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Inhibitory Effect of *Melastoma candidum* D. Don Acetone Extract on Foodborne Pathogenic Bacteria Survival in Food Products

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ABSTRACT

Melastoma candidum D. Don, a Taiwanese folk medicinal plant, has high levels of antibacterial and bactericidal activity. Our aim was to determine whether and to what extent an acetone extract of this plant inhibits the growth of foodborne pathogenic bacteria. *M. candidum* acetone extract had marked inhibitory effect on test bacteria introduced into sliced pork, which was then stored at 4°C. At the end of storage (day 12), the bacterial concentrations dropped by 1.59 to 2.91 log CFU/g compared with the control. In steamed rice stored at 30°C, a 0.2% extract decreased initial (before storage) concentrations of *Bacillus cereus* from 2.01 log CFU/g to an undetectable level, which remained for at least 24 h. After 72 to 168 h of storage, test bacterial concentrations were reduced by 2.59 to 5.66 log CFU/g. In fresh noodles stored at 30°C, both initial and final bacterial concentrations were decreased. At the end of storage (72 to 168 h), test bacteria concentrations were reduced by 1.85 to 2.88 log CFU/g. Overall, *M. candidum* acetone extract had an inhibitory effect on foodborne pathogenic bacteria in different food model systems.

Microorganismal contamination of food is the most frequent cause of food poisoning. *Salmonella, Listeria* spp., *Campylobacter* spp., *Escherichia coli* O157:H7, and *Staphylococcus aureus* have been reported to be the most common pathogens isolated from raw meat *(1, 4, 15, 28),* whereas *Vibrio* spp., *Aeromonas* spp., and *Clostridium botulinum* frequently have been isolated from seafoods *(8, 24). Bacillus cereus* has been found in rice-based foods *(10, 11),* and *Listeria monocytogenes, Salmonella, Shigella* spp., *Yersinia enterocolitica,* and *S. aureus* have been found on fresh fruits *(14). E. coli, Listeria* spp., *Salmonella,* and *S. aureus* have been isolated from 3,391 ready-to-eat (RTE) foods including cream cakes, custards, and egg mayonnaise sandwiches *(18).* Foodborne pathogenic bacteria including *S. aureus, Salmonella,* and *B. cereus* have been detected in fresh noodles *(12, 26, 30).*

Many natural substances have been investigated for their inhibitory effects on foodborne pathogenic bacteria in food products. The addition of 15% raisins to the formulation of beef jerky had a marked inhibitory effect on pathogenic bacteria *(5).* The presence of diallyl sulfide and diallyl disulfide (garlic-derived organosulfur compounds) in ground beef significantly reduced the total aerobic bacteria *(32).* The addition of antimicrobial protein derived from porcine leukocytes to ground ham and sausage posed a significant hurdle to formation of viable bacterial colonies *(31).* Organic acids such as acetic, lactic, and citric acids are frequently used to decontaminate meat *(4, 13, 21, 23).* Treatment with lactic acid, clove oil, and vitamin C extended the display life of buffalo meat steaks at 4°C *(21).* Lactoperoxidase treatment inhibited the growth of foodborne pathogenic bacteria in beef cubes *(9),* and growth of *L. monocytogenes* on chicken frankfurters was inhibited by the presence of 1 to 2% clove oil *(20).* The shelf life of fresh noodle products has been extended by Maillard reaction products *(12).*

Melastoma candidum D. Don is a plant of the Melastomataceae family that grows throughout southern China, Taiwan, Japan, and the Philippines *(17).* This folk medicinal plant is often used in Taiwan to eliminate atasis, clean the serum of toxins, treat traumatic injury, and cure bacterial dysentery *(17).* The three active compounds (castalagin, procyanidin B-2, and helichrysoside) isolated from the leaf have been reported to lower blood pressure through decreasing the sympathetic tone and causing direct vasodilatation in adult hypertensive rats *(6).* Four leaf-isolated flavonoids (quercitrin, isoquercitrin, rutin, and quercetin) exhibited an inhibitory effect on monoamine oxidase B *(16).*

In our previous study, the acetone extract of *M. candidum* had high levels of antibacterial and bactericidal activity against foodborne pathogenic bacteria and a broad effective pH range and was very stable under various temperature and pH conditions. In the present study, the usefulness of this extract as a preservative and inhibitor of foodborne pathogenic bacteria was tested in food models: sliced pork stored at 4°C and steamed rice and fresh noodles stored at 30°C.

