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Combined Efficacy of Nisin and Pediocin with Sodium Lactate, Citric Acid, Phytic Acid, and Potassium Sorbate and EDTA in Reducing the *Listeria monocytogenes* Population of Inoculated Fresh-Cut Produce†

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ABSTRACT

The inability of chlorine to completely inactivate human bacterial pathogens on whole and fresh-cut produce suggests a need for other antimicrobial washing treatments. Nisin (50 μg/ml) and pediocin (100 AU/ml) individually or in combination with sodium lactate (2%), potassium sorbate (0.02%), phytic acid (0.02%), and citric acid (10 mM) were tested as possible sanitizer treatments for reducing the population of *Listeria monocytogenes* on cabbage, broccoli, and mung bean sprouts. Cabbage, broccoli, and mung bean sprouts were inoculated with a five-strain cocktail of *L. monocytogenes* at 4.61, 4.34, and 4.67 log CFU/g, respectively. Inoculated produce was left at room temperature (25°C) for up to 4 h before antimicrobial treatment. Washing treatments were applied to inoculated produce for 1 min, and surviving bacterial populations were determined. When tested alone, all compounds resulted in 2.20- to 4.35-log reductions of *L. monocytogenes* on mung bean, cabbage, and broccoli, respectively. The combination treatments nisin–phytic acid and nisin–pediocin–phytic acid caused significant ($P < 0.05$) reductions of *L. monocytogenes* on cabbage and broccoli but not on mung bean sprouts. Pediocin treatment alone or in combination with any of the organic acid tested was more effective in reducing *L. monocytogenes* populations than the nisin treatment alone. Although none of the combination treatments completely eliminated the pathogen on the produce, the results suggest that some of the treatments evaluated in this study can be used to improve the microbial safety of fresh-cut cabbage, broccoli, and mung bean sprouts.

During the past decade, the frequency of reported outbreaks of illnesses due to foodborne pathogens has increased *(39)*. *Listeria monocytogenes* is a particular food safety concern, because it is widespread in the environment *(3, 10)*, grows under refrigeration conditions *(13, 26)*, and is a frequent resident in certain food processing establishments *(6, 8, 18)*. The microorganism has been isolated from soil, sewage sludge, vegetation, and water *(6, 10)* and, therefore, has the potential to contaminate produce surfaces. Many vegetables, including bean sprouts, cabbage, cucumber, potatoes, and radishes, have been found to be contaminated with *L. monocytogenes (1, 2, 10, 14, 18, 19)*. The pathogen has been reported to survive long-term storage on leafy vegetables *(8, 16)*, has been responsible for numerous product recalls of salads *(43)*, and was responsible for an outbreak of foodborne disease due to coleslaw prepared from contaminated raw cabbage *(2)*.

The level of sanitation and the populations of microorganisms are of primary importance to the quality, shelf stability, and safety of fresh produce *(7, 25, 26)*. The U.S. Food and Drug Administration and the U.S. Department of Agriculture, U.S. Food Safety Inspection Service have established a zero tolerance (no detectable level permitted) for *L. monocytogenes* in ready-to-eat foods, including processed fresh-cut fruits and vegetables. Chlorination of wash water up to 200 ppm is routinely applied to reduce microbial contamination in produce processing lines *(42)*. However, the use of chlorine water is of concern due to the potential formation of harmful by-products *(28)*, and only approximately 2- to 3-log reductions of native microflora have been reported *(36)*. Thus, much interest exists in developing safer and more effective sanitizers. Recently, Ukuku and Fett *(37)* reported that treatment of whole and fresh-cut cantaloupe and honeydew melon with nisin-EDTA significantly reduced the natural microflora and extended the shelf-life.

Nisin and pediocin are produced by lactic acid bacteria that are often found on produce *(15, 21, 24)*. There are several reports that nisin used in combination with a chelating agent exhibits a bactericidal effect toward both gram-positive and gram-negative bacteria *(5, 11, 30)*. In the current study, the efficacy of nisin and pediocin treatments in combination with EDTA, citric acid (CA), sodium lactate (NaL), potassium sorbate (KS), and phytic acid (Phy) in reducing populations of *L. monocytogenes* inoculated on fresh-cut produce was investigated.

