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Histamine formation by histamine-forming bacteria and yeast in mustard pickle products in Taiwan

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Abstract

The occurrence of histamine, histamine-forming bacteria and yeast were tested in 37 mustard pickle products sold in both retail markets and supermarkets in southern Taiwan. Aerobic plate count (APC), total coliform, and *Escherichia coli* were also tested for microbiological quality. Salt content, pH value, titratable acidity and sulphite content were determined for quality of mustard pickle products. Only one retail market sample and one supermarket sample had 8.9 and 7.4 mg histamine per 100 g products, although the average content for each of the nine biogenic amines was less than 2 mg/100 g. Ten histamine-forming bacterial strains and 6 histamine-producing yeast strains capable of producing 8.7 to 1260 ppm of histamine in trypticase soy broth (TSB) supplemented with 1% L-histidine (TSBH) were identified as *Staphylococcus capitis* (four strains),*Staphylococcus pasteuri* (two strains), *Enterobacter cloacae* (four strains), *Candida glabrata* (two strains) and *Candida rugosa* (four strains). *S. capitis*, which was previously reported to be halotolerant, was a potent histamine-former, capable of producing more than 1000 ppm of histamine in TSBH in the presence of 0.5–10% NaCl. The numbers of the aerobic plate count (APC) in all samples were below the Taiwanese regulatory level of 5 log CFU/g. None of the samples contained total coliform or *E. coli*. The values of pH, salt content, titratable acidity and sulphite content in all samples ranged from 3.8% to 5.0%, 2.0% to 10.0%, 0.21% to 1.18% and <2.0–1876 ppm, respectively.

Keywords: Histamine; Histamine-forming bacteria; *Staphylococcus*; *Candida*; Mustard pickle

1. Introduction

Biogenic amines are basic nitrogenous compounds occurring in meat, fish, cheese, and wine products 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).

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 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; Kung et al., 2005; Roig-Sagues, Hernandez-Herrero, Lopez-Sabater, Rodriguez-Jerez, & Mora-Ventura, 1996;Stratton, Hutkins, & Taylor, 1991; Stratton, Hutkins, Summer, & Taylor, 1992; Tsai et al., 2005a).

An incident of histamine poisoning that occurred in Europe was reported to be caused by sauerkraut that had

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a histamine content at near the toxic dose level (Mayer & Pause, 1972). 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 and 0.7 mg/100 g in two separate sauerkraut juice samples. High contents of histamine and other biogenic amines were detected in some kimchi products (Tsai et al., 2005a); the possible source of the biogenic amines was suspected to be fermented fish products, such as fish sauce or shrimp paste, which were added as ingredients during kimchi fermentation. Mower, Bhagavan, Pontinus, and McDermott (1989) found low levels of tyramine in kimchi and commercial samples of Japanese pickled vegetables (urume-zuke). Although higher levels of tyramine were detected in homemade kimchi and urume-zuke than their commercial counterparts, the tyramine levels were still too low to cause foodborne disease.

Mustard pickle, a specially fermented vegetable product, is an important dietary dish of the Taiwanese people. Preparation of mustard pickle involves soaking of the whole mustard vegetable (*Brassica juncea*) in 14% (wt/vol) NaCl in an appropriate vessel, pressing mechanically on the up most layer, and fermentation of the mixture for 4 months. After fermentation, the outer leaves of the mustard pickle product are cut off and the remaining portion soaked in sulphite solution for bleaching (Lee, Fan, & Lee, 1990). Lee et al. (1990) studied the chemical contents of mustard pickle sold in Taiwan.

Since some of the fermented vegetable products contained histamine and other biogenic amines, it was suspected that the mustard pickle products may also contain biogenic amines. As no information was available concerning the hygienic quality of the mustard pickle, this research was undertaken by testing 37 mustard pickle products sold in both the retail markets and supermarkets in Taiwan to better understand their safety, including the contents of total coliform, *Escherichia coli*, histamine and sulphites, for the purpose of better protecting the consumers. To our knowledge, this is the first finding ever reported to demonstrate the occurrence of halotolerant histamine-forming bacteria and histamine-forming yeast in mustard pickle products.