MATERIALS AND METHODS

***M. candidum* acetone extract preparation.** Samples of *M. candidum* were collected and identified by N. Y. Chiu (China Medical University, Taichung, Taiwan). A sample of this plant (voucher specimen no. 250482) has been deposited at the Institute of Ecology and Evolutionary Biology (College of Life Sciences,

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National Taiwan University, Taipei, Taiwan). The dried mixed stems and roots of *M. candidum* were used for the preparation of this herbal extract. Acetone (200 ml) was added to 30 g of ground specimen that was passed through a 60-mesh screen, stirred at room temperature for 1 h, and then centrifuged at 9,000 rpm for 15 min at 4°C. The residue was extracted twice more with 200 ml of acetone each time. All the supernatants were combined and concentrated to dryness in a rotary vacuum evaporator at less than 40°C. Before use, the dried extract was dissolved with propylene glycol and diluted with peptone-saline buffer (0.1% peptone and 0.85% sodium chloride) for treatment of sliced pork or with 10% propylene glycol for treatment of steamed rice and fresh noodles.

Bacteria strains and cultivation. *S. aureus* BCRC 12657, *B. cereus* BCRC 10603, and *Vibrio parahaemolyticus* BCRC 10806 were obtained from the Bioresources Collection and Research Center (Hsinchu, Taiwan). Each bacterial suspension (100 μl, 0.5 to 1.0 × 10^6 CFU/ml) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) and then incubated at 37°C for 12 h. For *V. parahaemolyticus* culture, the TSB was supplemented with 2.5% sodium chloride.

Sliced pork treatment. Frozen sliced pork (25-g slices, 6 by 4 by 1 cm each) from the local raw meat processing factory was transported frozen to the laboratory and stored at −30°C until use. Before use, the slices were thawed in a 45°C water bath for 15 min, immersed in a 1-liter beaker containing the *S. aureus* or *B. cereus* suspensions (0.5 to 1.0 × 10^7 CFU/ml) at room temperature for 1 min, and then placed in a laminar flow unit at room temperature for 30 min to facilitate bacterial attachment to the pork. Both inoculated and uninoculated pork slices were then immersed in 0.2 to 1.6% (wt/vol) *M. candidum* acetone extracts at room temperature for 60 min. As a control (0% extract), pork slices were immersed in peptone-saline buffer at room temperature for 60 min. After immersion, the slices were packed in aseptic bags and stored at 4°C for 12 days. At various intervals during storage, concentrations of viable cells were determined from inoculated pork for *S. aureus* and *B. cereus* and from uninoculated pork for total aerobic bacteria. Experiments were performed in triplicate.

Steamed rice treatment. Polished rice (200 g) was steamed in 240 ml of water. After cooling, *M. candidum* acetone extracts (16.7 ml of 1.2 to 4.8% extracts, wt/vol, for each 100 g of steamed rice) and *S. aureus* BCRC 12657, *B. cereus* BCRC 10603, or *V. parahaemolyticus* BCRC 10806 suspensions (0.4 ml of 10^5 CFU/ml concentrations for each 100 g of steamed rice) were immediately added and mixed well. The final rice mixture contained 0.2 to 0.8% extracts and initial bacterial concentrations of 0.8 to 4.0 × 10^2 CFU/g. Samples of inoculated and uninoculated steamed rice were packed into an aseptic bags (25 g per bag) and stored at 30°C for 72 to 168 h. At various intervals during storage, concentrations of viable cells were determined from inoculated rice for *S. aureus, B. cereus,* and *V. parahaemolyticus* and from uninoculated rice for total aerobic bacteria. In the steamed rice control samples (0% extract), extract was replaced by the same amount of 10% propylene glycol. Experiments were performed in triplicate.

