract No. NSC 94-2313-B-127-003).

References

Ababouch, L., Afilal, M. E., Rhafiri, S., & Busta, F. F. (1991). Identification of histamine-producing bacteria isolated from sardine (*Sardina pilchardus*) stored in ice and at ambient temperature (25 °C). *Food Microbiology, 8*, 127–136.

AOAC (1995). *Official methods of analysis of AOAC international* (16th ed.). Arlington, VA: AOAC International.

Arnold, S. H., & Brown, W. D. (1978). Histamine toxicity from fish products. *Advances in Food Research, 24*, 113–154.

Bjeldanes, L. F., Schutz, D. E., & Morris, M. M. (1978). On the aetiology of scombroid poisoning: cadaverine potentiation of histamine toxicity in the guinea pig. *Food and Cosmetics Toxicology, 16*, 157–159.

Chin, K. D. H., & Koehler, P. E. (1983). Identification and estimation of histamine, tryptamine, phenethylamine, and tyramine in soy sauce by thin-layer chromatography of dansyl derivatives. *Journal of Food Science, 48*, 1826–1828.

FDA (1992). *Bacteriological analytical manual*. Arlington, VA: AOAC International.

Hernandez-Herrero, M. M., Roig-Sagues, A. X., Rodriguez-Jerez, J. J., & Mora-Ventura, M. T. (1999). Halotolerant and halophilic histamine-forming bacteria isolated during the riping of salted anchovies. *Journal of Food Protection, 62*, 509–514.

Hesseltine, C. W., & Wang, H. L. (1972). Fermented soybean food products. In A. K. Smith & S. J. Circle (Eds.), *Soybeans: Chemistry and technology*. Westport, Conn USA: AVI Publishing, Co.

Hwang, D. F., Chang, S. H., Shiau, C. Y., & Chai, T. (1997). High-performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. *Journal of Chromatography B, 693*, 23–30.

Kim, S. H., Eun, J. B., Chen, T. Y., Wei, C. I., Clemens, R. A., & An, H. (2004). Evaluation of histamine and other biogenic amines and bacterial isolation in canned anchovies recalled by the USFDA. *Journal of Food Science, 69*, M157–M162.

Kuhnert, P., Capaul, S., Nicolet, J., & Frey, J. (1996). Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. *International Journal of Systematic Bacteriology, 46*, 1174–1176.

Kuhnert, P., Heyberger-Meyer, B., Nicolet, J., & Frey, J. (2000). Characterization of PaxA and Its operon: a cohemolytic RTX toxin determinant from pathogenic *Pasteurella aerogenes*. *Infection and Immunity, 68*, 6–12.

Kung, H. F., Tsai, Y. H., Hwang, C. C., Lee, Y. H., Hwang, J. H., Wei, C. I., et al. (2005). Hygienic quality and incidence of histamine-forming *Lactobacillus* species in natural and processed cheese in Taiwan. *Journal of Food Drug Analysis, 13*, 51–56.

Kung, H. F., Lee, Y. H., Teng, D. F., Hsieh, P. C., Wei, C. I., & Tsai, Y. H. (2006). Histamine formation by histamine-forming bacteria and yeast in mustard pickle products in Taiwan. *Food Chemistry, 99*, 579–585.

Kung, H. F., Tsai, Y. H., & Wei, C. I. (2007). Histamine and other biogenic amines and histamine-forming bacteria in miso products. *Food Chemistry, 101*, 351–356.

Lehane, L., & Olley, J. (2000). Histamine fish poisoning revisited. *International Journal of Systematic Microbiology, 58*, 1–37.

Lopez-Sabater, E. I., Rodriguez-Jerez, J. J., Hernandez-Herrero, M., Roig-Sagues, A. X., & Mora-Ventura, M. A. T. (1996). Sensory quality and histamine formation during controlled decomposition of tuna (*Thunnus thynnus*). *Journal of Food Protection, 59*, 167–174.