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† Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. A mixed bacterial cocktail that contained five strains of *L. monocytogenes* ATCC 43256 (American Type Culture Collection, Manassas, Va.) (Mexican-style cheese), ATCC 49594 (strain Scott A), JCM 7676 (Japan Collection of Microorganisms) (roast beef), JCM 7672 (salami sausage), and JCM 7671 (lax ham) was used for the study. Individual cell cultures were prepared by inoculating from stock cultures stored at −80°C into 5 ml of Trypticase soy broth (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) that contained 50 μg/ml of nalidixic acid and incubating for 18 h at 37°C. Two successive loop cultures were made with a final transfer of 0.2 ml into 20 ml of Trypticase soy broth that contained yeast extract and 50 μg/ml of nalidixic acid and incubation at 36°C for 18 h under static conditions. Populations of individual cultures before mixing ranged from 1.63 to 2.11 × 10^8 CFU/ml as determined by plating serial dilutions onto tryptic soy agar (Nissui) with incubation at 37°C for 24 h. The bacterial cells were harvested by centrifugation (3,000 × *g*, 10 min) at 20°C, and the cell pellets were resuspended in 5 ml of phosphate-buffered saline (pH 7.2). Plating the inocula on media that contained nalidixic acid greatly minimized interference with naturally occurring microorganisms, thus facilitating detection of the test pathogen on the media. The final bacterial concentration in the inoculum was approximately 6.40 to 6.65 × 10^7 CFU/ml by plating on tryptic soy agar that contained nalidixic acid. The inoculum was maintained at 21 ± 1°C and applied to fresh-cut produce within 1 h of preparation.

Pediocin preparation and purification. *Pediococcus acidilactici* strain LET 01 isolated from uncured ham (provided by Dr. Takashi Sameshima, PRIMA Meat Packers Ltd., Ibaraki, Japan) was used for the production of pediocin. Pediocin preparation and purification were performed as described by Cintas et al. *(9)* with modifications. *P. acidilactici* strains were grown in M-17 medium (1.5 liters; Difco, Becton Dickinson, Sparks, Md.) that contained 0.1% Tween 80 (Kishida Kagaku Co. Ltd., Osaka, Japan) and 10% glucose at 30°C until the stationary phase was reached (16 h). The culture was then heat treated at 70°C for 60 min to inactivate the cells, and the cells were removed by centrifugation at 10,000 × *g* for 10 min at 4°C, after which the supernatant fluid was adjusted to pH 5.7 using a 10% NaOH solution. The pediocin was precipitated from the supernatant fluid by using ammonium sulfate (final concentration of 60%, wt/vol) and was collected by centrifugation at 12,000 × *g* for 20 min at 4°C and resuspended in 50 ml of phosphate buffer (5.0 mM, pH 5.7).

The crude extract was filtered through a 0.45-μm-pore-size membrane filter (Millipore SA, Saint-Quentin-Yvelines, France), and the filtrate was then applied onto a 35-ml Sep-Pak Plus C_{18} column (Waters, Milford, Mass.) equilibrated with 5.0 mM phosphate buffer (pH 5.7). The column was washed with a 2-bed volume of 20% acetonitrile that contained 0.1% trifluoroacetic acid before addition of the filtered crude extract. Pediocin was eluted with 80% acetonitrile that contained 0.1% trifluoroacetic acid. Fractions (1.5 ml each) were collected, and a 2-μl portion of each fraction was tested for antimicrobial activity as described below. Active fractions that contained the purified pediocin were pooled or combined, dried under vacuum, and stored at −20°C until use. The specific activity of pediocin thus prepared was approximately 420 AU/mg, determined as described below.

Antimicrobial bioassay. The spot on-lawn method as described by van Reenen et al. *(41)* was used to determine the antimicrobial activity of pediocin against *L. monocytogenes.* A clear inhibition zone of at least 2 mm in diameter was recorded as positive. One arbitrary unit (AU) of pediocin activity was defined as the reciprocal of the highest dilution that produced an inhibition zone of at least 2.0 mm in diameter.

Preparation of fresh produce and inoculation. Commercial cabbage, mung bean sprouts, and broccoli used in these experiments were purchased from a local supermarket and stored at refrigerated temperature (4°C) for up to 6 h before use. Broccoli and cabbage were cut into pieces (3 by 3 cm) with a sterile knife on a cutting board. Five hundred grams of mung bean sprouts, broccoli, and cabbage was dipped in 2 liters of the *L. monocytogenes* cocktail suspension (approximately 10^8 CFU/ml) for 1 min. After the inoculum was decanted, produce was placed separately on a sterile perforated tray lined with four layers of cheesecloth and dried in a biosafety cabinet at room temperature (21 ± 1°C) for 2 h.