Fresh noodle treatment. Sixty milliliters of 6% sodium chloride and 26 ml of 2 to 8% (wt/vol) *M. candidum* acetone extracts were added to 200 g of wheat flour to make up fresh noodles, for a final concentration of 0.2 to 0.8% (wt/wt) extracts. *S. aureus* BCRC 12657, *B. cereus* BCRC 10603, or *V. parahaemolyticus* BCRC 10806 suspensions (0.4 ml of 10^5 CFU/ml for each 100 g of fresh noodles) were added to the fresh noodles and mixed well to produce initial bacterial concentrations of 0.8 to 4.0 × 10^2 CFU/g. Samples of inoculated and uninoculated noodles were packed into aseptic bags (25 g per bag) and stored at 30°C for 72 to 168 h. At various intervals during storage, concentrations of viable cells were determined from inoculated noodles for *S. aureus, B. cereus,* and *V. parahaemolyticus* and from uninoculated

TABLE 1. *Concentrations of viable test bacteria in sliced pork treated with acetone extracts of* Melastoma candidum *D. Don and stored at 4°C*

Extract treatment	Concn (log CFU/g)[a]				
	0 days	3 days	6 days	9 days	12 days
Total aerobic plate count					
Control	5.51 ± 0.03 A	7.84 ± 0.00 A	8.32 ± 0.09 A	9.71 ± 0.05 A	10.81 ± 0.02 A
0.2%	5.31 ± 0.03 B	7.53 ± 0.10 B	7.84 ± 0.03 B	9.05 ± 0.04 B	9.88 ± 0.04 B
0.4%	5.19 ± 0.02 C	6.80 ± 0.01 C	7.35 ± 0.08 C	8.37 ± 0.06 C	9.52 ± 0.04 C
0.8%	5.01 ± 0.05 D	6.46 ± 0.13 D	6.96 ± 0.05 D	7.95 ± 0.04 D	8.67 ± 0.11 D
1.6%	4.56 ± 0.05 E	6.05 ± 0.03 E	6.14 ± 0.06 E	7.24 ± 0.07 E	7.90 ± 0.04 E
Staphylococcus aureus BCRC 12657					
Control	5.45 ± 0.08 A	7.82 ± 0.03 A	9.49 ± 0.07 A	9.67 ± 0.01 A	9.72 ± 0.04 A
0.2%	5.13 ± 0.03 B	7.32 ± 0.28 B	9.17 ± 0.07 B	9.39 ± 0.06 B	9.61 ± 0.01 B
0.4%	4.77 ± 0.03 C	6.76 ± 0.07 C	8.74 ± 0.03 C	8.87 ± 0.05 C	9.22 ± 0.03 C
0.8%	4.38 ± 0.05 D	6.26 ± 0.06 D	8.01 ± 0.06 D	8.21 ± 0.06 D	8.75 ± 0.07 D
1.6%	3.98 ± 0.06 E	5.78 ± 0.09 E	7.02 ± 0.05 E	7.57 ± 0.03 E	8.13 ± 0.06 E
Bacillus cereus BCRC 10603					
Control	5.35 ± 0.02 A	5.74 ± 0.10 A	6.51 ± 0.17 A	7.69 ± 0.03 A	7.95 ± 0.12 A
0.2%	4.92 ± 0.06 B	5.28 ± 0.09 B	6.09 ± 0.06 B	6.92 ± 0.13 B	7.55 ± 0.06 B
0.4%	4.48 ± 0.11 C	5.08 ± 0.01 C	5.73 ± 0.12 C	6.66 ± 0.03 C	7.14 ± 0.17 C
0.8%	4.32 ± 0.09 D	4.85 ± 0.01 D	5.40 ± 0.08 D	6.06 ± 0.16 D	6.54 ± 0.02 D
1.6%	3.92 ± 0.02 E	4.45 ± 0.05 E	4.72 ± 0.02 E	5.22 ± 0.04 E	5.99 ± 0.10 E

[a] Values are means ± standard deviations ($n = 3$). Means followed by different letters are significantly different ($P < 0.05$).