2. Materials and methods

2.1. Materials

Twenty-three mustard pickle products sold in retail markets and 14 mustard pickle products sold in supermarkets were purchased from southern Taiwan from July to September, 2004. The retail market mustard pickle products were not packaged and were soaked in water at room temperature of 27–32 °C, while the supermarket mustard pickle products were vacuum packaged in plastic bags and stored at refrigerator temperature. After purchase, all mustard pickle samples were kept at 4 °C and immediately transported to the laboratory for analysis.

2.2. pH value, salt content, titratable acidity and sulphites

Samples of mustard pickle 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). The salt content and sulphite content of each sample were determined according to the AOAC procedures (1995). Titratable acidity, expressed as lactic acid, was determined by titration with 0.1 N NaOH to pH 8.1 (Zamora & Fields, 1979).

2.3. Microbiological analysis and isolation of histamine-forming bacteria

A 25 g portion of the mustard pickle 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 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 dilute of homogenates 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 mustard pickle samples were expressed as $\log_{10}$ colony forming units (CFU)/g.

To isolate histamine-forming bacteria, a 0.1 ml aliquot of the diluted sample was taken and 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 days at 35 °C, colonies with blue or purple colour on the plates were picked and further streaked on 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 mustard pickle samples were conducted using the methods described by FDA (1992).

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, 2000) and 600 bp of the 26S rDNA domain D1/D2 for yeast (Kurtzman, 2001; Kurtzman & Robnett, 1998). 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, Kuhnert, Heyberger-Meyer, Nicolet, & Frey, 2000). Bacterial cells were cultured overnight in 2 ml TSB at 35 °C and then centrifuged at 8000 rpm for 10 min. The cell pellet was washed and then resuspended in 0.5 ml of TE-buffer (10 mM Tris–HCl, 1 mM EDTA; pH 8.0). After the cells in the suspension were lysed by adding 38 μl of 20% sodium dodecyl sulfate (SDS), the solution was boiled for 20 min and the cellular debris discarded following centrifugation at 13,000*g* for 3 min. 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 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 (94 °C for 4 min) and a final extension (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, Calif. 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 previously described methods of Kurtzman and Robnett (1998) and Kurtzman (2001) for DNA extraction, PCR amplification of 26S rDNA domain D1/D2, and sequencing were followed to identity histamine-forming yeast. The sequences were analyzed with the BLAST (NCBI) for the identification of histamine-forming bacteria and yeast.

2.5. Biogenic amine analysis

Biogenic amines, including tryptamine hydrochloride (Trp), 2-phenylethylamine 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 solutions (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 mustard pickle 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 mustard pickle 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 mustard pickle 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, Darmstadt, 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.

A set of biogenic amine standards and their mixtures was 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 test samples.

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%, 3%, 10%, or 20% 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 5.70 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.

2.7. Statistical analysis

Pearson correlation was carried out to determine relationships between pH, salt content, titratable acidity, sulphite content, APC and histamine contents in the 37 samples collected from southern Taiwan. All statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 9.0 for windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

Values of the pH, salt content, titratable acidity, sulphite content, aerobic plate count (APC), total coliform, and *E. coli* in the mustard pickle products are presented in Table 1. The pH, salt content, and titratable acidity, in all samples, ranged from 3.8% to 5.0%, 2.0% to 10.0%, and 0.21% to 1.18%, respectively. These results are in agreement with those previously reported by Lee et al. (1990) for commercial mustard pickle in Taiwan. The average titratable acidities in mustard pickle obtained from retail markets (0.52%) were significantly lower than those samples from supermarkets (0.86%) ($p < 0.05$). The average contents of sulphites in mustard pickle obtained from retail markets and supermarkets were 535 and 551 ppm, respectively. Based on the Taiwanese regulatory standard of 30 ppm for sulphites, 82.6% (19/23) of the samples obtained in retail markets were unacceptable as compared to 85.7% (12/14) of the supermarket samples. The tested mustard pickle samples had <1.0–4.2 log CFU/g of APC, which are below the Taiwanese regulatory level of 5.0 log CFU/g. None of the samples used contained total coliform or *E. coli* (Table 1). In general, no correlation existed among the pH values, salt contents, titratable acidity, sulphites contents, APC, and histamine contents in the tested 37 samples. However, a negative correlation ($r = -0.60$, $p < 0.05$) was noted between the pH and titratable acidity values in the tested samples.