Preparation of wash solutions. A stock solution of nisin (10^6 IU, Sigma, St. Louis, Mo.) was prepared at a concentration of 2,500 μg/ml in 0.02 N hydrochloric acid. A stock solution of pediocin described above was prepared at a concentration of 48 mg/ml in double distilled water (ddH_2O). The CA (Kanto Chemical Co. Ltd., Tokyo, Japan) was prepared at a concentration of 100 mM in ddH_2O. A stock solution of 2 M disodium EDTA (Wako Chemical Co. Ltd., Tokyo, Japan) was prepared in ddH_2O, autoclaved at 121°C for 15 min, and then stored at room temperature until used. The NaL (60%, wt/vol; Nacalai Tesque, Inc., Kyoto, Japan) was prepared at a concentration of 20% in ddH_2O. The KS (Wako Chemical) was prepared at a concentration of 1% in ddH_2O. The Phy (50%, vol/vol; Wako Chemical) was prepared at a concentration of 2% in ddH_2O. All stock solutions were filter sterilized (0.22 μm; Millipore, Inc., Bedford, Mass.). Test solutions were prepared as follows: (i) CA was added to ddH_2O to give a final concentration of 10 mM, (ii) disodium EDTA was added to ddH_2O to give a final concentration of 0.02 M, (iii) nisin was prepared by dilution of the stock solution in 0.02 N hydrochloric acid to give 50 μg/ml of nisin, (iv) NaL was prepared by dilution of the stock solution in ddH_2O to give a 2% solution, (v) KS was prepared by dilution of the stock solution in ddH_2O to give a 0.02% solution, and (vi) Phy was also prepared by dilution of the stock solution in ddH_2O to give a 0.02% solution. The purified pediocin prepared as described previously was diluted 200 times in ddH_2O and used for the washing treatments.

Washing treatments. Final concentrations of the chemicals used alone or in combination were 0.02 M EDTA, 50 μg/ml of nisin, 2% NaL, 0.02% KS, 0.02% Phy, 10 mM CA, and 48 mg/ml of pediocin. Two hours after inoculation, 25 g each of inoculated sprouts, fresh-cut broccoli, and cabbage pieces was placed in a Ziploc bag that contained 50 ml of antimicrobial solutions and washed vigorously with agitation for 1 min. The produces were then transferred to a clean stomacher bag (ELMEX Co Ltd., Tokyo, Japan).

Microbiological analyses. One hundred milliliters of 0.1% peptone water was added to stomacher bags that contained individual fresh-cut pieces, and the bag contents were pummeled for 60 s in a stomacher (model CE-97, ILU Instruments, Barcelona, Spain) at medium speed. Serial decimal dilutions were prepared with 0.1% peptone water, and diluted and undiluted samples were surface plated (0.1 ml, in duplicate) onto tryptose phosphate agar and modified Oxford medium (Oxoid, CM 856, Unipath-Oxoid, Hampshire, England), both supplemented with 50 μg/ml of nalidixic acid for enumeration of *L. monocytogenes* with incubation at 37°C for 48 h. Representative presumptive colonies of *L. monocytogenes* were subjected to confirmation by use of API *Listeria*

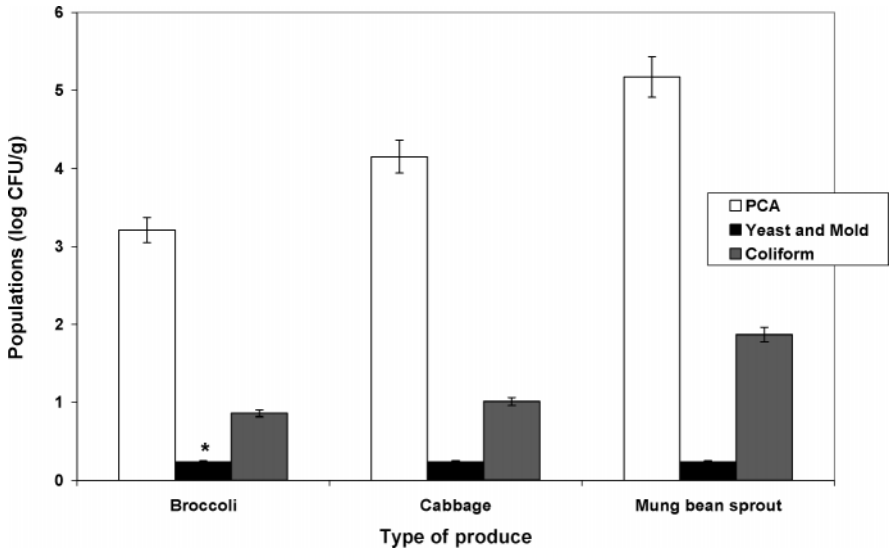

FIGURE 1. *Initial populations of natural microflora (plate count agar) of broccoli, cabbage, and mung bean sprouts before inoculation and treatments. Values are means ± standard deviations of three experiments with duplicate determinations. Asterisk indicates below detection limit of 1 CFU/g.*

test kits (bioMerieux, Inc., Marcy l'Etoile, France). Plate count agar (BBL, Becton Dickinson, Sparks, Md.) and potato dextrose agar (Nissui) with incubation at 30°C for 3 days were used for enumeration of mesophilic aerobes and yeasts and molds, respectively, before and after the treatments. Desoxycholate agar (Nissui) with incubation at 37°C for 48 h was used for the enumeration of coliforms.