TABLE 2. *Concentrations of viable test bacteria in steamed rice treated with acetone extracts of* Melastoma candidum *D. Don and stored at 30°C*

Extract treatment	Concn (log CFU/g)[a]					
	0 h	6 h	12 h	24 h	36 h	48 h
Total aerobic plate count						
Control	0 ± 0 A		1.97 ± 0.08 A	3.59 ± 0.05 A	5.11 ± 0.05 A	6.18 ± 0.12 A
0.2%	0 ± 0 A		1.28 ± 0.10 B	2.60 ± 0.09 B	2.91 ± 0.19 B	3.48 ± 0.08 B
0.4%	0 ± 0 A		1.06 ± 0.11 C	2.09 ± 0.12 C	2.73 ± 0.23 B	3.54 ± 0.33 B
0.8%	0 ± 0 A		0 ± 0 D	0.80 ± 0.16 D	1.74 ± 0.27 C	2.29 ± 0.12 C
Staphylococcus aureus BCRC 12657						
Control	2.40 ± 0.17 A	5.54 ± 0.13 A	8.54 ± 0.08 A	9.58 ± 0.04 A	10.15 ± 0.12 A	10.52 ± 0.05 A
0.2%	2.31 ± 0.10 A	3.81 ± 0.09 B	5.12 ± 0.13 B	6.06 ± 0.14 B	7.03 ± 0.18 B	8.24 ± 0.15 B
0.4%	1.79 ± 0.11 B	2.98 ± 0.10 C	4.64 ± 0.10 C	5.28 ± 0.15 C	6.17 ± 0.10 C	7.05 ± 0.17 C
0.8%	0 ± 0 C	1.92 ± 0.08 D	3.15 ± 0.12 D	3.43 ± 0.11 D	4.27 ± 0.13 D	5.13 ± 0.15 D
Bacillus cereus BCRC 10603						
Control	2.01 ± 0.13 A	3.86 ± 0.07 A	5.63 ± 0.37 A	7.46 ± 0.13 A	7.82 ± 0.16 A	8.82 ± 0.14 A
0.2%	0 ± 0 B	0 ± 0 B	2.57 ± 0.17 B	4.73 ± 0.09 B	5.46 ± 0.24 B	5.69 ± 0.11 B
0.4%	0 ± 0 B	0 ± 0 B	2.31 ± 0.09 C	4.22 ± 0.20 C	5.00 ± 0.25 C	5.45 ± 0.20 C
0.8%	0 ± 0 B	0 ± 0 B	0 ± 0 D	0 ± 0 D	2.63 ± 0.07 D	4.71 ± 0.11 D
Vibrio parahaemolyticus BCRC 10806						
Control	1.78 ± 0.10 A	2.98 ± 0.02 A	4.87 ± 0.07 A	6.82 ± 0.18 A	7.06 ± 0.06 A	7.29 ± 0.09 A
0.2%	1.61 ± 0.09 B	2.69 ± 0.09 B	3.36 ± 0.32 B	4.96 ± 0.19 B	5.76 ± 0.04 B	6.02 ± 0.05 B
0.4%	0 ± 0 C	1.98 ± 0.07 C	2.89 ± 0.05 C	4.45 ± 0.08 C	4.96 ± 0.07 C	5.58 ± 0.29 C
0.8%	0 ± 0 C	1.59 ± 0.09 D	2.44 ± 0.12 D	3.29 ± 0.04 D	3.75 ± 0.04 D	3.99 ± 0.08 D

[a] Values are means ± standard deviations ($n = 3$). Means followed by different letters are significantly different ($P < 0.05$).

noodles for total aerobic bacteria. In the noodle control samples (0% extract), extract was replaced by the same amount of 10% propylene glycol. Experiments were performed in triplicate.

Sample preparation for determination of bacterial survival. To evaluate bacterial survival, one sample bag (25 g) of sliced pork, steamed rice, or fresh noodles was homogenized in 225 ml of phosphate buffer solution (3.125×10^{-4} M, pH 7.2), and the mixture was diluted 10-fold with phosphate buffer solution.

Bacterial counts determination. The concentrations of total aerobes, *S. aureus,* and *B. cereus* were determined according to Chinese National Standards methods *(2).* An aliquot (1 ml) of sample suspension was added to a culture to form a plate, the plate was incubated at 37°C for 48 h (aerobic bacteria and *S. aureus*) or 30°C for 24 h (*B. cereus*), and colonies were counted. The media used (Difco, Becton Dickinson) were plate count agar for total aerobes, Baird-Parker medium enriched with egg yolk and tellurite for *S. aureus,* mannitol–egg yolk–polymyxin agar enriched with egg yolk and polymyxin B sulfate for *B. cereus,* and thiosulfate–citrate–bile salts–sucrose agar for *V. parahaemolyticus.*

Statistical analysis. Data for concentrations of viable aerobic bacteria, *S. aureus, B. cereus,* and *V. parahaemolyticus* were subjected to analysis of variance, and a *t* test was used to identify significant differences among the means ($P < 0.05$).