None of the 37 tested mustard pickle samples contained 2-phenylethylamine or spermidine (Table 2). Although the average content of each of the remaining seven biogenic amines in all samples was lower than 2.0 mg/100 g, one retail market sample and one supermarket sample had histamine levels (8.9 and 7.4 mg/100 g, respectively) greater than the 5.0 mg/100 g allowable limit suggested by the US Food and Drug Administration (USFDA, 2001). Therefore, based on the content of histamine in the test product, a 5.4% (2/37) unacceptable rate was obtained with these mustard pickle samples. These mustard pickle products did not contain as much histamine as the previously reported sauerkraut with an average content of 5.06 mg/100 g for 50 retail samples (Taylor et al., 1978a) and 4.07 mg/100 g in canned samples (Taylor et al., 1978b), or kimchi with an average content of 49.8 mg/100 g for 20 supermarket samples (Tsai et al., 2005a).

The mustard pickle samples produced 72 purple colonies on the differential HBI agar plates. Only 16 of them (22.2%) produced histamine in TSBH medium. The remaining 56 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 potential histamine-producers that were isolated from Niven's medium were actually false-positives when grown in a histidine-supplemented culture 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; Chen & Malison, 1987; Tsai, Kung, Lee, Lin, & Hwang, 2004). These 16 histamine-forming isolates were identified as *Staphylococcus capitis* (four strains), *Staphylococcus pasteuri* (two strains), *Enterobacter cloacae* (four strains), *Candida glabrata* (two strains) and *Candida rugosa* (four strains) by 16S rDNA sequencing for bacteria or 26S rDNA sequencing for yeast with PCR amplification (Table 3). All of them produced substantial amounts of histamine (8.7–1260 ppm) in TSBH medium. Some of these strains produced different amounts of putrescine, cadaverine, spermidine, spermine, and tyramine through the actions of their decarboxylase enzymes on various amino acids that existed in the TSBH medium (Table 3).

The four *S. capitis* strains isolated from the test products were potent histamine-formers, producing 854–1260 ppm of histamine in TSBH; whereas the two *S. pasteuri* strains were weak histamine-formers and produced 20.3 and 31.8 ppm of histamine (Table 3). *Staphylococcus* spp. were the most frequently reported histamine-formers in fermented salted fish, accounting for nearly 50% of histamine-forming microorganisms, and showed powerful

Table 1
Values of the pH, salt content, titratable acidity, sulphites, aerobic plate count (APC), total coliform (TC), and *E. coli* in tested mustard pickle products

Source of samples	No. of samples	pH	Salt content (%)	Titratable acidity (%)	Sulphites (ppm)	APC (log CFU/g)	TC (MPN/g)	*E. coli* (MPN/g)
Retail market	23	3.8–4.7 (4.1 ± 0.3)[a]A	2.0–8.0 (4.9 ± 1.4)A	0.21–0.95 (0.52 ± 0.20)A	ND[b]–1876 (535 ± 453)A	<1.0– 3.5 (3.0 ± 0.7)A	<3	<3
Supermarket	14	3.8–5.0 (4.2 ± 0.3)A	2.0–10.0 (5.1 ± 2.3)A	0.39–1.18 (0.86 ± 0.21)B	ND–1714 (551 ± 461)A	<1.0–4.2 (2.6 ± 1.1)A	<3	<3

[a] Mean ± SD. Values in the same column with different letters are statistically different ($p < 0.05$).
[b] ND, not detected (sulphites level less than 2 ppm).

Table 2
The levels of biogenic amines in tested mustard pickle products

Source	No. of samples	Range of amine level (mg/100 g)								
		Put[a]	Cad	Try	Phe	Spd	Spm	His	Tyr	Agm
Retail market	23	ND[b]–1.4 (0.12 ± 0.38)[c]	ND–1.7 (0.10 ± 0.36)	ND–7.2 (0.33 ± 1.54)	ND	ND	ND–9.5 (0.43 ± 2.03)	ND–8.9 (1.00 ± 2.65)	ND–4.3 (0.32 ± 1.07)	ND–3.7 (0.17 ± 0.79)
Supermarket	14	ND–13.1 (1.07 ± 2.03)	ND–2.4 (1.04 ± 1.76)	ND–15.5 (1.25 ± 2.56)	ND	ND	ND	ND–7.4 (0.45 ± 1.85)	ND–2.7 (0.17 ± 0.68)	ND