Statistical analyses. All experiments were repeated three times, and duplicate samples were analyzed at each sampling time. Data were subjected to the Statistical Analysis System (SAS Institute Inc., Cary, N.C.) for analysis of variance and the Bonferroni least significant difference method *(30)* to determine if there were significant differences ($P < 0.05$) among mean values of the number of cells recovered after each treatment.

RESULTS AND DISCUSSION

Natural microflora of fresh-cut cabbage, broccoli, and mung bean sprouts. The initial populations of natural microflora of the broccoli, cabbage, and mung bean sprouts determined after purchase varied among the produce (Fig. 1). Broccoli had the least population of aerobic mesophilic bacteria followed by cabbage and mung bean sprouts. No *L. monocytogenes* was detected in the produce, and the level of yeasts and molds was below the level of detection (<1.0 CFU/g). The level of total coliforms determined in all of the produce tested was approximately 0.86 CFU/g for broccoli, 1.01 CFU/g for cabbage, and 1.87 CFU/g for mung bean sprouts. All antimicrobial treatments significantly ($P < 0.05$) reduced the native microflora of the broccoli, cabbage, and mung bean sprout samples compared with control samples. Yeast and mold and total coliform of all treated fresh-cut produce were below detection (<1 CFU/g) (data not shown).

Effect of antimicrobial treatments on inoculated fresh-cut produce. The average populations of *L. monocytogenes* recovered from each inoculated fresh-cut produce sample using modified Oxford medium and tryptose phosphate agar were 4.43 and 4.54 log CFU/g, respectively. The addition of nalidixic acid into the agar media was to minimize interference from the natural microflora of produce on recovery of *L. monocytogenes.* Previous studies *(12, 32)* have shown that certain selective media do not support colony development of *L. monocytogenes* exposed to treatments with some antimicrobials. In the current study, populations of *L. monocytogenes* recovered using tryptose phosphate agar were slightly higher (approximately 0.10 log CFU/g) than those recovered on modified Oxford medium, but they were not significantly ($P > 0.05$) different. The slight difference in populations of recovered *L. monocytogenes* using the two selective media is in agreement with other reports where lower populations of *L. monocytogenes* were recovered from treated fresh-cut produce on selective medium *(4).*

Levels of *L. monocytogenes* populations recovered from fresh-cut produce washed with water were not significantly ($P > 0.05$) different from results of the untreated controls, with counts generally 0.10- to 0.60-log CFU/g lower with samples washed with water. Washing inoculated fresh-cut cabbage with nisin, pediocin, EDTA, NaL, CA, Phy, and KS individually caused approximately 1.5-log CFU/g reductions in *L. monocytogenes,* which was not significantly ($P > 0.05$) different, irrespective of the media used (data not shown). Population reductions of *L. monocytogenes* in fresh-cut produce washed with nisin or pediocin individually were not significantly ($P > 0.05$) different than when the bacteriocins were combined with EDTA, NaL, CA, Phy, and KS (Table 1). Nisin treatments alone caused more reduction of *L. monocytogenes* in broccoli and cabbage than in mung bean sprouts. Also, the efficacy of nisin treatment alone is better than pediocin in reducing the populations of *L. monocytogenes* in fresh-cut broccoli and cabbage but not in mung bean sprouts. Nisin treatment in combination with EDTA, lactate, sorbate, and Phy resulted in an approximately 3.00-log CFU/g reduction of *L. mono-*

TABLE 1. *Populations of* Listeria monocytogenes *recovered from cabbage following antimicrobial treatments*[a]

Treatment	Population recovered (log CFU/g)		Reduction (log CFU/g)[b]
	MOXN	TPAN	
Control	4.55 ± 0.15 A	4.61 ± 0.19 A	—
Distilled water	3.51 ± 0.05 B	3.75 ± 0.07 B	0.86
Pediocin	2.30 ± 0.30 C	2.67 ± 0.15 C	1.94
Pediocin + 10 mM CA	2.28 ± 0.17 C	2.40 ± 0.23 D	2.21
Pediocin + 0.02% KS	2.31 ± 0.09 C	2.65 ± 0.12 C	1.96
Pediocin + 0.02 M EDTA	1.61 ± 0.13 EF	2.17 ± 0.21 E	2.44
Pediocin + 2% NaL	2.43 ± 0.10 C	2.57 ± 0.11 CD	2.04
Pediocin + 0.02% Phy	2.06 ± 0.31 D	2.11 ± 0.20 E	2.50
Nisin	1.59 ± 0.10 EF	1.84 ± 0.15 F	2.77
Nisin + 10 mM CA	1.70 ± 0.12 E	2.14 ± 0.21 E	2.47
Nisin + 0.02% KS	1.43 ± 0.09 FG	2.18 ± 0.10 E	2.43
Nisin + 0.02 M EDTA	1.46 ± 0.12 FG	1.67 ± 0.13 F	2.94
Nisin + 2% NaL	1.36 ± 0.10 G	1.39 ± 0.14 G	3.22
Nisin + 0.02% Phy	0.24 ± 0.09 H	0.26 ± 0.10 I	4.35
Nisin + Pediocin + 0.02% Phy	0.72 ± 0.18 I	0.91 ± 0.09 H	3.70

[a] Values are means ± standard deviations of three replicate experiments. Means in each column not followed by the same letter are significantly ($P < 0.05$) different by the Bonferroni least significant difference means separation technique. MOXN, modified Oxford medium; TPAN, tryptose phosphate agar; CA, citric acid; KS, potassium sorbate; NaL, sodium lactate; Phy, phytic acid.