RESULTS AND DISCUSSION

Concentrations of viable test bacteria in sliced pork stored at 4°C. The effect of *M. candidum* acetone extract on the growth of foodborne pathogenic bacteria in sliced pork is shown in Table 1. All treatments (0.2 to 1.6% extracts) significantly inhibited the growth of total aerobes ($P < 0.05$). During the 12-day storage period, the growth of total aerobes decreased with increasing extract concentrations. All extracts (0.2 to 1.6%) reduced total aerobic plate counts by 0.20 to 0.95 log CFU/g on day 0 (before storage) and by 0.93 to 2.91 log CFU/g at the end of storage (day 12) compared with the controls.

S. aureus growth in sliced pork was inhibited by treatment with 0.2 to 1.6% extracts (Table 1). During the 12-day storage period, the growth of *S. aureus* significantly decreased ($P < 0.05$) with increasing extract concentration. All extracts (0.2 to 1.6%) reduced *S. aureus* concentrations by 0.32 to 1.47 log CFU/g on day 0 (before storage) and by 0.11 to 1.59 log CFU/g at the end of storage (day 12) compared with the controls.

Extracts at all concentrations (0.2 to 1.6%) significantly inhibited survival of *B. cereus* in sliced pork ($P < 0.05$) (Table 1). This effect increased with increasing extract concentration. All extracts (0.2 to 1.6%) reduced *B. cereus* concentrations by 0.43 to 1.43 log CFU/g on day 0 (before storage) and by 0.40 to 1.96 log CFU/g at the end of storage (day 12) compared with the controls.

Ogden et al. *(22)* found that a mixture of 2.26% propionic acid and 0.76% ascorbic acid reduced *Pseudomonas* concentrations by 5.2 log CFU/g in minced pork stored at 4°C. The aerobic plate counts in refrigerated pork loin were reduced 2.5 log CFU/g by treatment with 2% sodium lactate and 0.2% sodium tripolyphosphate *(3).* When an antimicrobial protein (160 μg/g) from porcine leukocytes was mixed with fresh ground ham and the mixture was stored

TABLE 2. *Extended*

Concn (log CFU/g)[a]					
60 h	72 h	96 h	120 h	144 h	168 h
	8.31 ± 0.15 A	8.59 ± 0.05 A	9.21 ± 0.23 A	9.30 ± 0.14 A	10.00 ± 0.18 A
	4.05 ± 0.36 B	5.40 ± 0.25 B	6.99 ± 0.33 B	7.48 ± 0.11 B	8.28 ± 0.24 B
	4.02 ± 0.38 B	5.08 ± 0.27 C	6.04 ± 0.24 C	7.14 ± 0.17 C	8.15 ± 0.21 B
	2.85 ± 0.14 C	4.52 ± 0.27 D	5.51 ± 0.11 D	6.37 ± 0.15 D	7.41 ± 0.16 C
10.61 ± 0.05 A	10.97 ± 0.06 A				
8.37 ± 0.05 B	8.35 ± 0.10 B				
7.15 ± 0.05 C	7.17 ± 0.05 C				
5.32 ± 0.04 D	5.31 ± 0.08 D				
9.66 ± 0.04 A	9.78 ± 0.04 A				
6.64 ± 0.05 B	6.82 ± 0.07 B				
5.71 ± 0.13 C	5.81 ± 0.08 C				
4.72 ± 0.05 D	4.75 ± 0.04 D				
7.46 ± 0.06 A	7.52 ± 0.12 A				
6.02 ± 0.09 B	6.17 ± 0.12 B				
5.68 ± 0.11 C	5.78 ± 0.06 C				
4.01 ± 0.10 D	4.07 ± 0.10 D				