[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
Histamine and other biogenic amines (ppm) produced in culture broth by histamine-forming bacteria and yeast isolated from tested mustard pickle products

Strain	Histamine former	His[a]	Put	Cad	Spd	Spm	Tyr
TS19-1	*Staphylococcus capitis*	1260	ND[b]	ND	ND	ND	ND
TS19-2	*S. capitis*	854	ND	ND	ND	48.6	ND
TS19-3	*S. capitis*	940	ND	ND	ND	ND	ND
TS19-4	*S. capitis*	883	ND	6.3	ND	19.6	ND
MH16-1	*S. pasteuri*	20.3	ND	ND	ND	ND	ND
MH16-2	*S. pasteuri*	31.8	ND	ND	ND	ND	ND
TS28-1	*Enterobacter cloacae*	11.3	86.7	19.8	ND	ND	ND
TS28-2	*E. cloacae*	25.6	17.8	25.8	ND	ND	ND
TS29-1	*E. cloacae*	11.3	25.3	48.1	ND	ND	ND
TS-29-2	*E. cloacae*	8.7	17.1	23.3	ND	ND	ND
MT8-3	*Candida glabrata*	18.8	ND	ND	7.8	ND	ND
MT19-4	*C. glabrata*	9.2	ND	ND	ND	ND	ND
MH15-1	*C. rugosa*	23.5	ND	ND	ND	22.4	4.2
MH15-5	*C. rugosa*	16.4	ND	ND	ND	ND	ND
MT15-7	*C. rugosa*	41.7	ND	2.7	10.9	18.0	9.5
MT23-2	*C. rugosa*	9.6	ND	ND	ND	ND	ND

[a] His, histamine; Put, Putrescine; Cad, cadaverine; Spd, spermidine; Spm, spermine and Tyr, tyramine.
[b] ND, not detected (amine level less than 1 ppm).

histamine-forming activity (Yatsunami & Echigo, 1991, 1992). *Staphylococcus epidermidis* and *Staphylococcus capitis*, isolated from salted Spanish anchovies, produced more than 1000 ppm and 400 ppm of histamine, respectively (Hernandez-Herrero, Roig-Sagues, Rodriguez-Jerez, & Mora-Ventura, 1999).

In this study, we also found that the four *E. cloacae* isolates from the test samples were weak histamine-formers, producing 8.7–25.6 ppm of histamine in TSBH medium. These findings were in agreement with the findings of Rodriguez-Jerez, Mora-Ventura, Lopez-Sabater, and Hernandez-Herrero (1994a, 1994b), Hernandez-Herrero et al. (1999), and Lopez-Sabater et al. (1996) that *E. cloacae* isolated from salted anchovies and tuna (*Thunnus thynnus*), were weak histamine-formers. Previously, Tsai et al. (2004, 2005b) also demonstrated that *E. cloacae,* isolated from commercial scombroid fish fillets and salted mackerel sold in Taiwan were weak histamine-formers, producing low levels of histamine in TSBH medium.

Although the yeasts, *C. glabrata* and *C. rugosa*, were never identified as histamine-formers, they accounted for 37.5% (6/16) of the total histamine-forming isolates in this study. Specifically, the *C. rugosa* strain MT15-7 produced 41.7 ppm of histamine in TSBH medium. To our knowledge, this is the fist report ever published to indicate that yeast, such as, *Candida* spp., was able to produce low levels of histamine. It is rarely reported that histamine-forming bacteria were isolated from fermented vegetables, except for *Lactobacillus* spp. and *Brevibacillus brevis* as weak histamine-formers from kimchi products (Tsai et al., 2005a). These findings indicate that the spectrum of histamine-forming bacteria in fermented vegetable products can change, depending on the vegetable species, their preparation procedures, salt contents and storage conditions.