[b] Within the same condition, log reduction compared with the number recovered from control on TPAN.

cytogenes. Antimicrobial treatment that contained nisin plus Phy or nisin plus pediocin plus Phy combinations were the most effective in reducing the populations of inoculated *L. monocytogenes* on fresh-cut broccoli and cabbage but not on mung bean sprouts.

Treatment with pediocin, pediocin-CA, pediocin-EDTA, pediocin-lactate, pediocin-sorbate, pediocin-lactate, and pediocin-Phy combinations resulted in an approximately 2.0-log CFU/g reduction of *L. monocytogenes* on cabbage and broccoli, irrespective of the combination used (Tables 1 and 2). All treatments were less effective in reducing the populations of *L. monocytogenes* on mung bean sprouts (Table 3) compared with broccoli or cabbage (Tables 1 and 2). The most effective antimicrobial treatment combination on mung bean sprouts was nisin plus pediocin plus Phy, which caused a 2.31-log CFU/g reduction.

Phytic acid naturally exists in the form of phytin and is traditionally extracted from rice bran or legumes. It has been used in food processing at 0.02 to 0.05% to improve the color of canned foods, soft drinks, and milk or to shorten fermentation time to improve color and aroma *(20, 35)*. However, Phytate is used in some countries at very low

TABLE 2. *Populations of* Listeria monocytogenes *recovered from broccoli following antimicrobial treatments*[a]

Treatment	Population recovered (log CFU/g)		Reduction (log CFU/g)[b]
	MOXN	TPAN	
Control	4.29 ± 0.12 A	4.32 ± 0.08 A	—
Distilled water	3.65 ± 0.08 B	3.80 ± 0.10 B	0.48
Pediocin	2.77 ± 0.16 CD	3.00 ± 0.10 C	1.11
Pediocin + 10 mM CA	1.90 ± 0.09 G	1.95 ± 0.13 G	2.35
Pediocin + 0.02% KS	2.87 ± 0.12 C	2.74 ± 0.10 D	1.43
Pediocin + 0.02 M EDTA	2.40 ± 0.07 F	2.42 ± 0.09 F	1.90
Pediocin + 2% NaL	2.76 ± 0.18 CD	2.80 ± 0.12 DE	1.53
Pediocin + 0.02% Phy	2.50 ± 0.18 EF	2.57 ± 0.14 E	1.70
Nisin	1.67 ± 0.15 H	1.73 ± 0.10 H	2.55
Nisin + 10 mM CA	2.51 ± 0.09 EF	2.57 ± 0.12 E	1.70
Nisin + 0.02% KS	2.68 ± 0.19 D	2.79 ± 0.18 DE	1.55
Nisin + 0.02 M EDTA	1.36 ± 0.10 I	1.62 ± 0.15 GH	2.47
Nisin + 2% NaL	2.64 ± 0.24 DE	2.69 ± 0.16 E	1.65
Nisin + 0.02% Phy	0.14 ± 0.05 K	0.16 ± 0.08 J	4.18
Nisin + Pediocin + 0.02% Phy	0.31 ± 0.03 J	0.44 ± 0.07 I	3.90

[a] Values are means ± standard deviations of three replicate experiments. Means in each column not followed by the same letter are significantly ($P < 0.05$) different by the Bonferroni least significant difference means separation technique. MOXN, modified Oxford medium; TPAN, tryptose phosphate agar; CA, citric acid; KS, potassium sorbate; NaL, sodium lactate; Phy, phytic acid.

[b] Within the same condition, log reduction compared with the number recovered from control on TPAN.