at 15°C, *S. aureus* concentrations were reduced by 3.9 log CFU/g and *E. coli* concentrations were reduced by 3.3 log CFU/g after 6 h *(31).* Addition of 0.6% chitosan to minced pork stored at 4°C for 18 days resulted in reductions of 1.5 and 2.5 log CFU/g for total aerobes and lactic acid bacteria, respectively *(27).* When 0.6% chitosan and 170 ppm sulfite was mixed with fresh pork sausages and the mixture stored at 4°C, total aerobe and lactic acid bacteria concentrations were reduced by 2.4 and 3.4 log CFU/g, respectively, after 24 days *(25).* Free or encapsulated nisin (1,000 IU/g) added to half-lean ground beef inoculated with *S. aureus* reduced *S. aureus* concentrations by 1.4 log CFU/g after 14 days of storage at 4°C *(19).* Lactoperoxidase treatment of beef cubes inoculated with pathogenic bacteria such as *S. aureus, Salmonella* Typhimurium, *L. monocytogenes,* and *E. coli* and stored at 37°C for 24 h reduced counts by 0.7 to 1.1 log CFU/cm^2. Lactoperoxidase treatment of the same beef cubes inoculated with *Pseudomonas aeruginosa* and *Y. enterocolitica* reduced counts by 4.1 and 2.6 log CFU/cm^2, respectively *(9).* Treatment with 2% lactic acid in combination with 0.1% clove oil reduced aerobic plate counts by 3.5 log CFU/g in buffalo steaks stored at 4°C *(21).* The inhibitory effect of the *M. candidum* extract on growth of bacteria in sliced pork stored at 4°C was similar to that described by some authors *(9, 22, 31)* and less than that described by others *(3, 19, 25, 27).*

Concentrations of viable test bacteria in steamed rice stored at 30°C. The growth of total aerobes in steamed rice was significantly inhibited ($P < 0.05$) by all extract concentrations (0.2 to 0.8%) during the 168-h storage period (Table 2). The 0.8% extract completely inhibited growth of total aerobes during the first 12 h of storage. After 168 h of storage, all extracts (0.2 to 0.8%) reduced concentrations of viable total aerobes by 1.72 to 2.59 log CFU/g. The effect of 0.2 and 0.4% extracts was similar ($P < 0.05$) during most of the storage period.

The growth of *S. aureus* in steamed rice was greatly inhibited by all extract concentrations during the 72-h storage period (Table 2). Before storage (hour 0), the 0.8% treatment decreased *S. aureus* concentrations from 2.40 ± 0.17 log CFU/g (control) to an undetectable level. After 72 h of storage, concentrations of viable *S. aureus* were reduced by 5.66 log CFU/g, and growth was significantly inhibited by both the 0.2 and 0.4% extracts during the 72-h storage period ($P < 0.05$).

The growth of *B. cereus* in steamed rice was inhibited by the extracts (Table 2). Initially, 0.2% extract decreased *B. cereus* concentrations from 2.01 ± 0.13 log CFU/g (control) to an undetectable level, and this level was maintained for at least 6 h. Significant inhibition of *B. cereus* growth ($P < 0.05$) persisted during the 72-h storage period. At the end of storage, concentrations of viable *B. cereus* were reduced by 2.96 to 5.03 log CFU/g by all extract concentrations.

The growth of *V. parahaemolyticus* in steamed rice also was significantly reduced ($P < 0.05$) by all extract concentrations during the 72-h storage period (Table 2). The 0.4% extract decreased the concentrations from 1.78 ± 0.10 log CFU/g (control) to an undetectable level at 0 h of storage. After 72 h of storage, the 0.2 to 0.8% extracts reduced concentrations by 1.35 to 3.45 log CFU/g.

Rice-based RTE foods containing meat, seafood, veg-

TABLE 3. *Concentrations of viable test bacteria in fresh noodles treated with acetone extracts of* Melastoma candidum *D. Don and stored at 30°C*