Niven, C. F., Jeffreg, M. B., & Corlett, D. A. (1981). Differential plating medium for quantitative detection of histamine-producing bacteria. *Applied and Environmental Microbiology, 41*, 321–322.

Nout, M. J. R., Ruiker, M. M. W., & Bouwmeester, H. M. (1993). Effect of processing conditions on the formation of biogenic amines and ethyl carbornate in soybean tempe. *Journal of Food Safety, 33*, 293–303.

Rodriguez-Jerez, J. J., Mora-Ventura, M. T., Lopez-Sabater, E. I., & Hernandez-Herrero, M. M. (1994). Histidine, lysine and ornithine decarboxylase bacteria in spanish salted semi-preserved anchovies. *Journal of Food Protection, 57*, 784–787.

Stratton, J. E., Hutkins, R. W., & Taylor, S. L. (1991). Biogenic amine in cheese and other fermented foods: a review. *Journal of Food Protection, 54*, 460–470.

Stratton, J. E., Hutkins, R. W., Summer, S. S., & Taylor, S. L. (1992). Histamine and histamine-producing bacteria in retail Swiss and low-salt cheeses. *Journal of Food Protection, 55*, 435–439.

Taylor, S. L. (1985). Histamine poisoning associated with fish, cheese, and other foods. Monograph, World Health Organization, Geneva. VPH/FOS/85.1.

Taylor, S. L. (1986). Histamine food poisoning: toxicology and clinical aspects. *Critical Reviews in Toxicology, 17*, 91–128.

Taylor, S. L. (1989). Histamine poisoning (scombroid fish poisoning): an allergy-like intoxication. *Clinical Toxicology, 27*, 225–240.

The 3M Products. (1999). *Manufacture's interpretation guide*. The 3M Microbiology Products, 3M Center, St. Paul, MN.

Tsai, Y. H., Kung, H. F., Lee, T. M., Lin, G. T., & Hwang, D. F. (2004). Histamine related hygienic qualities and bacteria found in popular commercial scombroid fish fillets in Taiwan. *Journal of Food Protection, 67*, 407–412.

Tsai, Y. H., Kung, H. F., Lin, Q. L., Hwang, J. H., Cheng, S. H., Wei, C. I., et al. (2005a). Occurrence of histamine and histamine-forming bacteria in kimchi products in Taiwan. *Food Chemistry, 90*, 635–641.

Tsai, Y. H., Lin, C. Y., Chang, S. C., Chen, H. C., Kung, H. F., Wei, C. I., et al. (2005b). Occurrence of histamine and histamine-forming bacteria in salted mackerel in Taiwan. *Food Microbiology, 22*, 461–467.

Tsai, Y. H., Lin, C. Y., Chien, L. T., Lee, T. M., Wei, C. I., & Hwang, D. F. (2006). Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria. *Food Chemistry, 98*, 64–70.

USFDA (US Food and Drug Administration) (2001). Scombrotoxin (histamine) formation. In *Fish and fishery products hazards and controls guide* (3rd ed., pp. 73–93). Washington, DC: Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood.

Yatsunami, K., & Echigo, T. (1991). Isolation of salt tolerant histamine-forming bacteria from commercial rice-bran pickle sardine. *Bulletin of the Japanese Society of Scientific Fisheries, 57*, 1723–1728.

Yatsunami, K., & Echigo, T. (1992). Occurrence of halotolerant and halophili histamine-forming bacteria in red meat fish products. *Bulletin of the Japanese Society of Scientific Fisheries, 58*, 515–520.

Yen, G. C. (1986). I. Determination of biogenic amines in fermented soybean foods by HPLC. *Journal of the Chinese Agricultural Chemical Society, 24*, 211–227.

Zhang, J. H., Tatsumi, E., Ding, C. H., & Li, L. T. (2006). Angiotensin I-converting enzyme inhibitory peptides in douchi, a Chinese traditional fermented soybean product. *Food Chemistry, 98*, 551–557.