TABLE 3. *Populations of* Listeria monocytogenes *recovered from mung bean sprout following antimicrobial treatments*[a]

Treatment	Population recovered (log CFU/g)		Reduction (log CFU/g)[b]
	MOXN	TPAN	
Control	4.45 ± 0.22 A	4.56 ± 0.17 A	—
Distilled water	3.52 ± 0.05 BC	3.77 ± 0.09 BC	0.90
Pediocin	3.00 ± 0.12 FG	3.05 ± 0.23 GH	1.54
Pediocin + 10 mM CA	2.96 ± 0.10 G	3.08 ± 0.16 H	1.59
Pediocin + 0.02% KS	3.46 ± 0.15 BCD	3.61 ± 0.24 CD	1.06
Pediocin + 0.02 M EDTA	3.13 ± 0.13 EFG	3.29 ± 0.09 FGH	1.38
Pediocin + 2% NaL	3.28 ± 0.10 E	3.47 ± 0.16 DE	1.20
Pediocin + 0.02% Phy	3.27 ± 0.17 E	3.31 ± 0.13 EFG	1.36
Nisin	3.18 ± 0.08 EF	3.36 ± 0.21 EF	1.31
Nisin + 10 mM CA	3.29 ± 0.12 DE	3.44 ± 0.09 DE	1.23
Nisin + 0.02% KS	3.55 ± 0.25 B	3.85 ± 0.17 B	0.82
Nisin + 0.02 M EDTA	3.01 ± 0.16 FG	3.17 ± 0.10 FGH	1.50
Nisin + 2% NaL	3.31 ± 0.31 CDE	3.61 ± 0.22 CD	1.06
Nisin + 0.02% Phy	2.95 ± 0.15 FG	3.10 ± 0.13 GH	1.57
Nisin + Pediocin + 0.02% Phy	2.29 ± 0.10 H	2.36 ± 0.14 I	2.31

[a] Values are means ± standard deviations of three replicate experiments. Means in each column not followed by the same letter are significantly ($P < 0.05$) different by the Bonferroni least significant difference means separation technique. MOXN, modified Oxford medium; TPAN, tryptose phosphate agar; CA, citric acid; KS, potassium sorbate; NaL, sodium lactate; Phy, phytic acid.

[b] Within the same condition, log reduction compared with the number recovered from control on TPAN.

concentrations and may not be approved for use in all countries. Although certain food matrices have been shown to inhibit the efficacy of nisin *(27)*, the emergence of nisin tolerance in certain bacteria such as *L. monocytogenes* has been reported *(29, 38)*. Nevertheless, a strategy that would involve combining nisin with organic acids or chelators might assist in overcoming some of these concerns and further extend the range of applications of nisin in food processing. Ukuku and Fett *(36)* reported reductions in the surface microflora of whole melon and extension of the shelf-life of fresh-cut pieces using nisin-EDTA. In addition, nisin in combination with NaL, EDTA, and/or KS caused a significant reduction of inoculated *Salmonella* spp. populations on whole melon surfaces and reduced the populations of the pathogen transferred to fresh-cut pieces during cutting *(37)*. Nisin and some of the organic acids, such as sorbate, both act on the cytoplasmic membrane of bacteria, leading to an additive or synergistic effect, suggesting that both compounds may be used as antimicrobials in foods at lower levels without diminishing their inhibitory effects. Of the many bacteriocins produced by lactic acid bacteria, only nisin has been approved for use as a food preservative in 50 countries, and it is the only purified bacteriocin that is commercially available *(17, 33)*. The bactericidal activity of nisin increases at pH levels below 5, due to its greater solubility at low pH *(31, 40)*. This makes nisin suitable for use on fruit and vegetables with pH levels in the range of 3 to 6.

TABLE 4. *The pH of the treatment solutions*[a]

Treatment solution	pH
Control (ddH_2O)	6.9 ± 0.01
Pediocin	3.4 ± 0.02
Pediocin + 10 mM CA	1.8 ± 0.02
Pediocin + 0.02% KS	5.0 ± 0.03
Pediocin + 0.02 M EDTA	2.1 ± 0.04
Pediocin + 2% NaL	5.7 ± 0.03
Pediocin + 0.02% Phy	2.6 ± 0.02
Nisin	2.8 ± 0.03
Nisin + 10 mM CA	1.8 ± 0.01
Nisin + 0.02% KS	4.8 ± 0.01
Nisin + 0.02 M EDTA	2.0 ± 0.02
Nisin + 2% NaL	5.8 ± 0.01
Nisin + 0.02% Phy	2.5 ± 0.02
Nisin + Pediocin + 0.02% Phy	2.3 ± 0.04

[a] Values are means ± standard deviations of three replicate experiments. ddH_2O, double distilled water, Ca, citric acid; KS, potassium sorbate; NaL, sodium lactate; Phy, phytic acid.

The pH levels of all antimicrobial solutions used in this study are presented in Table 4. Sterile tap water (control) had the highest pH value. The pH of pediocin or nisin individually or in combination with CA, EDTA, lactate, sorbate, and Phy varied among the different solutions but was below 5.0. The pH of pediocin and nisin in combination with CA was the lowest, but this combination did not give the highest reductions. Populations of *L. monocytogenes* recovered from inoculated fresh-cut produce were below 2.0 log units. These reductions on fresh-cut vegetables are similar to or greater than those achieved with aqueous chemical sanitizers or by washing with water. Foods that have been implicated in *Listeria* outbreaks generally contain more than 3.0 log units of bacteria per g or ml *(34)*. The combined treatments of nisin or pediocin with short chain organic acids tested in this study are consistent with the hurdle concept to ensure the safety of food *(22)*. The reduced efficacy of nisin or pediocin alone or in combination with any of the organic acids tested on mung bean sprouts compared with cabbage and broccoli may be attributed to the

presence of inaccessible sites for the antimicrobial solutions to inactivate bacteria attached to mung bean sprouts. Any of these treatment combinations can be combined with other control measures, such as modified atmosphere, water activity, pH, and temperature, to maximize protection from foodborne pathogens on fresh-cut produce *(23)*.