Extract treatment	Concn (log CFU/g)[a]					
	0 h	6 h	12 h	24 h	36 h	48 h
Total aerobic plate count						
Control	1.25 ± 0.16 A		2.46 ± 0.14 A	4.42 ± 0.11 A	5.50 ± 0.09 A	6.26 ± 0.06 A
0.2%	0.94 ± 0.15 B		2.28 ± 0.10 B	2.88 ± 0.03 B	4.30 ± 0.06 B	5.48 ± 0.05 B
0.4%	0.93 ± 0.07 BC		1.73 ± 0.15 C	2.64 ± 0.14 C	4.10 ± 0.01 C	5.14 ± 0.08 C
0.8%	0.80 ± 0.15 C		1.35 ± 0.12 D	2.31 ± 0.09 D	3.33 ± 0.06 D	4.68 ± 0.08 D
Staphylococcus aureus BCRC 12657						
Control	1.92 ± 0.04 A	2.97 ± 0.12 A	4.57 ± 0.05 A	5.08 ± 0.09 A	6.33 ± 0.07 A	7.62 ± 0.03 A
0.2%	1.70 ± 0.09 B	2.36 ± 0.03 B	2.72 ± 0.11 B	3.38 ± 0.05 B	4.27 ± 0.09 B	5.47 ± 0.05 B
0.4%	1.37 ± 0.13 C	2.13 ± 0.07 C	2.43 ± 0.06 C	3.06 ± 0.08 C	4.01 ± 0.15 C	5.07 ± 0.18 C
0.8%	0.19 ± 0.11 D	0.91 ± 0.24 D	1.16 ± 0.09 D	1.52 ± 0.09 D	2.95 ± 0.13 D	4.10 ± 0.13 D
Bacillus cereus BCRC 10603						
Control	1.87 ± 0.15 A	2.00 ± 0.13 A	2.34 ± 0.15 A	3.74 ± 0.07 A	5.38 ± 0.10 A	7.35 ± 0.12 A
0.2%	1.80 ± 0.14 A	1.98 ± 0.09 A	2.27 ± 0.06 A	3.61 ± 0.05 B	4.49 ± 0.08 B	5.94 ± 0.10 B
0.4%	1.75 ± 0.17 A	1.98 ± 0.11 A	2.07 ± 0.11 A	2.96 ± 0.16 C	4.06 ± 0.07 C	5.70 ± 0.05 C
0.8%	0.73 ± 0.67 B	1.36 ± 0.28 B	1.40 ± 0.73 B	2.43 ± 0.20 D	3.64 ± 0.06 D	5.49 ± 0.11 D
Vibrio parahaemolyticus BCRC 10806						
Control	2.13 ± 0.07 A	3.41 ± 0.20 A	4.40 ± 0.08 A	5.83 ± 0.13 A	5.76 ± 0.12 A	5.74 ± 0.14 A
0.2%	2.03 ± 0.04 B	2.63 ± 0.08 B	3.50 ± 0.07 B	4.70 ± 0.06 B	4.78 ± 0.07 B	4.87 ± 0.06 B
0.4%	1.88 ± 0.12 C	2.22 ± 0.13 C	3.00 ± 0.10 C	4.13 ± 0.14 C	4.42 ± 0.10 C	4.77 ± 0.08 B
0.8%	1.80 ± 0.14 C	2.00 ± 0.17 D	2.60 ± 0.02 D	2.95 ± 0.12 D	3.31 ± 0.09 D	3.57 ± 0.13 C

[a] Values are means ± standard deviations ($n = 3$). Means followed by different letters are significantly different ($P < 0.05$).

etable, and other items are popular in Taiwan. RTE sushi products and box meals are considered equivalent to sandwiches in the West. The use of gamma radiation to inhibit pathogens in such foods has been studied. Chung et al. *(7)* reported that 1 kGy of radiation decreased concentrations of pathogens (*Salmonella* Typhimurium*, E. coli, S. aureus,* and *Listeria ivanovii*) by 2 to 3 log CFU/g in Kimbab. The radiation doses needed to inactivate 1 log unit of pathogen in RTE sandwiches and other multicomponent RTE products were 0.61, 0.54, 0.47, 0.36, and 0.15 kGy for *Salmonella, S. aureus, L. monocytogenes, E. coli* O157:H7, and *Y. enterocolitica,* respectively *(29).* Oil of clove (*Syzgium aromaticum,* 1 to 2%) inhibited *L. monocytogenes* growth in RTE poultry products, reducing concentrations by 2.5 to 8.0 log CFU/g after 7 days of storage at 15°C *(20).* The inhibitory effect of our *M. candidum* acetone extracts in steamed rice stored at 30°C was similar to effects of other such inhibitors, i.e., better than the effect of radiation reported by Sommers and Boyd *(29)* and similar to the effect of clove oil reported by Mytle et al. *(20).* These results suggest that our extract has great potential as a preservative in RTE rice and related products.