The growth and histamine production of *S. capitis* strain TS19-1 in TSBH medium containing 0.5%, 3%, 10%, or 20% of NaCl are shown in Fig. 1. At 0.5% and 3% NaCl, histamine production was accelerated, along with bacterial growth; the histamine levels exceeded 1000 ppm in two days. At 10% NaCl, the bacterial counts increased gradually and eventually reached

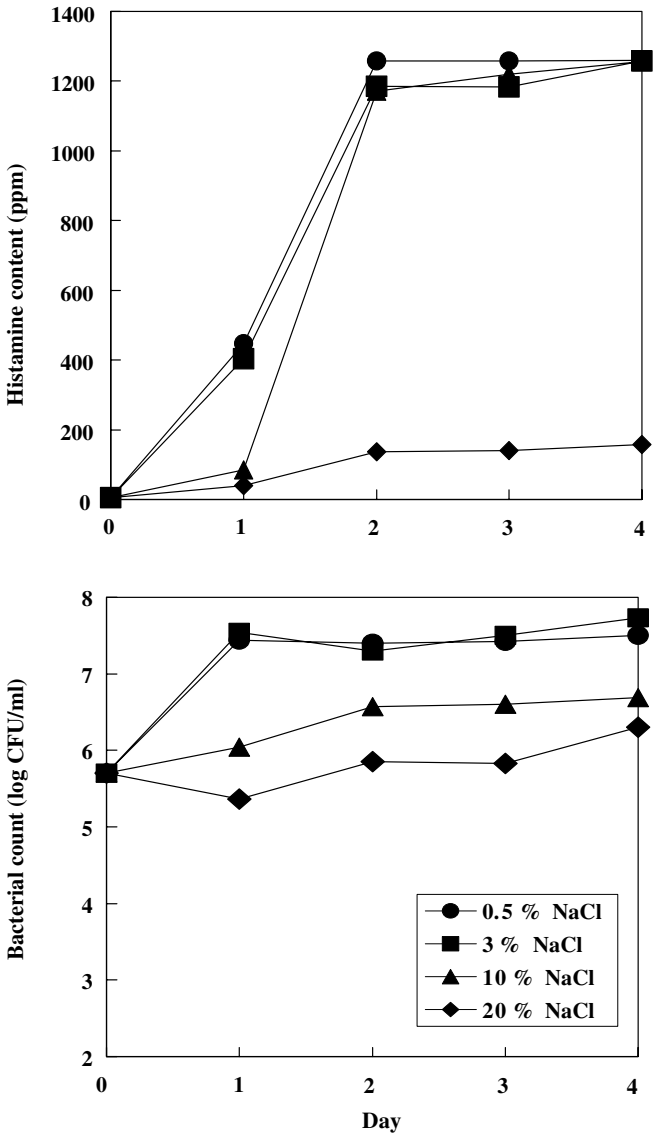

Fig. 1. Histamine production and growth of *Staphylococcus capitis* strain TS19-1 at 35 °C in TSBH medium containing 0.5%, 3%, 10%, or 20% of NaCl.

at about 6.7 log CFU/ml after four days. The levels of histamine were below 100 ppm on day one of incubation, but increased to above 1000 ppm on day two. However, when the NaCl content in TSBH medium was increased to 20%, the growth of the bacteria was inhibited and histamine formation increased only slightly, reaching about 158 ppm in four days. Hernandez-Herrero et al. (1999) reported that NaCl concentrations, in the range of 0.5–10%, had a stimulatory effect on histamine formation of *S. capitis* and *S. epidermidis*, whereas levels of NaCl in excess of 20% inhibited their growth and histamine formation. Therefore, our results on histamine formation by *S. capitis* strain TS19-1 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.

4. Conclusion

This study, to determine the safety of 37 mustard pickle products sold in Taiwan, showed that they had satisfactory bacterial quality with no total coliform, no *E. coli*, and a legally allowable content of APC. However, this study found that 82% of the test samples failed to meet Taiwanese regulatory standards for sulphite content. The average content of each of the nine tested biogenic amines in these samples was less than 2 mg/100 g, although two of them had histamine contents of 8.9 and 7.4 mg/100 g. While the isolates of *S. pasteuri, E. cloacae, C. glabrata* and *C. rugosa* were identified to be weak histamine-formers, the *S. capitis* isolates were proven to be prolific histamine-formers with a consistent ability to produce >1000 ppm of histamine at elevated NaCl concentration to 10% in TSBH medium.

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