In conclusion, pediocin and nisin applications in combination with organic acids caused a significant reduction of native microflora and inoculated populations of *L. monocytogenes* on fresh produce. Nisin is a natural antimicrobial compound and may provide a novel, environmentally safe alternative for control of bacterial contamination of produce. Although total inactivation of *L. monocytogenes* on produce surfaces tested was not achieved by the individual or the combined antimicrobial agents, nisin in combination with Phy significantly reduced the population of *L. monocytogenes* on cabbage and broccoli. Therefore, this combination could be useful in controlling the population of *L. monocytogenes* on cabbage and broccoli.

REFERENCES

1. Berrang, M. E., R. E. Brackett, and L. R. Beuchat. 1989. Growth of *Listeria monocytogenes* on fresh vegetables stored under a controlled atmosphere. *J. Food Prot.* 52:702–705.
2. Beuchat, L. R. 1996. *Listeria monocytogenes*: incidence on vegetables. *Food Control* 7:223–228.
3. Beuchat, L. R., and R. E. Brackett. 1991. Behavior of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. *Appl. Environ. Microbiol.* 57:1367–1371.
4. Beuchat, L. R., T. E. Ward, and C. A. Pettigrew. 2001. Comparison of chlorine and a prototype produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. *J. Food. Prot.* 64:152–158.
5. Blackburn, P., J. Polack, S. Gusik, and S. D. Rubino. 1989. Nisin compositions for use as enhanced, broad range bacteriocins. International patent application number PCT/US89/02625; international publication number W089/12399. Applied Microbiology, Inc., New York.
6. Brackett, R. E. 1988. Presence and persistence of *Listeria monocytogenes* in food and water. *Food Technol.* 42:162–164.
7. Brackett, R. E. 1992. Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *J. Food Prot.* 55:808–814.
8. Carlin, F., and C. Nguyen-The. 1996. Fate of *Listeria monocytogenes* on four types of minimally processed green salads. *Lett. Appl. Microbiol.* 18:222–226.
9. Cintas, L. M., J. M. Rodriguez, M. F. Fernandez, K. Sletten, I. F. Nes, P. E. Hernandez, and H. Holo. 1995. Isolation and characterization of pediocin L50, a new bacteriocin from *Pediococcus acidilactici* with a broad inhibitory spectrum. *Appl. Environ. Microbiol.* 61:2643–2648.
10. Cox, L. J., T. Kleiss, J. L. Cordier, C. Cordellana, P. Konkel, C. Pedrazzini, R. Beumer, and A. Siebenga. 1989. *Listeria* spp. in food processing, non-food and domestic environments. *Food Microbiol.* 6:49–61.
11. Cutter, C. N., and G. R. Siragusa. 1995. Population reductions of gram negative pathogens following treatments with nisin and chelators under various conditions. *J. Food Prot.* 58:977–983.
12. Delaquis, P. J., P. L. Sholberg, and K. Stanich. 1999. Disinfection of mung bean seeds with gaseous acetic acid. *J. Food Prot.* 62:953–957.
13. Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55:476–511.
14. Farber, J. M., G. W. Sanders, and M. A. Johnston. 1989. A survey of various foods for the presence of *Listeria* species. *J. Food Prot.* 52:456–458.
15. Fleming, H. P., J. L. Etchells, and R. N. Costilow. 1975. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. *Appl. Microbiol.* 30:1040–1042.
16. Francis, G. A., and D. O'Beirne. 1997. Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *Listeria innocua* and *Listeria monocytogenes* on minimally processed lettuce. *Int. J. Food Sci. Technol.* 32:141–151.
17. Gould, G. W. 1996. Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. *J. Food Prot.* 1996(Suppl.):82–86.
18. Heisick, J. E., L. Rosas-Marty, and S. R. Tatini.1995. Enumeration of viable *Listeria* species and *Listeria monocytogenes* in foods. *J. Food Prot.* 58:733–736.
19. Heisick, J. E., D. E. Wagner, M. L. Nierman, and J. T. Peeler. 1989. *Listeria* spp. found on fresh market produce. *Appl. Environ. Microbiol.* 55:1925–1927.
20. Hiasa, Y., Y. Kitahori, J. Morimoto, N. Konishi, S. Nakaoka, and H. Nishioka. 1992. Carcinogenicity study in rats of phytic acid 'Daiichi,' a natural food additive. *Food Chem. Toxicol.* 30:117–125.