Concentrations of viable test bacteria in fresh noodles stored at 30°C. Extracts at all concentrations significantly decreased ($P < 0.05$) the growth of total aerobes in fresh noodles during the 168-h storage period (Table 3). At all except the initial point, growth of total aerobes decreased with increasing extract concentration. Initially, all extracts (0.2 to 0.8%) reduced the concentrations of total aerobes by 0.31 to 0.45 log CFU/g, but the difference in reduction between the three extract concentrations was not significant ($P < 0.05$). After 168 h of storage, all extracts (0.2 to 0.8%) reduced the concentrations of total aerobes by 0.90 to 2.88 log CFU/g.

The growth of *S. aureus* in fresh noodles was significantly inhibited ($P < 0.05$) by all extract concentrations during the 72-h storage period (Table 3). Concentrations of viable *S. aureus* decreased with increasing extract concentration. Initially, all extracts (0.2 to 0.8%) reduced *S. aureus* concentrations by 0.22 to 1.73 log CFU/g, and at the end of storage concentrations were reduced by 2.56 to 2.86 log CFU/g.

Except during the first 12 h of the 72-h storage period, concentrations of viable *B. cereus* in fresh noodles significantly decreased with increasing extract concentration ($P < 0.05$). At 0.8%, *B. cereus* concentrations were reduced by 1.14 and 2.27 log CFU/g at the beginning and end of storage, respectively. At the end of storage (hour 72), all extracts (0.2 to 0.8%) reduced concentrations by 1.24 to 2.27 log CFU/g.

Growth of *V. parahaemolyticus* in fresh noodles decreased significantly ($P < 0.05$) with increasing extract concentration during most of the storage period (Table 3). Growth increased slowly after treatment with 0.8% extract (1.80 ± 0.14 log CFU/g at hour 0 and 3.89 ± 0.25 log CFU/g at hour 72). Initially, all extracts (0.2 to 0.8%) reduced *V. parahaemolyticus* concentrations by 0.10 to 0.33 log CFU/g, and after 72 h of storage, concentrations were reduced by 0.50 to 1.85 log CFU/g.

Fresh noodles contain a high level of moisture and

TABLE 3. *Extended*

Concn (log CFU/g)[a]					
60 h	72 h	96 h	120 h	144 h	168 h
	7.41 ± 0.26 A	7.98 ± 0.16 A	8.23 ± 0.06 A	9.08 ± 0.07 A	10.02 ± 0.06 A
	6.43 ± 0.11 B	6.99 ± 0.10 B	7.91 ± 0.10 B	8.45 ± 0.07 B	9.12 ± 0.06 B
	6.30 ± 0.10 B	6.88 ± 0.06 C	7.14 ± 0.10 C	8.08 ± 0.10 C	8.58 ± 0.07 C
	6.09 ± 0.12 C	6.36 ± 0.06 C	6.44 ± 0.03 D	6.96 ± 0.14 D	7.14 ± 0.17 D
8.96 ± 0.18 A	9.76 ± 0.30 A				
6.12 ± 0.13 B	7.20 ± 0.04 B				
5.91 ± 0.09 C	7.11 ± 0.07 B				
4.98 ± 0.11 D	6.90 ± 0.15 C				
8.32 ± 0.11 A	8.54 ± 0.07 A				
6.50 ± 0.10 B	7.30 ± 0.10 B				
6.13 ± 0.10 C	6.43 ± 0.13 C				
6.15 ± 0.12 C	6.27 ± 0.27 D				
5.71 ± 0.05 A	5.74 ± 0.08 A				
5.03 ± 0.10 B	5.24 ± 0.10 B				
4.92 ± 0.06 C	5.06 ± 0.08 C				
3.70 ± 0.12 D	3.89 ± 0.25 D				

therefore are easily attacked by foodborne pathogenic bacteria such as *S. aureus, Salmonella,* and *B. cereus (12, 26, 30).* Maillard reaction products prepared from chitosan and xylose have been active against *Bacillus subtilis* in fresh noodles. Addition of 0.5% acetic acid (0.05 g per 100 ml) to fresh noodle formulations stored at 4°C extended the shelf life by 6 days *(12).* No other study on fresh noodle preservation has been published. Our 0.8% extract dramatically decreased the growth of *S. aureus* and *V. parahaemolyticus* in fresh noodles.

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