21. Jack, R. W., G. Bierbaum, and H.-G. Sahl. 1998. Biological activities, p. 157–190. *In* Lantibiotics and related peptides. Springer-Verlag and Landes Bioscience, Georgetown, Tex.
22. Leistner, L. 1992. Food preservation by combined methods. *Food Res. Int.* 25:151–158.
23. Leverentz, B., W. J. Janisiewicz, and W. S. Conway. 2002. Biological control of minimally processed fruits and vegetables, p. 319–332. *In* J. S. Novak, G. M. Sapers, and V. K. Juneja (ed.), Microbial safety of minimally processed foods. CRC Press, Boca Raton, Fla.
24. Li, J., M. L. Chikindas, R. D. Ludescher, and T. J. Montville. 2002. Temperature- and surfactant-induced membrane modifications that alter *Listeria monocytogenes* nisin sensitivity by different mechanisms. *Appl. Environ. Microbiol.* 68:5904–5910.
25. Madden, J. M. 1992. Microbial pathogens in fresh produce-the regulatory perspective. *J. Food Prot.* 55:821–823.
26. Marston, E. V. 1995. Fresh-cut fruits: maximizing quality. *Cutting Edge* 9:3–5.
27. Pol, I. E., and E. J. Smid. 2000. Combined action of nisin and carvacrol on *Bacillus cereus* and *Listeria monocytogenes. Lett. Appl. Microbiol.* 29:166–170.
28. Richardson, S. D., A. D. Thurston, Jr., T. V. Caughran, T. W. Collette, K. S. Patterson, and B. W. Lykins, Jr. 1998. Chemical by-products of chlorine and alternative disinfectants. *Food Technol.* 52:58–61.
29. Schillinger, U., and F. K. Lucke. 1989. Antibacterial activity of *Lactobacillus* sake isolated from meat. *Appl. Environ. Microbiol.* 55: 1901–1906.
30. Stevens, K. A., N. A. Klapes, B. W. Sheldon, and T. R. Klaenhammer. 1992. Effect of treatment conditions on nisin inactivation of gram-negative bacteria. *J. Food Prot.* 55:763–766.
31. Stiles, M. E. 1994. Potential for biological control of agents of foodborne disease. *Food Res. Int.* 27:245–250.
32. Taormina, P. J., and L. R. Bauchat. 1999. Behavior of enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa sprouts during the sprouting process as influenced by treatments with various chemicals. *J. Food Prot.* 62:850–856.
33. Thomas, L. V., M. R. Clarkson, and J. Delves-Broughton. 2000. Nisin, p. 463–524. *In* A. S. Naidu (ed.), Natural food antimicrobial systems. CRC Press, Boca Raton, Fla.
34. Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. *J. Food Prot.* 65:709–725.
35. Torre, M., A. R. Rodriguez, and F. Saura-Calixto. 1991. Effects of dietary fiber and phytic acid on mineral availability. *Crit. Rev. Food Sci. Nutr.* 0.30:1–22.
36. Ukuku, D. O., and W. F. Fett. 2002. Effectiveness of chlorine and nisin-EDTA treatments of whole melons and fresh-cut pieces for reducing native microflora and extending shelf life. *J. Food Saf.* 22: 231–253.
37. Ukuku, D. O., and W. F. Fett. 2004. Effect of nisin in combination with EDTA, sodium lactate and potassium sorbate for reducing *Salmonella* on whole and fresh-cut cantaloupe. *J. Food Prot.* 67:2143–2150.
38. Ukuku, D. O., and L. A. Shelef. 1997. Sensitivity of six strains of *Listeria monocytogenes* to Nisin. *J. Food Prot.* 60:867–869.

39. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2001. Direct food substances affirmed as generally recognized as safe, p. 462–464. *In* Title 21 of the Code of Federal Regulations—Food and Drugs, chapter 1, part 184. U.S. Food and Drug Administration, U.S. Department of Health and Human Services, Washington, D.C.
40. Vandenbergh, P. A. 1993. Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiol. Rev.* 12:221–238.
41. van Reenen, C. A., L. M. Dicks, and M. L. Chikindas. 1998. Isolation, purification and partial characterization of plantaricin 423, a bacteriocins produced by *Lactobacillus plantarum. J. Appl. Microbiol.* 84:1131–1137.
42. Wei, C. I., D. L. Cook, and J. R. Kirk. 1985. Use of chlorine compounds in the food industry. *Food Technol.* 39:107–115.
43. Wong, S., D. Street, S. I. Delgado, and K. C. Klontz. 2000. Recalls of foods and cosmetics due to microbial contamination reported to the U.S. Food and Drug Administration. *J. Food Prot.* 63:1113–1116.